Benzouracil–Coumarin–Arene Conjugates as Inhibiting Agents for Chikungunya Virus

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ABSTRACT. Chikungunya virus (CHIKV) causes an arboviral disease and was first recognized in epidemic form in East Africa in 1952–1953. The virus is primarily transmitted through mosquitoes and the resulting disease, chikungunya fever, is found in nearly 40 countries. Neither an effective vaccine nor a specific antiviral drug exists for treatments of Chikungunya fever. Thus 25 new triply conjugated compounds of uracil–coumarin–arene were designed and synthesized as potential inhibiting agents. Their chemical structures were determined unambiguously by spectroscopic methods, including single-crystal X-ray diffraction crystallography. The three nuclei in these conjugates were connected by specially designed $-SCH_2-$ and $-OSO_2-$ joints. Five of these new conjugates were found to inhibit CHIKV Vero

cells with significant potency (EC_{50} = 10.2–19.1 μM) and showed low toxicity (CC_{50} = 75–178 μM). The selective index values were 9.3–11.5 for three conjugates. By analysis of the data from the anti-viral assays, the structure–activity relationship is derived on the basis of the nature of the uracil nucleus, the joint between the nuclei, and the functional group attached to the arene nucleus.

Introduction

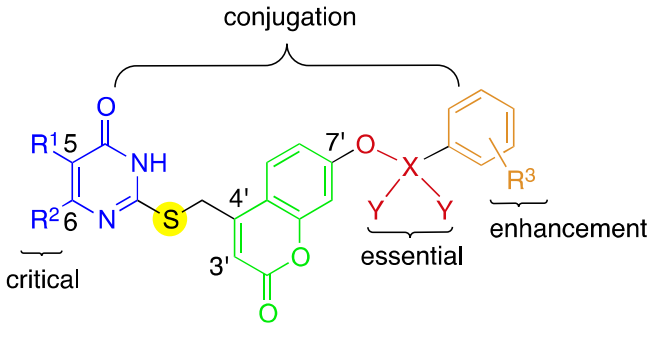
Chikungunya (CHIK) fever was first recognized in an epidemic form in East Africa in 1952–1953[1] and is mainly transmitted by mosquitoes. A number of CHIK cases have emerged in more than 40 countries in Africa and Asia over the past half century.[2,3] Chikungunya virus (CHIKV) belongs to the family Togaviridae and the genus Alphavirus, which includes enveloped and positive single-stranded RNA viruses.[1,4] Unfortunately, the pathogenesis of CHIK disease still remains unknown. Presently, there is neither an effective vaccine nor a specific antiviral drug available for this disease.[5] Consequently, the development of drugs with activity against CHIKV is becoming a global concern of the utmost importance.

S-Adenosyl homocysteine hydrolase, inositol monophosphate dehydrogenase, and orotidine 59-phosphate decarboxylase are broad-spectrum antiviral agents. Nevertheless, their application to against CHIKV infection is inadequate.[6] Several chemical compounds have been reported to exhibit weak anti-CHIKV activity. They include apigenin,[6] 6-azauridine,[6] chloroquine,[7] chrysin,[6] (5,7-dihydroxy)flavones,[6] interferon, mycophenolic acid,[8] prothipendyl,[6] ribavirin,[9] silibin,[6] etc. In 2011, arbidol and its two derived metabolites were tested in vitro on the CHIKV by use of two cell lines in pre- and post-infection conditions.[10] Arbidol was found to have an IC_{50} of 12.2 μM. In 2012, D'Hooghe et al.[11] reported that two purine-β-lactam hybrids possess anti-CHIKV activity with EC_{50} of 17.11 and 13.01 μM, respectively, and selectivity

indices (SIs) of >5.75 and >4, respectively. Additionally, purine-aminopropanol exhibits an EC_{50} of 11.51 μM with an SI > 6.15. In 2013, Bassetto et al.[12] reported an EC_{50} of 5 μM with an SI of 14 for benzylidene(cyclopropane)carbohydrazide, which inhibited the virus-induced cytopathic effect.

Many efforts have sought to identify novel inhibitors of CHIKV from natural sources. For example, Bourjot et al.[13] performed a phytochemical study on the leaves of Anacolosa pervilleana (a Madagascan plant) in a virus-cell-based assay. They isolated the triterpenoids lupenone and β-amyrone, which show moderate anti-CHIKV activity.

The aim of this study was to develop new leads that inhibit emerging viruses, including CHIKV. Accordingly the molecular structure **1** was designed, from which a new compound library would be established. The resultant compounds were primarily composed of a "triply" conjugated skeleton of uracil–coumarin–arene; three nuclei therein were linked through thiomethylene (–SCH_2–) and sulfo (–OSO_2–) joints. In some of these triply conjugated compounds, the –OSO_2– joint was replaced by –OCH_2–. The structure–activity relationship (SAR) would be established on the analysis of the derivatives containing various substituents on the uracil and the arene nuclei.



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The uracil, thymine (i.e., 5-methyluracil), and benzouracil (i.e., quinazolinone) moieties exist in many biologically active compounds.[14] Recently, Wang et al.[15] revealed that the substituents on the C-5 and C-6 positions (i.e., R^1 and R^2, respectively, in **1**) of the uracil nucleus are important to anti-viral activity. Abdel–Aal et al.[16] synthesized compounds with two hydrophobic residues at the C-5 and C-6 positions. These compounds exhibited potent activity against hepatitis B virus (HBV). Kumar et al.[17] prepared various 5-substituted uracils containing a 1-[(2-hydroxyethoxy)methyl]glycosyl moiety at the N-1 position. These compounds show potent in vitro activity toward both chronically infected primary duck hepatocytes and a human HBV DNA transfected human hepatoblastoma cell line (HepG2 2.2.15). Among acyclic pyrimidine nucleosides, substituents at the C-5 position are influential determinants against duck HBV, wild-type human HBV, and lamivudine-resistant HBV in vitro at non-cytotoxic concentrations. Ordonez et al.[18] found that the introduction of a substituent at the C-6 position of uracil resulted in elevated anti-HIV-1 activity.

Benzouracil and its derivatives have been extensively studied because of their wide range of pharmacological activities. Medicinally, many of them display anti-cancer,[19] anti-convulsant,[20] anti-depressant,[21] anti-inflammatory,[22] anti-fungal,[23] anti-HIV,[24] anti-microbial,[25] anti-ulcer,[26] analgesic,[27] hypolipidemic,[28] or immunotropic activity.[29] Some of them act as inhibitors of thymidylate synthase,[30] poly(ADP-ribose) polymerase (PARP),[31] or protein tyrosine kinase.[32] Alogliptin, which contains a benzouracil moiety, is a potent selective inhibitor of serine protease.[33] Moreover, the benzouracil nucleus is widely present in biologically active natural products.[34]

Results from recent studies indicate the prominent activity of coumarins toward proteases. The two hydroxyl groups attached to the nucleus and the α,β-unsaturated carbon to

carbon double bond therein make coumarin as one of the most potent protease inhibitors.[35] Coumarin ・ type molecules also display high inhibitory activity towards various serine proteases, human leukocyte elastase, porcine pancreatic elastase, thrombin, urokinase, and human plasmin.[36] Additionally, isocoumarins are effective as mechanism-based inhibitors of serine proteases.[36] Coumarins conjugated with various benzimidazoles[37] or heterobicycles[38] have been synthesized by our research group recently. These conjugates exhibit appealing anti-viral activity and become promising leads for further development.

