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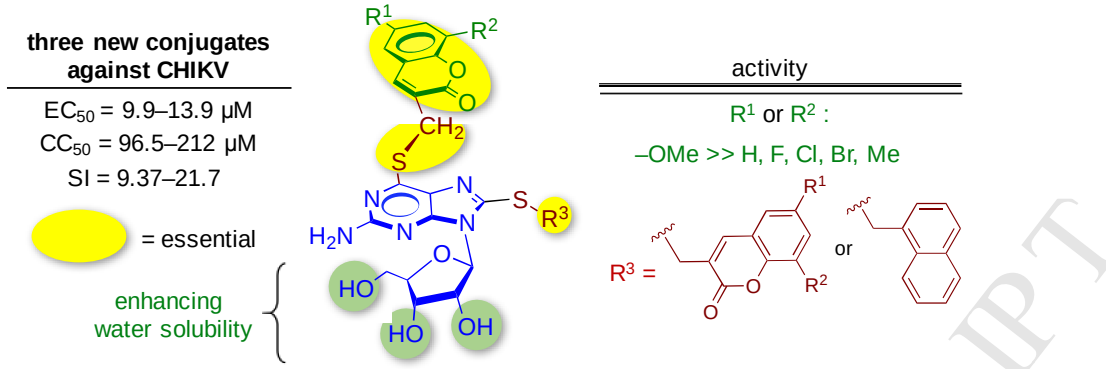
Graphical abstract



Chikungunya Virus Inhibition by Synthetic Coumarin–Guanosine Conjugates

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ABSTRACT

Since its discovery in Tanganyika, Africa in 1952, chikungunya virus (CHIKV) outbreaks have occurred in Africa, Asia, Europe, and America. Till now chikungunya fever has spread in nearly 40 countries. Because of lack of effective vaccines and antiviral drugs to intervene this disease, 21 new conjugated compounds were designed and synthesized by coupling of 6,8-dithioguanosine at its C-6 position with 3-(chloromethyl)coumarins bearing an F, Cl, Br, Me, or –OMe substituent through the $–SCH_2–$ joint. Meanwhile, an organic "dummy" ligand (e.g., methyl, benzyl, and naphthylmethyl) or a coumarinyl moiety was attached at the C-8 position. By high through-put screening, three of these new conjugates were found to inhibit CHIKV in Vero cells with significant potency (EC_{50} = 9.9–13.9 μM) and showed low toxicity (CC_{50} = 96.5–212 μM). The selectivity index values were 9.37–21.7. Their structure–activity relationship was deduced, which indicates that the coumarin moiety is essential and the presence of a –OMe group enhances the antiviral activity.

1. Introduction

As an emerging disease, chikungunya (CHIK) fever causes a major medical problem at the present time. In 1952, this arboviral disease was first recognized in East Africa [1]. There were >146,900 confirmed cases in 2016, including those from Africa, Argentina, Brazil, Bolivia, and Colombia. The CHIK illness is characterized by abrupt onset of fever, headache, fatigue, nausea, vomiting, rash, myalgia, and severe arthralgia. This disease often incurs serious economic and social impact on the individual and even the communities [2]. Unfortunately, current treatments of CHIK fever are merely for symptoms with no effective licensed vaccine [3]. Even worse is that the current world still has no specific antiviral drug for the treatment of the CHIK fever.

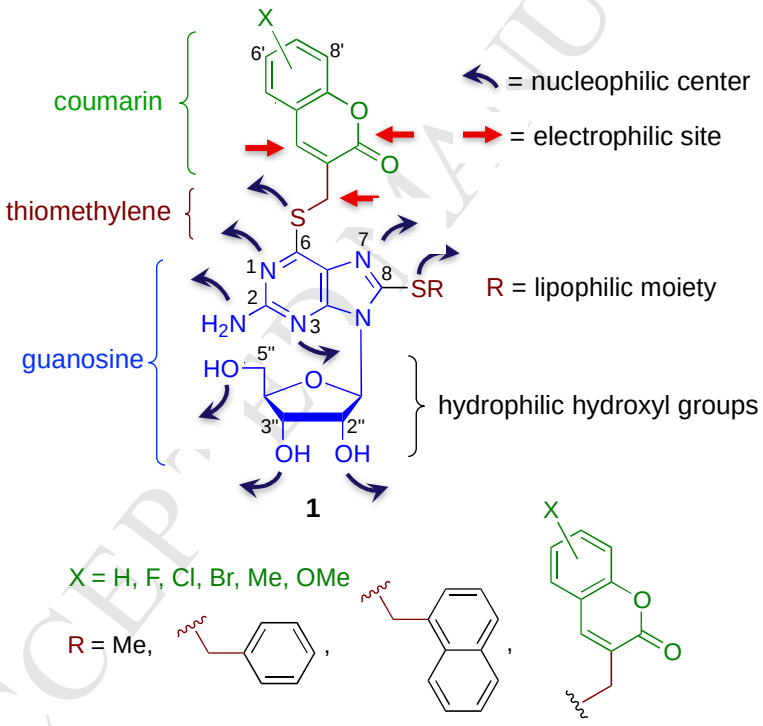
In addition to the antiviral agents described in a recent review article [4], the drug chloroquine [5] exhibits cellular anti-viral effect on chikungunya virus (CHIKV) infection. Nevertheless, its utilization has been proved to be poorly active *in vivo*. Scientists also devoted numerous efforts to develop β-amyrone, apigenin, chrysin, 5,7-dihydroxy flavones, lupenone, mycophenolic acid, prothipendyl, silibin, et al. as anti-CHIKV agents. Nevertheless, these compounds only show weak efficacy [6]. Moreover, trigocherrierin A was found with an EC_{50} value of 0.6 ± 0.1 μM and an SI value of 71.7 [6e].