The connection of a benzimidazole nucleus to a coumarin nucleus produces a conjugated compound, which exhibit activity against hepatitis C virus (HCV).[37] Replacement of the benzimidazole nucleus with nucleobases results in the related conjugates with enhanced anti-HCV acitivity.[39] Anti-HCV compound libraries have been established and include conjugated compounds, in which coumarin moieties are in connection with various heterocycles, including benzothiazole,[38] benzoxazole,[38] and imidazopyridine.[40] These findings indicate the importance of the thiomethylene joint for the connection. [41]

Compounds containing a sulfo (R–OSO_2–R') joint have been identified as potent protease inhibitors.[42,43] The reaction of sulfonate compounds with the hydroxyl site of an active protease residue can form a stable enzyme derivative.[44] Substantial hypoxic selectivity and antitumor activity have been also observed for many sulfonates.[45] Nitrophenyl esters of benzenesulfonic acid and phenylmethanesulfonic acid bearing various positively charged groups on the benzene ring act as inactivators of trypsin-like proteases.[44] For example, *p*-nitrophenyl *p*-(amidinothiomethyl)benzenesulfonate inactivates thrombin. As five-membered cyclic sulfonates, sultones are highly reactive toward chymotrypsin.[44] 2-Hydroxy-3,5-dinitro-*R*-toluenesulfonic acid sultone and *o*-hydroxy-*R*-toluenesulfonic acid sultone react with α-

chymotrypsin rapidly to form catalytically inactive sulfonyl enzymes that decompose slowly over time. Recently, Udommaneethanakit et al.[46] reported the inhibition of avian influenza A virus with sulfonate drugs, such as oseltamivir, peramivir–sulfonate, and zanamivir. Moreover, Cravatt, Sorensen, et al.[47] successfully identified the targets of sulfonate ester probes that belong to several mechanistically distinct enzyme classes. They include dehydrogenases, epoxide hydrolases, glutathione *S*-transferases, sugar kinases, and transglutaminases. These sulfonate ester labeling events occur in enzyme active sites that exhibit heat sensitivity, competition between the substrates and cofactors, and dependency on endogenous activators.[48] They also discovered the potent and selectively reversible inhibitors of enzymes in complex proteomes and analyzed the compounds in competitive screens using a class of probes with a sulfonate ester group. These sulfonate probes profile several distinct enzyme classes, including aldehyde dehydrogenases, enoyl CoA-hydratases, and glutathione *S*-transferases.[49]

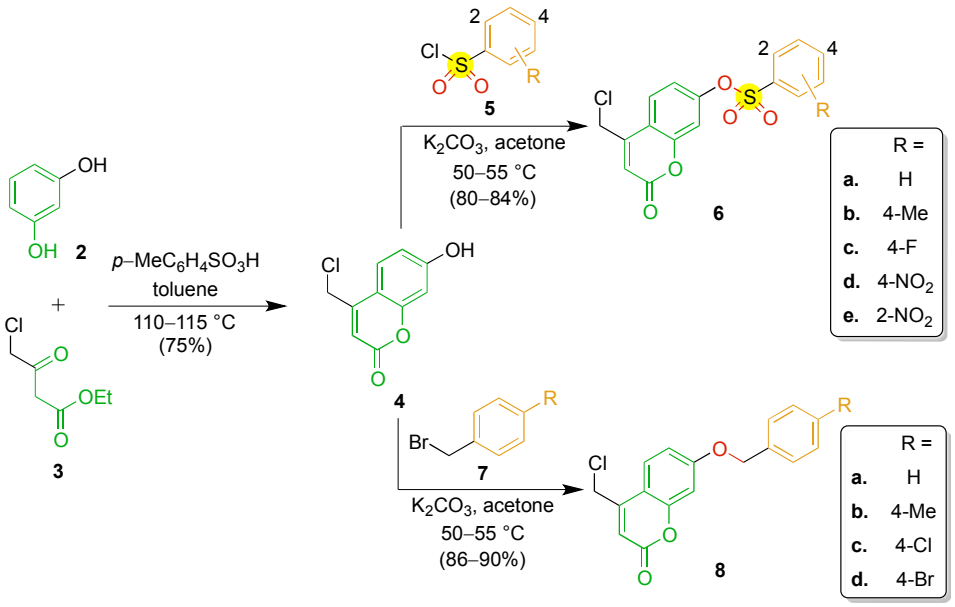
Herein we report the scope of uracil–coumarin–arene conjugates as an unprecedented class of anti-CHIKV agents. Furthermore, various substituents were linked to the uracil and arene nuclei. In total, 25 compounds with the common skeleton **1** were synthesized, five of which exhibited significant anti-CHIKV activity. Accordingly, their structure–activity relationship was deduced.

Results and Discussion

For the synthesis of "triply" conjugated compounds with the scaffold **1** (i.e., uracil–coumarin–arenes), three challenges had to be overcome. First, the labile sulfo group connecting the coumarin and arene moieties had to remain intact throughout the synthetic steps. Second, the conditions to form the S–CH_2 single bond had to be mild enough to maintain the bond. Third,

the reagents could not induce a Michael addition to the α,β-unsaturated ester group or ring-opening of the δ-lactone moiety in **1**. Given these three concerns, the sequential synthetic routes shown in Schemes 1−4 were developed.

Synthesis of Doubly Conjugated (4-Chloromethyl)coumarin–Arene Intermediates (Scheme 1). For the construction of the triply conjugated scaffold **1**, the intermediates (arenesulfonyl)coumarins **6** and benzylcoumarins **8** were prepared by use of resorcinol **(2)** and 4-chloroethyl acetoacetate **(3)** as the starting materials. Through the Pechmann condensation[50] in the presence of *p*-toluenesulfonic acid as the catalyst in dry toluene, 7-hydroxycoumarin **4** was generated at an elevated temperature. Its sulfonylation with benzenesulfonyl chlorides 5 in the presence of K_2CO_3 (anhydrous) at 50–55 °C afforded the corresponding 7-*O*-sulfonylated coumarins **6** in good yields. Under similar reaction conditions, 7-*O*-benzylated coumarins **8** were obtained by benzylation of 7-hydroxycoumarin **4** with the benzyl bromides **7**. Accordingly, two series of coumarins, **6** and **8**, were obtained, in which the substituents R included Me, F, Cl, Br, Me, and NO_2 in the *ortho* or *para* position.

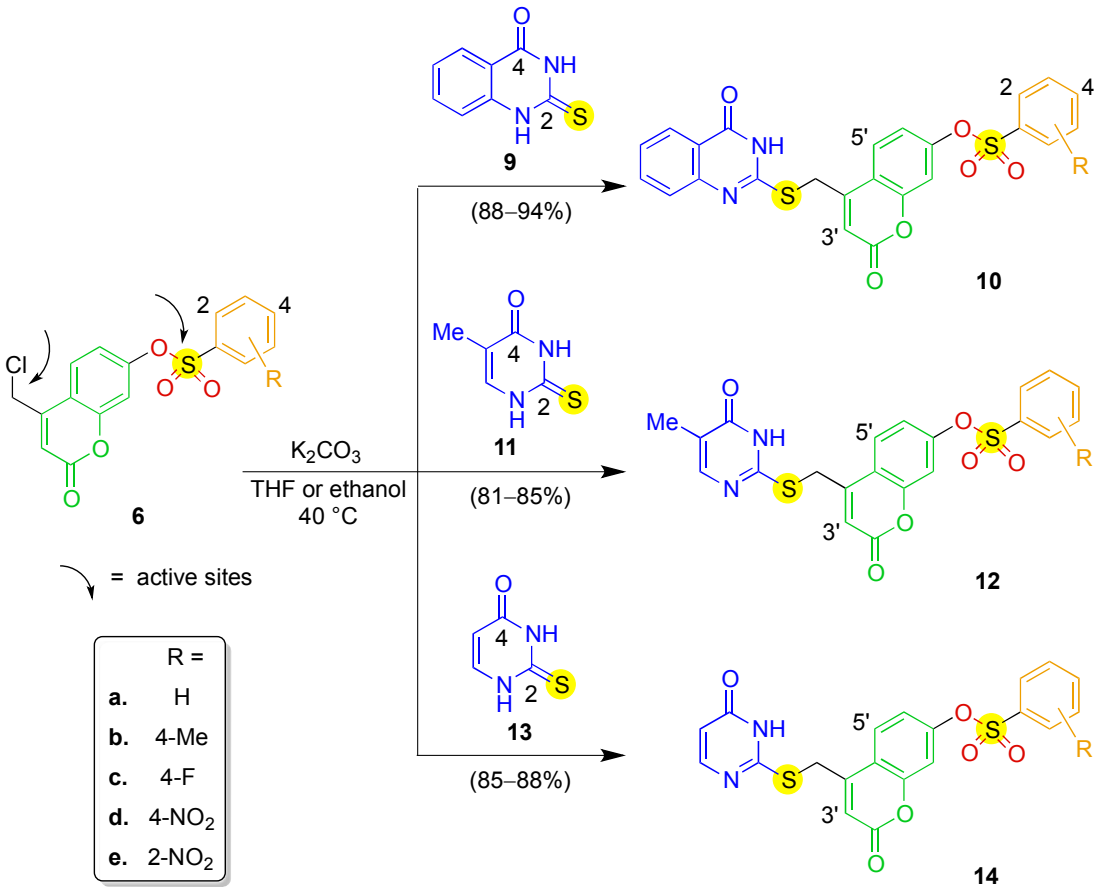


Scheme 1.

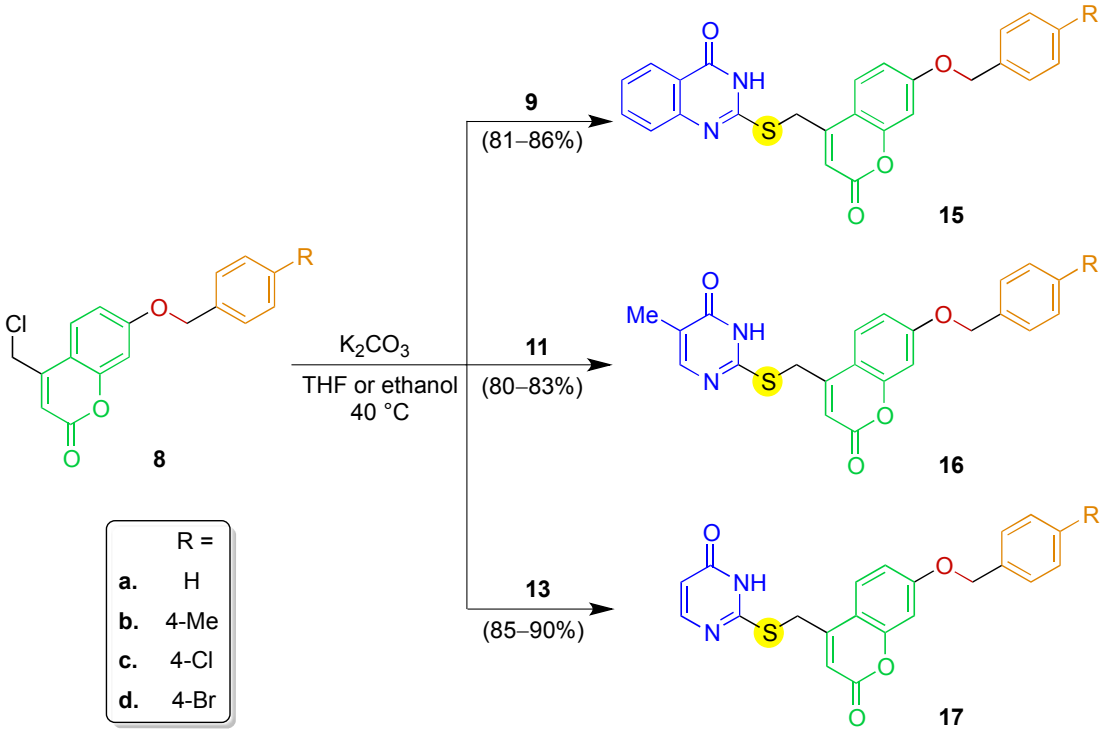
Syntheses of coumarin derivatives with benzenesulfonyl or benzyl fragments

Syntheses of Triply Conjugated Uracil−Coumarin−Arene Targets and Their Analogs (Schemes 2 and 3). The coupling of 2-thiobenzouracil **9** with various (4-chloromethyl)coumarins **6** to generate uracil–coumarin–arene conjugates **10a–e** were performed under different alkaline conditions. At elevated temperatures (>65 °C), the sulfo group (i.e., $–OSO_2–$) in **10** was destroyed. The use of bases, such as (*N*,*N*-diisopropyl)ethylamine and (4-dimethylamino)pyridine for the generation of a nucleophile from 2-thiobenzouracil **9** resulted in problems with either ring opening of the coumarin moiety or substitution at the allylic chloride or both. Workup conditions with appropriate pH values between 10.0 and 11.5 were found to be critical for the maintenance of the $S–CH_2$ single bond in **10**. Accordingly, the desired triply conjugated compounds **10a**–**e** were synthesized through alkylation at the C-2 thione center of **9**, which resulted from the presence of a preferred negative charge on the exocyclic sulfur atom instead of the exocyclic oxygen atom.[51] This *S*-alkylation was carried out at 40 °C in the presence of anhydrous K_2CO_3 in THF or ethanol. A slightly alkaline solution with pH 8.0–9.0 was then applied during the workup. Accordingly, the triply conjugated compounds **10a**–**e**, **12a**–**e**, and **14a**–**e**, which contained nuclei of benzouracil, methyluracil, and uracil, were obtained in 81–94% yields (see Scheme 2). Potential by-products from the additions of uracil to the lactone carbonyl group of coumarins **6** or to the α,β-unsaturated carbon to carbon double bond were not detected.

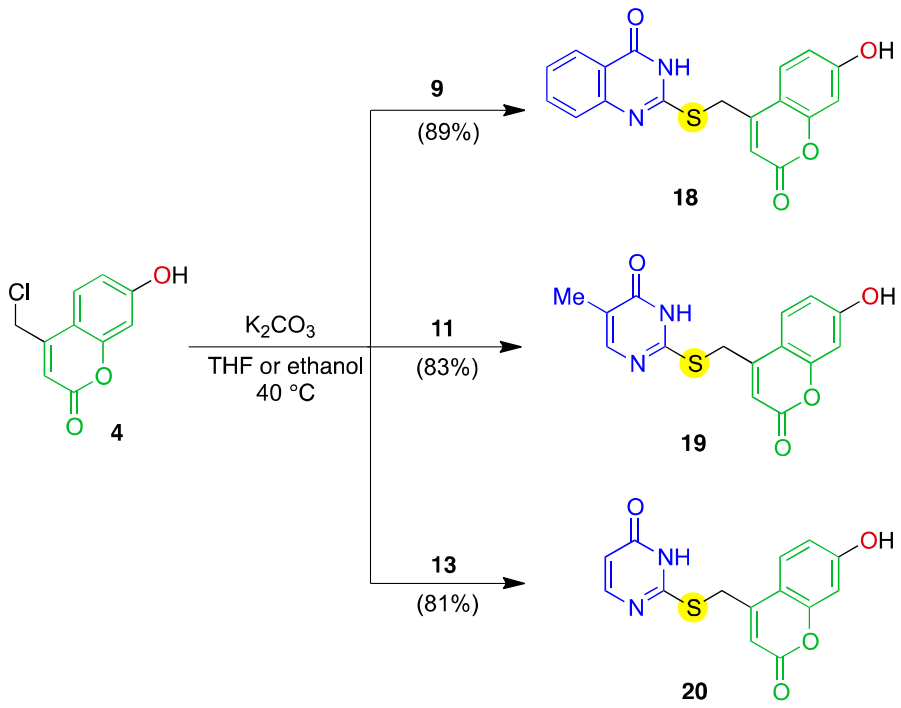
Under similar reaction conditions, the triply conjugated compounds **15a–d**, **16a–d**, and **17a–d** with $–SCH_2–$ and $–OCH_2–$ joints were also obtained as shown in Scheme 3. The corresponding doubly conjugated compounds **18**–**20** without the arene moiety were produced (see Scheme 4) as the reference compounds for deduction of the structure–activity relationship.