Recently, a thorough review article on >20 classes of antiviral agents has been reported by da Silva-Júnior and co-workers [7]. Pérez–Pérez et al. disclosed their synthetic triazolopyrimidinones and Chai et al. disclosed new thienopyrroles with significant anti-CHIKV activity [8a,b]. van Hermert et al. also found that bis(benzofuran–thiazolidinone)s and bis(benzofuran–thiazinanone)s inhibit CHIKV replication in Vero E6 cells with an EC_{50} value as low as 1.9 μM and SI value as high as 75 [8c]. At present, scientific community is eager to obtain new compounds with favorable potency and SI values so that an effective anti-CHIKV drug may emerge. It is our plan to develop new leads to inhibit CHIKV by synthesizing a series of nucleoside derivatives with the general structures **1**. The conjugative frameworks were designed to contain three essential parts: a coumarin nucleus, a guanosine moiety, and a thiomethylene (–SCH_2–) joint.

Coumarins exist in a diverse range of plant sources as well as certain microorganisms and animals [9]. These natural products display an extraordinarily wide range of biological activities with therapeutic values. An example is 7-hydroxy-4-methylcoumarin, which was investigated as a lead in cancer-drug development [10]. Synthetic coumarins are found to possess different pharmacological or biological activities or both, such as antiviral [11–14], anticoagulant [15], anti-inflammatory [16], antimutagenic [17], antitumor [18], antitubercular [19], CNS stimulant [20], fungicidal [21], scavenger of oxygen species [22], and vasodilator

activities [23]. Some planar coumarin derivatives function as DNA intercalators and have been used in cancer treatment [24]. Recently, coumarins conjugated with various organic moieties have been synthesized [12,13]. These moieties include benzimidazoles, benzothiazoles, benzoxazoles, benzouracils, heterobicycles, imidazoles, imidazopyridines, and purine ribofuranosides. The resultant conjugates exhibit appealing anti-HCV activity and become promising leads for their further development as drugs [13].

The frameworks of **1** as shown below contain a 6-thioguanine moiety in common. 6-Thioguanine is a medication for the treatment of several types of leukemia [25a], ulcerative colitis, autoimmune diseases [25b], and HIV [25c]. Herdewijn et al. [26] synthesized 8-thiol-6-thioguanine derivatives, which act as potent inhibitors of human nucleotide pyrophosphatase/phosphodiesterase 1. These enzymes can hydrolyze phosphodiester bonds in a wide variety of substrates. Accordingly, inhibitors of these enzymes hold promise for the treatment of diabetes and tumors.



Guanosine provides multiple functional groups for medicinal chemists to manipulate during the development of their derivatives as drug leads. The C-6 and C-8 positions can be used for its attachment to a conjugated component as shown in the structures **1**. The three hydroxyl groups at the C-2″, C-3″, and C-5″ positions of a ribose would improve the solubility of the conjugates in the aqueous medium. Furthermore, the free amino group at the C-2 position of guanine may form hydrogen bonds with substrates. The thiomethylene ($–SCH_2–$) unit therein serves the purpose of a joint, which performs better efficacy than others [4], such as methylene ($–CH_2–$), aminoethylene ($–NHCH_2–$), amido (–CONH–), urea (–NHCONH–), ureide (–CONHCONH–), and directly hinged (–) units. This is based on

our results during the development of new conjugated compounds with antiviral activities [13d].

Herein the synthesis of 21 new conjugated compounds with the frameworks of **1** is reported. Their anti-CHIKV activity was evaluated and a structure–activity relationship was elucidated. The results indicate that the lowest EC_{50} value of coumarin–guanosine conjugates was 9.9 μM and the highest SI value reached 21.7.