Scheme 2. Synthesis of the benzouracil−, thymine−, and uracil−coumarin−arene conjugates bearing the –SCH_2– and –OSO_2– joints



Scheme 3. Synthesis of uracil−coumarin−arene conjugates bearing the –SCH_2– and –OCH_2– joints



Scheme 4. Syntheses of doubly conjugated compounds containing benzouracil−, thymine–, and uracil–coumarin nuclei

Identification of the Structures of Conjugated Compounds. The structures of all new compounds were determined according to their spectroscopic characteristics. For example, the mass spectrum of **14a** from FABMS analysis exhibited 442.0290, which indicates a molecular formula of $C_{20}H_{14}N_2O_6S_2$ with a theoretical value of 442.0293. Its ^{13}C NMR spectrum showed 18 signals, which is in accordance with the theoretical number. Resonance at δ 164.91 and 154.88 ppm were assigned to the two C=O carbons; resonance at δ 159.61 and 29.99 ppm were assigned to the N=C−N and the SCH_2 carbons, respectively.

Furthermore, the ^1H NMR spectrum of **14a** displayed two characteristic singlets at δ · 4.48 and 6.58 ppm for the SCH_2 and H-3′ protons, respectively. A multiplet appeared at δ 6.23−6.21 ppm for the H-5 proton. Its IR spectrum showed two medium absorption bands at 1718 and 1653 cm^{-1}, which were attributed to the two carbonyl stretching vibrations.[39] A medium absorption band at 1353 cm^{-1} was attributed to the S=O stretching vibration.

These spectroscopic data did not provide sufficient information to establish the connection of the uracil nucleus to the coumarin nucleus through the C=O or the C=S site. Therefore, molecular framework of the uracil−coumarin−arene conjugate **14a** was determined by single X-ray diffraction analysis (Figure). Its monoclinic crystals possessed the space group *P*1 2_1/c 1 with a = 21.413(4) Å, b = 6.2583(12) Å, c = 14.392(3) Å, and · = 90°, · = 99°, and γ = 90°. These results indicate that the alkylation took place unequivocally at the C-2 position of the uracil and thus confirm the formation of an NCS−CH_2 single bond.

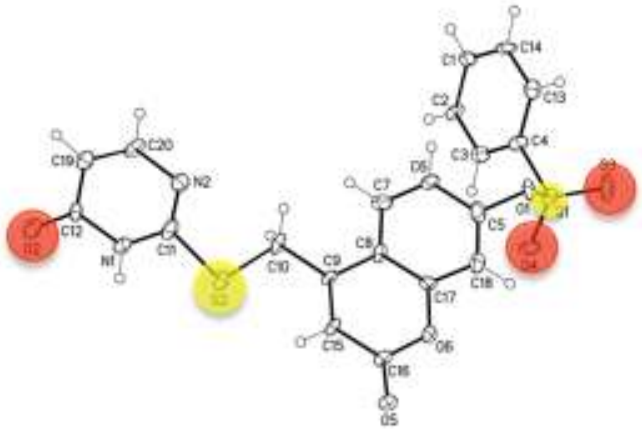


Figure. Molecular frameworks: ORTEP diagram of **14a** obtained by X-ray diffraction analysis.

The ^{13}C NMR spectra of all triply conjugated compounds exhibited the expected number of carbon peaks, with the exception of compound **12e**. Its spectrum exhibited one less carbon resonance than expected because the peaks associated with the two quaternary carbons C-7′ and C-2′′ were overlapped at δ150.19 ppm.

Evaluation of the Anti-CHIKV Activity and Measurement of Lipophilicity

The biological activity of the conjugated compounds against the CHIKV (899 strain) in Vero cells subtype A was evaluated according to established methods.[12] The compound concentration that inhibited virus replication by 50% (i.e., EC_{50}) and that reduced host cell metabolism by 50% (i.e., CC_{50}) were calculated from the dose-response curves. Subsequently, these values were used to calculate the selectivity index (i.e., SI = CC_{50}/EC_{50}), which is a measure for the therapeutic window of the compound in an assay system. Compounds were only considered as selective inhibitors in the replicon assay when virus RNA replication was significantly inhibited (>70%) at concentrations that did not adversely affect the host cell

metabolism. The antiviral effect of 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 25 new conjugated compounds, **10a**, **10b**, **10e**, **12e**, and **14e** inhibited CHIKV Vero cells with appealing potency (Table) with EC_{50} values ranging from 10.2 to 19.1 μM. They displayed a significant window of selectivity with SI values between 5.8 and 11.5.

Molecular lipophilicity, usually quantified as log *P*, of chemical entities plays an important role in the development of drug leads[52] and is related to the structure–activity relationship. The apparent partition coefficient (*P*) is the ratio of the concentration of conjugated compounds in *n*-octanol to the concentration of the same species in the aqueous phase. The log *P* coefficient is one of the principal parameters for the estimation of lipophilicity/hydrophobicity of chemical compounds and determines their pharmacokinetic properties. Consequently, the ''shake–flask method'' was applied by use of *n*-octanol and water to obtain the log *P* values[53] of the triply conjugated compounds **10a,b,e**, **12b,e**, **14b**, and **15a,b** as well as the doubly conjugated compound **18** for comparison (Table).

The log *P*s of thousands of drugs and potential drugs have been measured. Nevertheless, only a limited open pilot study is carried out with chloroquine as a treatment for chronic Chikungunya arthritis on patients and is reported an improvement in symptoms such as morning stiffness. Results from many reports indicate antiviral activity of chloroquine *in vitro,* in particular the inhibition of the CHIKV-related virus Mayaro. This led to a clinical trial in La Re´union Island to test the efficiency of chloroquine *in vivo*.[7] However, chloroquine with a log *P* value of 5.3 does not follow the Lipinski rule of lipophilicity. Poor absorption or permeation is more likely to occur when the calculated log *P* is greater than 5.[54]

The data presented in Table indicate that the molecular lipophilicity of compounds **10–18** fell into the range of –2.74 to 3.57, which follows the Lipinski rule for lipophilicity.[54] The log *P* value of 2.13 for benzouracil sulfonate **10b** with promising EC_{50} and SI values was less than that (5.3) for chloroquine.

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

conjugates	CC_{50}[a] (μM)	EC_{50}[b] (μM)	SI[c]	log *P*
10a	178	19.1	9.3	1.91
10b	117	10.2	11.5	2.13
10c	30	18.4	1.6	–
10d	117	54.5	2.2	–
10e	144	17.2	8.8	−1.07
12a	126	58	2.2	–
12b	114	26.4	4.3	0.611
12c	86.4	116	–	–
12d	–	>199	–	–
12e	107	19.0	5.6	−2.60
14a	–	57.4	–	–
14b	60.2	23.1	2.6	0.467
14c	111	128	–	–
14d	–	>205	–	–
14e	75.2	13	5.8	−2.74
15a	–	>45.2	–	3.35
15b	102	>219	–	3.57

16a	–	>246	–	–
16b	–	>48	–	–
16c	104	45.1	2.3	–
17a	–	>255	–	–
17d	13.8	4.6	3.0	–
18	>284	192	>1.5	1.32
19	–	>316	–	–
20	–	>331	–	–

[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.

Structure–Activity Relationship: Essential Moieties and Functional Groups. In this new compound library, the triply conjugated compounds primarily contain three primary components: coumarin, uracil (including thymine and benzouracil), and arene (with different substituents, including Me, F, Cl, Br, Me, and NO_2). Meanwhile, a $–SCH_2–$ unit was used to link the uracil and the coumarin moieties; then a $–OSO_2–$ or $–OCH_2–$ unit was used to link the coumarin and arene moieties. Through analysis of their anti-CHIKV activities and lipophilicities shown in Table, we deduce the following SAR by scrutinizing their EC_{50}, CC_{50}, SI, and log *P* values.

(1) Introduction of a benzenesulfonyl moiety to the thiomethylene-linked benzouracil–coumarin conjugates led to triply hybrid compounds **10**, which exhibited desirable anti-CHIKV activity. Successful examples include **10a**, **10b**, and **10e** with EC_{50} = 10.2–19.1 μM and SI = 8.8–11.5. The benzouracil–coumarin conjugates without a benzenesulfonyl moiety (e.g., **18**–**20**) did not exhibit significant activity.

(2) The triply conjugated compounds with the –OSO_2– joint between coumarin–arene moieties exhibited greater anti-CHIKV activity than those with the –OCH_2– joint (cf. **10a** *versus* **15a**, **10b** *versus* **15b**, **12a** *versus* **16a**, **12b** *versus* **16b**, and **14a** *versus* **17a**).

(3) Addition of the Me electron-releasing group to the benzenesulfonyl moiety often increased the potency and SI value by a factor of 1.2–2.3 (cf. **10b**, **12b**, and **14b** *versus* **10a**, **12a**, and **14a**, respectively). Compounds with the NO_2 electron-withdrawing group at the *ortho* position showed greater activity (>3.16–15.8 fold) than those with the same group at the *para* position (cf. **10e** > **10d**, **12e** > **12d**, and **14e** > **14d**).

(4) The conjugated compounds with the benzouracil moiety showed a higher selectivity (i.e., the SI value) than those with thymine and uracil moieties (cf. **10a–e** > **12a–e** > **14a–e**). As the size of this moiety increases (i.e., benzouracil > thymine > uracil), the inhibition of CHIKV generally increases. This relationship also exist with the lipophilicity of the conjugates as exhibited by the log *P* values shown in Table (cf. **10b** > **12b** > **14b** and **10e** > **12e** > **14e**).

(5) Anti-CHIKV activity (as reflected by the EC_{50} value) was greater for the triply conjugated compounds with higher lipophilicity (as indicated by a lager value of log *P*). For example, the methylated **10b** (EC_{50} = 10.2 μM, log *P* = 2.13) was more active than the parent compound **10a** (EC_{50} = 19.1 μM, log *P* = 1.91). Compound **10b** containing a benzouracil moiety was more potent and lipophilic than compound **12b** containing a thymine moiety (EC_{50} = 26.4 μM, log *P* = 0.611) and compound **14b** containing a uracil moiety (EC_{50} = 23.1 μM, log *P* = 0.467). Similar phenomena were observed among compounds **10e**, **12e**, and **14e.**

Conclusions

For the development of new compounds with anti-CHIKV activity, a series of triply conjugated uracil−coumarin−arenes were designed and synthesized. Among 25 new compounds produced, five conjugates were found to inhibit CHIKV (899 strain) in Vero cells subtype A. Promising results were associated with the five compounds **10a**, **10b**, **10e**, **12e**, and **14e**, which impeded CHIKV replication at EC_{50} values of 19.1, 10.2, 17.2, 19.0, and 13.0 μM, respectively.

Moreover, guidelines of SAR were deduced by the analysis of the scaffold of the conjugated compounds **10**, **12**, **14**–**20** and their anti-CHIKV activities. The coumarin moiety in the conjugated compounds was required for antiviral activity. This central moiety was attached to a pyrimidine nucleus at the C-2 position through a $–SCH_2–$ joint on one side and an arene group through a $–OSO_2–$ joint on the other side. The triply conjugated scaffold, as in compounds **10**, provided higher anti-CHIKV activity than other analogs such as conjugates **15**–**20**. These guidelines will be useful for the future design and synthesis of new conjugated compounds. The mode of actions of these new leads towards enzymes associated with CHIKV is currently under investigation and the results will be reported in due course.

Experimental

General. All reactions were carried out in oven-dried glassware (120 °C) under an atmosphere of nitrogen unless as indicated otherwise. Acetone, dichloromethane, ethanol, ethyl acetate, hexanes, methanol, and THF were purchased from Mallinckrodt Chemical Co. Ethyl acetate was dried and distilled from CaH_2. Tetrahydrofuran was dried by distillation from sodium and benzophenone under an atmosphere of nitrogen. 2-Thiouracil and triethylamine was purchased from Tedia Company Inc. 5-Methyl-2-thiouracil and quinazolinone were purchased from Alfa

Aesar Chemical Co. Benzyl bromide, 4-bromobenzyl bromide, 4-chlorobenzyl bromide, and 4-methylbenzyl bromide were purchased from Acros Organics. Benzenesulfonyl chloride, *p*-toluenesulfonyl chloride, 4-methylbenzenesulfonyl chloride, 4-fluorobenzenesulfonyl chloride, 4-nitrobenzenesulfonyl chloride, and 2-nitrobenzenesulfonyl chloride were purchased from Sigma-Aldrich Chemical Co. Potassium carbonate and *p*-toluenesulfonic acid were purchased from Showa Chemical Co. 7-Hydroxycoumarin was prepared according to the reported methods.[50]

Melting points were obtained with a Fargo MP-2D melting point apparatus. Analytical thin layer chromatography (TLC) was performed on precoated plates (silica gel 60 F-254), purchased from Merck Inc. High performance liquid chromatography (HPLC) was performed on two Waters 515 HPLC pumps equipped with a Waters 2489 UV/visible detector and a Thermo 5 μm Hypersil ODS (250 mm 4.6 mm i.d.). Purity of all compounds was >98.0%, as checked by HPLC.

Infrared (IR) spectra were measured on a Perkin–Elmer Model Spectrum 100 spectrophotometer. Absorption intensities are recorded by the following abbreviations: s = strong; m = medium; and w = weak. Proton NMR spectra were obtained on a Varian Mercury-400 (400 MHz) spectrometer or Bruker AC-400 (400 MHz) spectrometer by use of chloroform-*d* ($CDCl_3$) and dimethylsulfoxide-d_6 (DMSO-d_6) as the solvents. Proton NMR chemical shifts were referenced to residual protonated solvents (δ 7.24 for chloroform and δ 2.49 for dimethylsulfoxide). Carbon-13 NMR spectra were obtained on a Varian Mercury-400 (100 MHz) spectrometer by use of chloroform-*d* ($CDCl_3$) and dimethylsulfoxide-d_6 (DMSO-d_6) as the solvents. Carbon-13 chemical shifts are referenced to the center of the $CDCl_3$ triplet (δ 77.0 ppm) and DMSO septet (δ 39.5 ppm). Multiplicities are recorded by the following

abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; *J*, coupling constant (hertz). High-resolution mass spectra were obtained by means of a JEOL JMS-700 mass spectrometer.

Standard Procedure 1 for the Preparation of Couamrin Intermediates 6 and 8. To a solution containing 7-hydroxycoumarin[50] (**4**, 1.0 equiv) in anhydrous acetone (6.0–15 mL) was added potassium carbonate (1.5 equiv) and a sulfonyl chloride **5** or benzyl bromide **7** (1.2 equiv). After the reaction mixture was stirred at 55 °C for 1.0–1.5 h, it was cooled down to room temperature. Inorganic solids were filtered off and the filtrate was concentrated under reduced pressure to afford the residue. It was then purified by use of gravity column chromatography on silica gel (various ratio of EtOAc to hexanes) to give the desired coupled derivatives **6** or **8**.

4-(Chloromethyl)-2-oxo-2*H*-chromen-7-yl Benzenesulfonate (6a).[55] The Standard Procedure **1** was followed by use of **4** (500.4 mg, 2.376 mmol, 1.0 equiv) in acetone (15 mL), K_2CO_3 (492.6 mg, 3.564 mmol, 1.5 equiv), and benzenesulfonyl chloride (**5a**, 503.5 mg, 2.851 mmol, 1.2 equiv). After workup, the residue was purified by use of gravity column chromatography on silica gel (20% EtOAc in hexanes) to give the desired coumarin **6a** (728.1 mg, 1.996 mmol) in 84% yield as white solids. The spectroscopic data were found to be in accordance with literature data.[55]

4-(Chloromethyl)-2-oxo-2*H*-chromen-7-yl 4-Methylbenzenesulfonate (6b). The Standard Procedure **1** was followed by use of **4** (500.1 mg, 2.374 mmol, 1.0 equiv) in acetone (15 mL), K_2CO_3 (492.3 mg, 3.562 mmol, 1.5 equiv), and 4-methylbenzenesulfonyl chloride (**5b**, 543.2 mg, 2.849 mmol, 1.2 equiv). After workup, the residue was purified by use of gravity column chromatography on silica gel (20% EtOAc in hexanes) to give the desired coumarin **6b** (710.3 mg, 1.947 mmol) in 82% yield as white solids: mp (recrystallized from CH_2Cl_2)

130.3–131.2 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.72 (d, J = 8.0 Hz, 2 H, 2 × ArH), 7.61 (d, J = 8.8 Hz, 1 H, H-5), 7.33 (d, J = 8.0 Hz, 2 H, 2 × ArH), 7.11–7.08 (m, 1 H, H-6), 6.89 (d, J = 2.4 Hz, 1 H, H-8), 6.52 (s, 1 H, H-3), 4.60 (s, 2 H, CH_2Cl), 2.45 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.46 (C=O), 154.19, 151.80, 148.84, 146.12, 131.76, 130.06, 128.38, 125.43, 119.03, 116.09, 115.85, 111.26, 41.02 (CH_2Cl), 21.73 (CH_3); IR (neat) 1734 (s, C=O), 1376 (m, S=O), 1177 (s), 1121 (s), 996 (s), 755 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{17}H_{13}ClO_5S$): 364.0172; found 364.0179.