2. Results

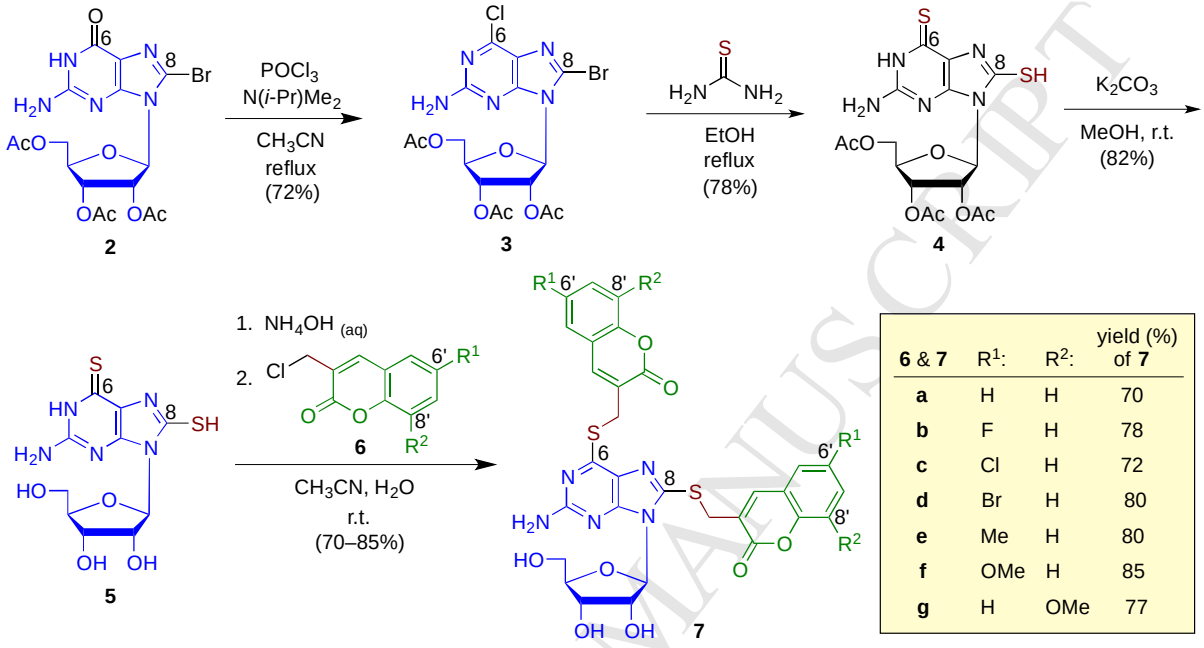
During the synthesis of target molecules with the chemical scaffolds **1**, three challenges were encountered when performed a coupling reaction in the key step. These challenges were related to the active sites of the starting materials thioguanosine substrates and coumarin reagents, as well as those in the desired conjugated products. The thioguanosine nucleus has nine nucleophilic centers, including one NH_2 group attached to the C-2 position, three nitrogen atoms at the C-1, C-3, and C-7 positions, two sulfur atoms attached to the C-6 and C-8 positions, and three OH groups attached to the C-2″, C-3″, and C-5″ positions. Meanwhile, coumarin reagents have three electrophilic sites, including an allyl halide, a lactone, and an α,β-unsaturated ester. Finally, when the desired conjugated products **7** and **14** are formed, they may not be stable under the conditions for their generation. Thus a coumarin moiety needs to be incorporated to the thioguanosine nucleus in an appropriate step during their synthesis. The reaction conditions have also to be mild enough so that a competitive Michael addition would not take place at the α,β-unsaturated ester group. Moreover, a 1,2-addition would not occur either to induce a ring-opening of the δ-lactone moiety. Given all these concerns, two different sequential synthetic routes as shown in Schemes 1 and 2 were developed for the production of the conjugated targets **7** and **14**.

*2.1. Synthesis of new biscoumarin–dithioguanosine conjugates **7** (Scheme 1)*

For the incorporation of sulfur atoms at the C-6 and C-8 positions of 8-bromoguanosine **2** [27], it was first activated by use of phosphorus oxychloride in the presence of (*N*,*N*-dimethyl)isopropylamine in acetonitrile. The resultant chloride **3** was then treated with an excess of thiourea in ethanol to give dithio intermediate **4**. The three acetyl protecting groups of **4** were then removed by potassium carbonate in methanol to give triol **5**.

The last step was the coupling of triol **5** (1.0 equiv) with 3-(chloromethyl)coumarins **6a**–**g** (2.0 equiv); the latter compounds were prepared in three steps by use of the established procedures [14a]. Various substituents, including F, Cl, Br, Me, and –OMe, were attached at the C-6′ or C-8′ position of the coumarins **6**. Among numerous trials of the conditions for

the coupling reaction, the desired biscoumarin–dithioguanosine conjugates **7a**–**g** were obtained as yellow solids. These reaction conditions were optimized to give the best yields (70–85%) by use of 35% ammonium hydroxide in aqueous acetonitrile at room temperature for 40 min to 3.0 h. This synthetic route led to the target conjugates with two identical coumarin moieties attached to the central guanosine nucleus through a thiomethylene joint.