4-(Chloromethyl)-2-oxo-2*H*-chromen-7-yl 4-Fluorobenzenesulfonate (6c). The Standard Procedure **1** was followed by use of **4** (400.2 mg, 1.900 mmol, 1.0 equiv) in acetone (15 mL), K_2CO_3 (393.9 mg, 2.850 mmol, 1.5 equiv), and 4-fluorobenzenesulfonyl chloride (**5c**, 443.7 mg, 2.280 mmol, 1.2 equiv). After workup, the residue was purified by use of gravity column chromatography on silica gel (20% EtOAc in hexanes) to give the desired coumarin **6c** (581.6 mg, 1.577 mmol) in 83% yield as white solids: mp (recrystallized from CH_2Cl_2) 133.5–134.5 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.91–7.87 (m, 2 H, 2 × ArH), 7.64 (d, J = 8.8 Hz, 1 H, H-5), 7.26–7.22 (m, 2 H, 2 × ArH), 7.08 (dd, J = 8.8, 2.2 Hz, 1 H, H-6), 6.97 (d, J = 2.2 Hz, 1 H, H-8), 6.55 (s, 1 H, H-3), 4.62 (s, 2 H, CH_2Cl); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.83 (C−F), 165.26 (C=O), 159.61, 154.58, 151.80, 149.00, 131.68, 131.58, 125.87, 119.12, 117.34, 117.11, 116.58, 116.37, 111.58, 41.29 (CH_2Cl); IR (neat) 1734 (s, C=O), 1381 (m, S=O), 1260 (s, C−F), 764 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{16}H_{10}ClFO_5S$): 367.9922; found 367.9926.

4-(Chloromethyl)-2-oxo-2*H*-chromen-7-yl 4-Nitrobenzenesulfonate (6d).[55] The Standard Procedure **1** was followed by use of **4** (500.2 mg, 2.375 mmol, 1.0 equiv) in acetone (15 mL), K_2CO_3 (492.4 mg, 3.562 mmol, 1.5 equiv), and 4-nitrobenzenesulfonyl chloride (**5d**, 631.6 mg,

2.850 mmol, 1.2 equiv). After workup, the residue was purified by use of gravity column chromatography on silica gel (20% EtOAc in hexanes) to give the desired coumarin **6d** (778.2 mg, 1.966 mmol) in 83% yield as light yellow. The spectroscopic data were found to be in accordance with literature data.[55]

4-(Chloromethyl)-2-oxo-2*H*-chromen-7-yl 2-Nitrobenzenesulfonate (6e). The Standard Procedure **1** was followed by use of **4** (500.2 mg, 2.375 mmol, 1.0 equiv) in acetone (15 mL), K_2CO_3 (492.4 mg, 3.562 mmol, 1.5 equiv), and 2-nitrobenzenesulfonyl chloride (**5e**, 631.6 mg, 2.850 mmol, 1.2 equiv). After workup, the residue was purified by use of gravity column chromatography on silica gel (20% EtOAc in hexanes) to give the desired coumarin **6e** (789.6 mg, 1.995 mmol) in 84% yield as light yellow: mp (recrystallized from CH_2Cl_2) 137.4–138.2 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.02 (d, J = 7.6 Hz, 1 H, ArH), 7.88–7.87 (m, 2 H, 2 × ArH), 7.75–7.67 (m, 2 H, H-5 + ArH), 7.27 (dd, J = 8.8, 2.4 Hz, 1 H, H-6), 7.19 (d, J = 2.4 Hz, 1 H, H-8), 6.56 (s, 1 H, H-3), 4.62 (s, 2 H, CH_2Cl); ^{13}C NMR (DMSO, 100 MHz) δ 158.97 (C=O), 153.88, 150.33, 149.77, 147.90, 137.32, 133.31, 131.84, 127.25, 125.92, 125.67, 118.17, 116.74, 115.98, 110.70, 41.11 (CH_2Cl); IR (neat) 1734 (s, C=O), 1541 (m, N=O), 1381 (m, S=O), 996 (m), 837 (m) cm^{-1}; HRMS (FAB) calcd for ($C_{16}H_{10}ClNO_7S$): 394.9870 found 394.9875.

7-(Benzyloxy)-4-(chloromethyl)-2*H*-chromen-2-one (8a).[56] The Standard Procedure **1** was followed by use of **4** (360.4 mg, 1.711 mmol, 1.0 equiv) in acetone (7.0 mL), K_2CO_3 (354.7 mg, 2.567 mmol, 1.5 equiv), and benzyl bromide (**7a**, 351.2 mg, 2.053 mmol, 1.2 equiv). After workup, the residue was purified by use of gravity column chromatography on silica gel (15% EtOAc in hexanes) to give the desired coumarin **8a** (458.0 mg, 1.523 mmol) in 89% yield as white solids. The spectroscopic data were found to be in accordance with literature data.[56]

4-(Chloromethyl)-7-[(4-methylbenzyl)oxy]-2*H*-chromen-2-one (8b). The Standard Procedure **1** was followed by use of **4** (350.2 mg, 1.663 mmol, 1.0 equiv) in acetone (7.0 mL), K_2CO_3 (344.7 mg, 2.494 mmol, 1.5 equiv), and 4-methylbenzyl bromide (**7b**, 369.2 mg, 1.995 mmol, 1.2 equiv). After workup, the residue was purified by use of gravity column chromatography on silica gel (15% EtOAc in hexanes) to give the desired coumarin **8b** (471.0 mg, 1.496 mmol) in 90% yield as white solids: mp (recrystallized from CH_2Cl_2) 209.4–210.2 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.53 (d, J = 8.6 Hz, 1 H, H-5), 7.30 (d, J = 7.2 Hz, 2 H, 2 × ArH), 7.19 (d, J = 7.2 Hz, 2 H, 2 × ArH), 6.93 (d, J = 8.6 Hz, 1 H, H-6), 6.88 (s, 1 H, H-8), 6.37 (s, 1 H, H-3), 5.07 (s, 2 H, OCH_2), 4.58 (s, 2 H, CH_2Cl), 2.34 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.02 (C=O), 160.75, 155.55, 149.60, 138.26, 132.49, 129.39, 127.62, 125.11, 113.26, 112.54, 110.82, 102.19, 70.45 (OCH_2), 41.27 (CH_2Cl), 21.16 (CH_3); IR (neat) 1719 (s, C=O), 1612 (s), 1397 (m), 1265 (m), 1148 (m), 1058 (m) cm^{-1}; HRMS (FAB) calcd for ($C_{18}H_{15}ClO_3$): 314.0710; found 314.0716.