6 & 7	R^1:	R^2:	yield (%) of 7
a	H	H	70
b	F	H	78
c	Cl	H	72
d	Br	H	80
e	Me	H	80
f	OMe	H	85
g	H	OMe	77

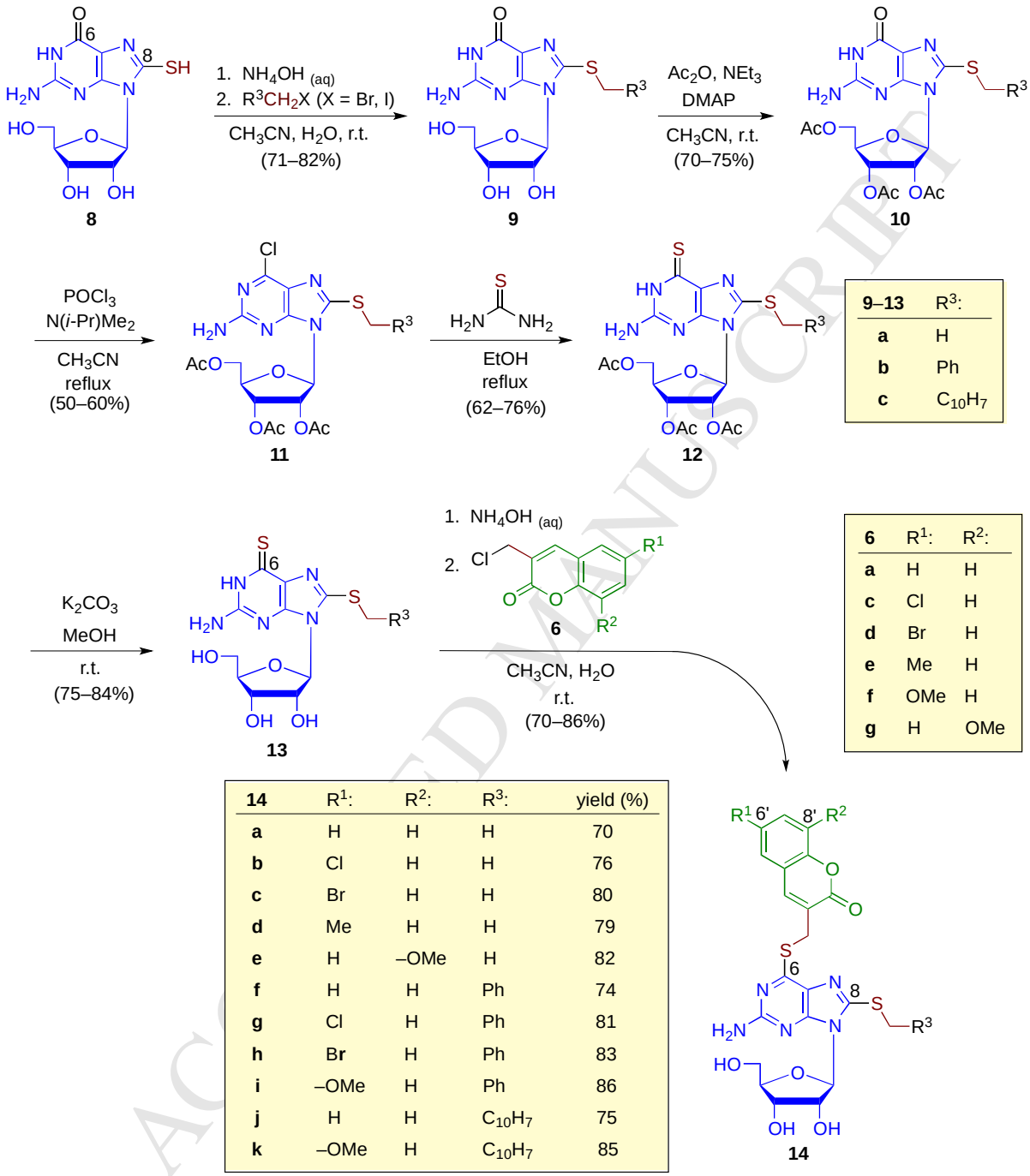
Scheme 1. Synthetic procedure for the generation of conjugated compounds **7** containing two coumarin moieties with different substituents

*2.2. Synthesis of new monocoumarin–dithioguanosine conjugates **14** (Scheme 2)*

Alternative synthetic routes were developed to produce monocoumarin–dithioguanosine conjugates **14**. In all of these 11 target conjugates, a coumarin moiety was attached at the C-6 position through a thiomethylene joint. The S atom at the C-8 position was attached with a "dummy" ligand, including alkyl and aryl groups.