7-[(4-Chlorobenzyl)oxy]-4-(chloromethyl)-2*H*-chromen-2-one (8c).[57] The Standard Procedure **1** was followed by use of **4** (320.2 mg, 1.520 mmol, 1.0 equiv) in acetone (6.0 mL), K_2CO_3 (315.2 mg, 2.280 mmol, 1.5 equiv), and 4-chlorobenzyl bromide (**7c**, 374.8 mg, 1.824 mmol, 1.2 equiv). After workup, the residue was purified by use of gravity column chromatography on silica gel (12% EtOAc in hexane) to give the desired coumarin **8c** (453.5 mg, 1.353 mmol) in 89% yield as white solids. The spectroscopic data were found to be in accordance with literature data.[57]

7-[(4-Bromobenzyl)oxy]-4-(chloromethyl)-2*H*-chromen-2-one (8d). The Standard Procedure **1** was followed by use of **4** (300.1 mg, 1.425 mmol, 1.0 equiv) in acetone (6.0 mL), K_2CO_3 (295.4 mg, 2.137 mmol, 1.5 equiv), and 4-bromobenzyl bromide (**7d**, 427.3 mg, 1.710 mmol, 1.2

equiv). After workup, the residue was purified by use of gravity column chromatography on silica gel (12% EtOAc in hexane) to give the desired coumarin **8d** (476.0 mg, 1.254 mmol) in 88% yield as white solids: mp (recrystallized from CH_2Cl_2) 203.3−204.2 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.56–7.49 (m, 3 H, H-5 + 2 × ArH), 7.29–7.27 (m, 2 H, 2 × ArH), 6.92 (dd, J = 6.0, 2.4 Hz, 1 H, H-6), 6.86 (d, J = 2.4 Hz, 1 H, H-8), 6.38 (s, 1 H, H-3), 5.06 (s, 2 H, OCH_2), 4.59 (s, 2 H, CH_2Cl); ^{13}CNMR ($CDCl_3$, 100 MHz) δ 161.66, (C=O), 160.61, 155.61, 149.52, 134.63, 131.90, 129.10, 125.29, 122.39, 113.22, 112.87, 111.13, 102.25, 69.73 (OCH_2), 41.28 (CH_2Cl); IR (neat) 1725 (s, C=O), 1611 (s), 1386 (m), 1265 (m), 1148 (m), 1058 (m) cm^{-1}; HRMS (FAB) calcd for ($C_{17}H_{12}ClBrO_3$): 377.9658; found 377.9654.

Standard Procedure 2 for the Preparation of Conjugated Compounds 10, **12**, **and 14–20**. To a solution containing 2-thio-5,6-benzopyrimidine or 2- thiopyrimidine (**9**, **11**, or **13**, 1.0 equiv) in THF or ethanol (4.0–6.0 mL) was added K_2CO_3 (1.5 equiv) and the suitable 4-(chloromethyl)coumarin (**4**, **6**, or **8**, 1.2 equiv). After the solution was stirred at 40 °C for 3.0–4.0 h, it was cooled down to room temperature and diluted with CH_2Cl_2 (4.0–6.0 mL). Inorganic solids were filtered off and the filtrate was concentrated under reduced pressure to afford the residue. It was then purified by use of gravity column chromatography on silica gel (various ratio of methanol to CH_2Cl_2) to give the desired conjugates **10**, **12**, and **14–20.**

2-Oxo-4-([(4-oxo-3,4-dihydroquinazolin-2-yl)thio]methyl)-2*H*-chromen-7-yl Benzenesulfonate (10a). The Standard Procedure **2** was followed by use of **9** (40.1 mg, 0.225 mmol, 1.0 equiv) in THF (5.0 mL), K_2CO_3 (46.6 mg, 0.337 mmol, 1.5 equiv), and 4-(chloromethyl)coumarin-7-yl benzenesulfonate (**6a**, 94.7 mg, 0.270 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired

conjugated compound **10a** (99.6 mg, 0.202 mmol) in 90% yield as white solids: mp (recrystallized from methanol/CH_2Cl_2) 201.7–202.6 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.05–8.00 (m, 2 H, H-5 + ArH), 7.93 (d, J = 7.2 Hz, 2 H, 2 × ArH), 7.84 (t, J = 7.3 Hz, 1 H, H-7), 7.77–7.74 (m, 1 H, H-8), 7.70–7.66 (m, 2 H, 2 × ArH), 7.50 (d, J = 8.0 Hz, 1 H, H-5′), 7.42 (t, J = 7.3 Hz, 1 H, H-6), 7.13–7.11 (m, 2 H, H-6′ + H-8′), 6.72 (s, 1 H, H-3′), 4.67 (s, 2 H, SCH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.25 (C=O), 159.09 (N=C–S), 154.14 (C=O), 153.75, 150.73, 150.69, 149.19, 148.02, 135.39, 134.71, 133.93, 129.99, 128.33, 127.07, 126.09, 125.95, 120.09, 118.29, 117.33, 116.00, 110.69, 29.26 (SCH_2); IR (neat) 3007 (w), 1734 (s, C=O), 1653 (s, C=O), 1559 (m), 1457 (m), 1275 (s, S=O), 1260 (s), 764 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{24}H_{16}N_2O_6S_2$): 492.0450; found 492.0450.

2-Oxo-4-([(4-oxo-3,4-dihydroquinazolin-2-yl)thio]methyl)-2*H*-chromen-7-yl 4-Methylbenzenesulfonate (10b). The Standard Procedure **2** was followed by use of **9** (30.1 mg, 0.169 mmol, 1.0 equiv) in THF (4.0 mL), K_2CO_3 (35.0 mg, 0.253 mmol, 1.5 equiv), and 4-(chloromethyl)coumarin-7-yl 4-methylbenzenesulfonate (**6b**, 73.9 mg, 0.203 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **10b** (75.3 mg, 0.148 mmol) in 88% yield as off white solids: mp (recrystallized from methanol/CH_2Cl_2) 215.1–216.2 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.13 (d, J = 7.8, 1.6 Hz, 1 H, H-5), 7.73–7.66 (m, 4 H, H-7 + H-8 + 2 × ArH), 7.50 (d, J = 8.4 Hz, 1 H, H-5′), 7.37 (t, J = 7.8 Hz, 1 H, H-6), 7.30 (d, J = 8.0 Hz, 2 H, 2 × ArH), 7.08 (d, J = 8.4 Hz, 1 H, H-6′), 6.83 (s, 1 H, H-8′), 6.63 (s, 1 H, H-3′), 4.58 (s, 2 H, SCH_2), 2.41 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.33 (C=O), 159.69 (N=C–S), 154.25 (C=O), 152.22, 151.78, 149.50, 148.55, 146.10, 135.33, 131.87, 130.08, 128.42, 126.86, 126.55, 126.35, 125.58, 119.84, 119.12,

117.08, 116.49, 111.23, 30.04 (SCH_2), 21.76 (CH_3); IR (neat) 3008 (w), 1734 (s, C=O), 1674 (s, C=O), 1379 (m, S=O), 1261 (s), 1179 (w), 764 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{25}H_{18}N_2O_6S_2$): 506.0610; found 506.0612.

2-Oxo-4-([(4-oxo-3,4-dihydroquinazolin-2-yl)thio]methyl)-2*H*-chromen-7-yl 4-Fluorobenzenesulfonate (10c). The Standard Procedure **2** was followed by use of **9** (50.2 mg, 0.282 mmol, 1.0 equiv) in THF (6.0 mL), K_2CO_3 (58.4 mg, 0.422 mmol, 1.5 equiv), and 4-(chloromethyl)coumarin-7-yl 4-fluorobenzenesulfonate (**6c**, 0.125 g, 0.338 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **10c** (0.131 g, 0.256 mmol) in 91% yield as white solids: mp (recrystallized from methanol/CH_2Cl_2) 252.3–253.1 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.08 (d, J = 8.0 Hz, 1 H, H-5), 7.83–7.80 (m, 2 H, 2 × ArH), 7.71 (d, J = 8.0 Hz, 1 H, H-6), 7.64 (t, J = 8.0 Hz, 1 H, H-7), 7.46 (d, J = 8.2 Hz, 1 H, H-5′), 7.34 (t, J = 8.0 Hz, 1 H, H-6), 7.19–7.15 (m, 2 H, 2 × ArH), 7.01 (d, J = 8.2 Hz, 1 H, H-6′), 6.89 (s, 1 H, H-8′), 6.62 (s, 1 H, H-3′), 4.55 (s, 2 H, SCH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.56 (C−F), 164.99 (C=O), 161.52, 159.53 (N=C–S), 154.91, 154.28, 151.41, 149.62, 134.96, 131.40, 131.31, 130.37, 129.87, 129.14, 127.45, 126.53, 126.08, 125.69, 118.76, 117.04, 116.82, 116.52, 111.17, 32.01 (SCH_2); IR (neat) 2988 (w), 2322 (w), 1750 (s, C=O), 1683 (s, C=O), 1276 (s, C−F), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{24}H_{15}FN_2O_6S_2$): 510.0360; found 510.0365.