Selective mono-alkylation of thioguanosine **8** [28] was performed at its C-8 thiol group before the coumarin moiety was incorporated at the C-6 position in the final conjugated targets (Scheme 2). The methylation with MeI took place preferentially at the C-8 thiol group by use of 35% aqueous ammonia in acetonitrile for 6.0 h. Under these conditions, the desired 8-(methylthio)guanosine (**9a**) was generated with a yield better than the reported one (82% versus 70%) [29]. Its IR spectrum showed a strong absorption band at 1697 cm^{-1} very close to the reported 1696 cm^{-1}, which was associated with the C-6 carbonyl stretching vibration of the thioguanosine moiety in **8**. The spectroscopic data clearly indicate that the

C-6 carbonyl group of thioguanosine **8** remained intact and its alkylation took place at the C-8 thiol group to produce the methylthioether **9a**.



Scheme 2. Synthetic procedure for the generation of conjugated compounds **14** containing one coumarin moiety and a "dummy" ligand

For activation of the C-6 position and subsequent incorporation of a sulfur atom, phosphorus oxychloride was used. Thus the three free hydroxyl groups of triol **9a** were protected by use of acetic anhydride to give the corresponding triacetate **10a**, which was then

chlorinated with phosphorus oxychloride to produce the intermediate **11a**. Reaction of the chloride **11a** with thiourea yielded the thione **12a**, in which the three acetyl protecting groups were then removed under the same conditions (i.e., K_2CO_3 in methanol) applied previously for the conversion of **4** to **5**. In the final step, *S*-alkylation was carried out at the C-6 position of thione **13a** (1.0 equiv) with various coumarins **6** (1.5 equiv) to give the desired monocoumarin–guanosine conjugates **14a**–**e** in 70–82% yields.

Applied the same synthetic strategy and reaction conditions shown in Scheme 2, a benzyl (from benzyl bromide) and a naphthylmethyl (from 1-naphthylmethyl bromide) groups were also incorporated into thioguanosine **8** to produce the corresponding aralkylated thioguanosines **9b** and **9c** [30]. These intermediates were then converted to the desired coumarin-conjugated compounds **14f**–**k**, which were subjected to biological assays.

The total syntheses of coumarin–thioguanosine conjugates shown in Schemes 1 and 2 shared a common strategy: the coupling reactions between coumarins **6** and thionguanosines (**5** and **13a**–**c**) were performed in the final step. It was due to the instability of the conjugated targets **7a**–**g** and **14a**–**k**. The purity of all new compounds was ≥98.1%, which was obtained by gravity chromatography and then determined by HPLC.

2.3. Structural identification of new conjugated compounds

The structures of all new compounds were determined on the basis of their spectroscopic characteristics. For example, the mass spectrum of biscoumarin–guanosine **7f** obtained by fast atom bombardment mass spectrometry had a molecular mass of 707.1363 for the species M^+ with the molecular formula $C_{32}H_{29}N_5O_{10}S_2$, which is close to the theoretical value of 707.1356. Its ^{13}C NMR spectrum contained 32 peaks and had resonance at δ 31.71 and 27.23 ppm for the two SCH_2 carbons. The two peaks at 160.43 and 160.21 ppm were associated with the carbons of the two –O–C=O functionalities in the coumarin moieties. The resonance at 88.08 ppm belonged the glycosidic C-1″ carbon. In its ^1H NMR spectrum, the characteristic doublet with J = 6.4 Hz at 5.63 ppm was attributed to the glycosidic proton [31]. Its IR spectrum showed a strong absorption band at 1708 cm^{-1}, which was associated with the carbonyl stretching vibration of the coumarin moiety [32].

The target **14k** represents an example for the structural identification of monocoumarin–guanosine conjugates. Its ^{13}C NMR spectrum had one peak at around 36.66 ppm, which was correlated to the carbon of the C-8 SCH_2 unit; it is comparable with the peak at δ 36.88 ppm for compound **13c**. The peak at δ 28.20 ppm was assigned to the carbon of the C-6 SCH_2–coumarin moiety; it is comparable with the peak at δ 27.23 ppm for the compound **7f**.

Moreover, the spectrum of conjugated compound **14k** had only one peak around 160.36 ppm, which was attributed to the –O–C=O carbon of the exclusive coumarin moiety.

2.4. Biological activity against CHIKV

The biological activity of the conjugated coumarin–guanosine against the CHIKV (899 strain) was primarily evaluated in Vero cells subtype A by the Bassetto procedures [33]. The concentrations of conjugates that inhibited virus replication by 50% (i.e., EC_{50}) were calculated on the basis of the obtained dose-response curves and listed in Table 1. The concentrations to reduce host cell metabolism by 50% (i.e., CC_{50}) were obtained only for promising compounds that exhibited significantly low EC_{50} values. The selectivity index ($SI = CC_{50}/EC_{50}$) was then calculated. It is a measure for the therapeutic window of the compound in the assay system. The antiviral effect of conjugated compounds that adversely affected the host cell metabolism was likely as a result of a pleiotropic or non-specific effect on the host cell.

Among these 21 new compounds tested through high through-put screening, we found that **7f**, **7g**, and **14k** exhibited compelling potency in CHIKV Vero cells with EC_{50} values ranging from 9.9 to 13.9 μM (Table 1). They displayed a significant window of selectivity with SI values between 9.37 and 21.7.

2.5. Measurement of Lipophilicity

Molecular lipophilicity, often quantified as log *P* [34], is a major factor to be considered during the development of chemical entities as drug leads. A good range for the log *P* values is between –0.4 and 5.6 for drug-like molecules [35]. When the calculated log *P* is greater than 5, poor absorption or permeation is likely to occur [36]. An example is chloroquine with a log *P* value of 5.3; it is a medication used to prevent and to treat malaria. Hence the ‘‘shake–flask method’’ [37] was applied to obtain the log *P* values of the newly synthesized conjugated compounds **7e**, **7f**, **7g**, **14i**, and **14k** as well as dithioguanosine **5** for comparison (Table 1). Our data show that the molecular lipophilicity values of the five coumarin–guanosine conjugates were between 2.78 and 3.77, which are considered in the good range for drug-like molecules.

3. Discussion

Coumarinyl, methyl, benzyl, and naphthylmethyl groups were introduced onto the dithioguanosine nucleus to form the conjugated compounds shown in Schemes 1 and 2 for the study of their structure–activity relationship toward CHIKV. Different substituents,

Table 1

Inhibitory effects of conjugated compounds on CHIKV (899 strain) in Vero cells subtype A and their lipophilicity.

compound	CC_{50}[a] (μM)	EC_{50}[b] (μM)	SI[c]	log *P*
5	ND	>302	NA	–0.13
7a	ND	>154	NA	–
7b	ND	>146	NA	–
7c	ND	>140	NA	–
7d	ND	>124	NA	–
7e	81.3	40.9	1.98	3.22
7f	>212	9.9	>21.7	3.01
7g	96.5	10.3	9.37	2.97
13b	ND	>237	NA	–
13c	ND	>212	NA	–
14a	ND	>198	NA	–
14b	ND	>186	NA	–
14c	ND	>172	NA	–
14d	ND	>194	NA	–
14e	ND	>188	NA	–
14f	ND	>173	NA	–
14g	ND	>163	NA	–
14h	ND	>152	NA	–
14i	ND	>164	NA	2.78
14j	ND	>159	NA	–
14k	>227	13.9	>16.3	3.77
3-[(methylthio)-methyl]coumarin	NA	>485	NA	–

ND = not detected; NA = not available

[a] The concentration of a compound with an adverse effect of 50% was observed on the host cell metabolism, as determined by the MTS method; [b] The concentration of a compound at which virus replication was inhibited by 50% was observed, as determined by real-time quantitative RT–PCR; [c] Selectivity index.

including F, Cl, Br, Me, and –OMe, were attached to the coumarin moiety to investigate their influence on the antiviral activity. According to the EC_{50}, CC_{50}, SI, and log *P* values of all new compounds listed in Table 1, the following SAR guidelines are deduced for their activity against CHIKV.

(1) The parent dithioguanosine **5** (EC_{50} >302 μM) did not show antiviral activity toward CHIKV. After its C-8 thione functionality was alkylated with a benzyl or naphthylmethyl group, the resultant compounds **13b** and **13c** still did not show anti-CHIKV activity. After a coumarin moiety was attached to these dithioguanosines **5** and **13** through a $–SCH_2–$ joint, the conjugated compounds **7f**, **7g**, and **14k** exhibited potency with EC_{50} values <13.9 μM. Thus the coumarin is an essential moiety for these new conjugated compounds to have antiviral activity toward CHIKV.

(2) When the C-8 coumarinyl moiety in biscoumarin–guanosine conjugate **7f** was replaced by a biologically "dummy" naphthyl moiety (i.e., **14k**), its anti-CHIKV activity retained. The size and the planarity of a naphthyl moiety are similar to those of the coumarinyl moiety. Nevertheless, the biological activity diminished in a great extent when the coumarinyl moiety in conjugate **7f** was replaced by a smaller benzyl group (i.e., **14i**).

(3) Among various electron-withdrawing and -donating groups, including F, Cl, Br, Me, and –OMe, the coumarin–guanosine conjugates containing the –OMe group in the coumarin moiety (i.e., **7f**, **7g**, and **14k**) exhibited much greater anti-CHIKV activity than conjugates bearing other substituents.

(4) Lipinski [38] reported the requirement of a minimum aqueous solubility of 5.2 mg/100 mL for a compound with medium intestinal permeability to achieve oral absorption. 6-Methoxycoumarin derivative **6f** coupled with guanosine derivative **5** gave the conjugated compound **7f**, which had three free hydroxyl groups. Its solubility was measured as 7.2 mg/100 mL, which fits into the Lipinski's requirement for oral absorption.

4. Conclusions

Twenty one new compounds, including 19 coumarin–thioguanosine conjugates, were synthesized and their antiviral activity toward CHIKV was investigated. Conjugates **7f**, **7g**,

and **14k** were found to impede CHIKV replication at EC_{50} values of 9.9, 10.3, and 13.9 μM, respectively. Their SI values ranged from 9.37 to 21.7. The coumarin moiety in these conjugated compounds was proven to be essential. By possessing three free hydroxyl groups, the three conjugate drug leads showed lipophilicity and water solubility that are fitted into the suggested physical properties of pharmaceuticals. Moreover, our guidelines of SAR provide valuable information for design and synthesis of new antivirals. Development of new coumarin-containing conjugates with antiviral activities on a broad spectrum against other essential, emerging, and neglected RNA viruses is in progress. The results will be reported in due course.

5. Experimental Section

*5.1. Standard Procedure for the Synthesis of Guanosine–Coumarin Conjugates **7f**, **7g**, and **14k***

To a solution containing a thione (**5** or **13c**, 1.0 equiv) in water (2.5 mL) and acetonitrile (1.5 mL) was added saturated aqueous ammonium hydroxide (0.10–0.15 mL). To the solution stirred at room temperature for 30 min was added a coumarin **6f** or **6g** (2.0 equiv for **5**; 1.5 equiv for **13c**). The reaction mixture was stirred at room temperature for 1.0 h. After acetonitrile was removed under reduced pressure, the residue was treated with 20% aqueous $NaHCO_3$ solution (30 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 25 mL), and the combined organic layers were then washed with brine (30 mL), dried over $MgSO_4$ (s), filtered, and concentrated under reduced pressure to afford a residue. The residue was purified by use of column chromatography packed with silica gel to give the desired product **7f**, **7g**, or **14k**.

*5.1.1.2-Amino-6,8-bis[(6′-methoxycoumarin-3′-yl)methylthio]-9-(β-D-ribofuranos-1″-yl)purine (**7f**)*

The standard procedure was followed by use of guanosine-6,8-dithione (**5**, 35.3 mg, 0.107 mmol, 1.0 equiv), aqueous ammonium hydroxide (0.15 mL), and coumarin **6f** (46.7 mg, 0.208 mmol, 2.0 equiv). After the solution was stirred at room temperature for 1.0 h and then worked up, the residue was purified by use of column chromatography (5.0% MeOH in EtOAc as the eluent) to give biscoumarin **7f** (64.2 mg, 45.5 μmol) in 85% yield as yellow solids: mp (recrystallized from CH_2Cl_2/MeOH) 270.2–272.0 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.30 (s, 1 H, H-4′), 8.07 (s, 1 H, H-4′), 7.34–7.31 (m, 2 H, 2 × ArH), 7.23 (s, 1 H, ArH), 7.16–7.12 (m, 2 H, ArH), 711–7.09 (m, 1 H, ArH), 6.65 (s, 2 H, NH_2), 5.63 (d, J = 6.4 Hz, 1 H, H-1″), 5.38–5.36 (m, 1 H, OH), 5.12–5.09 (m, 1 H, OH), 4.98–4.93 (m, 2 H, OH + H-2″), 4.34–4.31 (m, 4 H, 2 × SCH_2), 4.11–4.07 (m, 1 H, H-3″), 4.01–3.98 (m, 1 H, H-4″), 3.74 (s, 3 H CH_3), 3.66 (s, 3 H, CH_3), 3.66–3.59 (m, 1 H, H-5″), 3.51–3.49 (m, 1 H, H-5″);

^{13}C NMR (DMSO-d_6, 100 MHz) δ160.43 (C=O), 160.21 (C=O), 158.53, 156.35, 155.68, 155.48, 152.46, 147.50, 147.29, 147.15, 141.73, 141.28, 124.99, 124.34, 123.84, 119.47, 119.40, 119.13, 118.94, 117.21, 117.12, 110.45, 110.23, 88.08, 85.93, 70.55, 70.36, 61.95, 55.66 (OCH_3), 55.63 (OCH_3) 31.71 (SCH_2), 27.23 (SCH_2); IR (neat) 3349 (br, NH + OH), 2924 (w), 1708 (s, C=O), 1609 (m), 1478 (m), 1273 (m), 1190 (m) cm^{-1}; HRMS (FAB+) calcd *m/z* $[M]^+$ for $C_{32}H_{29}N_5O_{10}S_2$ 707.1356, found 707.1363.

*5.1.2.2-Amino-6,8-bis[(8′-methoxycoumarin-3′-yl)methylthio]-9-(β-D-ribofuranos-1″-yl)purine (**7g**)*

The standard procedure was followed by use of guanosine-6,8-dithione (**5**, 32.0 mg, 96.6 µmol, 1.0 equiv), aqueous ammonium hydroxide (0.15 mL), and coumarin **6g** (43.4 mg, 193 µmol, 2.0 equiv). After the solution was stirred at room temperature for 1.0 h and then worked up, the residue was purified by use of column chromatography (5.0% MeOH in EtOAc as the eluent) to give biscoumarin **7g** (52.5 mg, 74.3 µmol) in 77% yield as yellow solids: mp (recrystallized from CH_2Cl_2/MeOH) 270.8–272.2 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.31 (s, 1 H, H-4′), 8.06 (s, 1 H, H-4′), 7.24–7.21 (m, 3 H, 3 × ArH), 7.20–7.17 (m, 3 H, 3 × ArH), 6.65 (s, 2 H, NH_2), 5.59–5.58 (m,1 H, H-1″), 5.34–5.33 (m, 1 H, OH), 5.09–5.08 (m, 1 H, OH), 4.96–4.93 (m, 2 H, OH + H-2″), 4.33–4.31 (m, 4 H, 2 × SCH_2), 4.08–4.04 (m, 1 H, H-3″), 4.00–3.98 (m, 1 H, H-4″), 3.88 (s, 3 H CH_3), 3.84 (s, 3 H, CH_3), 3.60–3.54 (m, 1 H, H-5″), 3.46–3.42 (m, 1 H, H-5″); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.26 (C=O), 160.04 (C=O), 158.36, 156.18, 155.51, 155.29, 152.29, 147.33, 141.56, 141.11, 124.89, 124.17, 123.67, 119.30, 119.23, 118.96, 118.77, 117.04, 116.94, 110.27, 110.06, 87.91, 85.76, 70.53, 70.09, 61.78, 55.64 (OCH_3), 55.36 (OCH_3), 31.54 (SCH_2), 27.06 (SCH_2); IR (neat) 3349 (br, NH + OH), 2924 (w), 1708 (s, C=O), 1609 (m), 1478 (m), 1273 (m), 1190 (m) cm^{-1}; HRMS (FAB+) *m/z* $[M]^+$ calcd for $C_{32}H_{29}N_5O_{10}S_2$ 707.1356, found 707.1371.

*5.1.3.2-Amino-6-(6′-methoxycoumarin-3′-yl)methylthio-8-(1″-naphthylmethylthio)-9-(β-D-ribofuranos-1‴-yl)purine (**14k**)*

The standard procedure was followed by use of thione **13c** (20.2 mg, 42.8 µmol, 1.0 equiv), aqueous ammonium hydroxide (0.10 mL), and coumarin **6f** (14.41 mg, 643 µmol, 1.5 equiv). After the solution was stirred at room temperature for 1.0 h and then worked up, the residue was purified by use of column chromatography (5.0% MeOH in EtOAc as the eluent) to give conjugate **14k** (23.9 mg, 36.3 µmol) in 85% yield as yellow solids: mp (recrystallized from CH_2Cl_2/MeOH) 178.2–179.5 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.36 (s, 1 H, H-4′), 7.84–7.83 (m, 1 H, ArH), 7.82–7.81 (m, 1 H, ArH), 7.61–7.56 (m, 2 H, 2 × ArH), 7.43–7.41 (m, 3 H, 3 × ArH), 7.29–7.26 (m, 3 H, 3 × ArH), 6.65 (br, 2 H, NH_2), 5.63–5.60 (m, 1 H, H-1‴), 5.37–5.36 (m, 1 H, OH), 5.13–5.12 (m, 1 H, OH), 5.09–4.98 (m, 1 H, OH), 4.93–4.91 (m, 1 H, H-2‴), 4.51 (m, 2 H, $ArCH_2S$), 4.33–4.31 (br s, 2 H, SCH_2CCO), 4.32–4.28 (m, 2 H, H-3‴ + H-4‴), 3.89 (s, 3 H, OCH_3), 3.59–3.56 (m, 1 H, H-5‴), 3.48–3.46

(m, 1 H, H-5‴); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.36 (C=O), 159.25, 157.09, 153.12, 152.23, 152.15, 147.97, 145.27, 142.33, 138.56, 138.22, 135.62, 134.17, 131.73, 131.23, 131.20, 129.38, 129.18, 128.74, 126.90, 126.33, 126.04, 125.72, 123.91, 86.90, 79.75, 72.11, 70.47, 62.93, 55.62 (OCH_3), 36.66 ($ArCH_2S$), 28.20 (SCH_2CCO); IR (neat) 3335 (br, NH + OH), 2922 (w), 1720 (s, C=O), 1625 (m), 1475 (m), 1270 (m), 1120 (m) cm^{-1}; HRMS (FAB+) *m/z* $[M]^+$ calcd for $C_{32}H_{29}N_5O_7S_2$ 659.1058, found 659.1071.

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Appendix A. Supporting Information

Supplementary data associated with this article can be found in the online version.

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Highlights

- New coumarin–guanosine conjugates were designed and synthesized.
- Three new conjugates showed potent inhibitory activity towards chikungunya virus.
- Coumarin is an essential moiety for new conjugated compounds with activity against chikungunya virus.
- Attachment of a methoxy group to the coumarin moiety enhanced the activity against chikungunya virus.