2-Oxo-4-([(4-oxo-3,4-dihydroquinazolin-2-yl)thio]methyl)-2*H*-chromen-7-yl 4-Nitrobenzenesulfonate (10d). The Standard Procedure **2** was followed by use of **9** (30.1 mg, 0.169 mmol, 1.0 equiv) in THF (4.0 mL), K_2CO_3 (35.0 mg, 0.253 mmol, 1.5 equiv), and 4-(chloromethyl)coumarin-7-yl 4-nitrobenzenesulfonate (**6d**, 80.2 mg, 0.203 mmol, 1.2 equiv).

After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **10d** (80.8 mg, 0.150 mmol) in 89% yield as pale yellow solids: mp (recrystallized from methanol/CH_2Cl_2) 252.1–253.3 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.45 (d, J = 8.8 Hz, 2 H, 2 × ArH), 8.21 (d, J = 8.8 Hz, 2 H, 2 × ArH), 8.06 (d, J = 8.0 Hz, 1 H, H-5), 8.01 (d, J = 8.0 Hz, 1 H, H-8), 7.76 (t, J = 8.0 Hz, 1 H, H-7), 7.52 (d, J = 8.4 Hz, 1 H, H-5′), 7.42 (t, J = 8.0 Hz, 1 H, H-6), 7.27 (s, 1 H, H-8′), 7.19 (d, J = 8.4 Hz, 1 H, H-6′), 6.74 (s, 1 H, H-3′), 4.68 (s, 2 H, SCH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.29 (C=O), 159.03 (N=C–S), 153.83 (C=O), 151.20, 150.59, 150.36, 149.46, 148.02, 139.10, 134.65, 130.12, 127.24, 126.07, 125.91, 125.14, 123.34, 120.08, 118.32, 117.63, 116.21, 110.83, 29.26 (SCH_2); IR (neat) 3005 (w), 1735 (s, C=O), 1675 (s, C=O), 1525 (w, N=O), 1275 (m, S=O), 1194 (m), 1119 (m), 997 (m), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{24}H_{15}N_3O_8S_2$): 537.0301; found 537.0300.

2-Oxo-4-([(4-oxo-3,4-dihydroquinazolin-2-yl)thio]methyl)-2*H*-chromen-7-yl 2-Nitrobenzenesulfonate (10e). The Standard Procedure **2** was followed by use of **9** (30.2 mg, 0.169 mmol, 1.0 equiv) in THF (4.0 mL), K_2CO_3 (35.1 mg, 0.254 mmol, 1.5 equiv), and 4-(chloromethyl)coumarin-7-yl 2-nitrobenzenesulfonate (**6e**, 80.4 mg, 0.203 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **10e** (85.6 mg, 0.159 mmol) in 94% yield as pale yellow solids: mp (recrystallized from methanol/CH_2Cl_2) 261.4–260.2 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.23 (d, J = 7.6 Hz, 1 H, H-5), 8.10–8.07 (m, 3 H, 3 × ArH), 8.01 (d, J = 8.0 Hz, 1 H, ArH), 7.92–7.88 (m, 1 H, H-8), 7.75 (t, J = 7.6 Hz, 1 H, H-7), 7.51 (d, J = 8.4 Hz, 1 H, H-5′), 7.42 (t,

J = 7.6 Hz, 1 H, H-6), 7.28 (d, J = 2.2 Hz, 1 H, H-8′), 7.23 (dd, J = 8.4, 2.2 Hz, 1 H, H-6′), 6.75 (s, 1 H, H-3′), 4.69 (s, 2 H, SCH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.27 (C=O), 159.02 (N=C–S), 154.17 (C=O), 153.87, 150.65, 150.27, 148.02, 147.94, 137.37, 134.71, 133.34, 131.88, 127.38, 126.09, 125.93, 125.85, 125.71, 125.46, 120.10, 118.16, 117.79, 116.25, 110.70, 29.29 (SCH_2); IR (neat) 3007 (w), 1735 (s, C=O), 1683 (s, C=O), 1260 (m, S=O), 1193 (m), 997 (m), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{24}H_{15}N_3O_8S_2$): 537.0301; found 537.0300.

4-([(5-Methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]methyl)-2-oxo-2*H*-chromen-7-yl Benzenesulfonate (12a). The Standard Procedure **2** was followed by use of **11** (35.2 mg, 0.247 mmol, 1.0 equiv) in ethanol (4.0 mL), K_2CO_3 (51.0 mg, 0.369 mmol, 1.5 equiv), and 4-(chloromethyl)coumarin-7-yl benzenesulfonate (**6a**, 0.104 g, 0.297 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **12a** (89.2 mg, 0.195 mmol) in 82% yield as white solids: mp (recrystallized from methanol/CH_2Cl_2) 196.3–197.4 °C; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.83–7.81 (m, 2 H, H-6 + ArH), 7.69–7.65 (m, 2 H, 2 × ArH), 7.62 (d, J = 8.8 Hz, 1 H, H-5′), 7.54–7.51 (m, 2 H, 2 × ArH), 7.06–7.03 (m, 1 H, H-6′), 6.86 (d, J = 1.6 Hz, 1 H, H-8′), 6.55 (s, 1 H, H-3′), 4.44 (s, 2 H, SCH_2), 1.95 (s, 3 H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.49 (C=O), 159.05 (N=C–S), 157.83 (C=O), 153.70, 150.78, 150.57, 149.17, 135.35, 133.96, 129.96, 128.31, 126.89, 119.05, 118.30, 117.26, 115.35, 110.69, 29.44 (SCH_2), 12.44 (CH_3); IR (neat) 3007 (w), 1718 (s, C=O), 1654 (s, C=O), 1559 (m), 1353 (m, S=O), 1276 (s), 836 (m), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{21}H_{16}N_2O_6S_2$): 456.0450; found 456.0459.

4-([(5-Methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]methyl)-2-oxo-2*H*-chromen-7-yl 4-Methylbenzenesulfonate (12b). The Standard Procedure **2** was followed by use of **11** (30.2 mg,

0.212 mmol, 1.0 equiv) in ethanol (4.0 mL), K_2CO_3 (44.0 mg, 0.319 mmol, 1.5 equiv), and 4-(chloromethyl)coumarin-7-yl 4-methylbenzenesulfonate (**6b**, 92.9 mg, 0.255 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **12b** (79.9 mg, 0.170 mmol) in 81% yield as white solids: mp (recrystallized from methanol/CH_2Cl_2) 210.1–211.2 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.69–7.65 (m, 3 H, H-6 + 2 × ArH), 7.61 (d, J = 8.6 Hz, 1 H, H-5′), 7.31–7.29 (m, 2 H, 2 × ArH), 7.06 (dd, J = 8.6, 2.2 Hz, 1 H, H-6′), 6.84 (d, J = 2.2 Hz, 1 H, H-8′), 6.54 (s, 1 H, H-3′), 4.44 (s, 2 H, SCH_2), 2.41 (s, 3 H, CH_3), 1.94 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.96 (C=O), 159.98 (N=C−S), 155.93 (C=O), 154.08, 151.63, 151.43, 149.59, 146.13, 131.64, 130.02, 128.32, 125.45, 120.95, 118.99, 117.01, 115.94, 111.15, 29.84 (SCH_2), 21.64 (CH_3), 12.57 (CH_3); IR (neat) 3005 (w), 1718 (s, C=O), 1653 (s, C=O), 1558 (m), 1353 (m, S=O), 1260 (s), 836 (m), 764 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{22}H_{18}N_2O_6S_2$): 470.0606; found 470.0600.

4-([(5-Methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]methyl)-2-oxo-2*H*-chromen-7-yl 4-Fluorobenzenesulfonate (12c). The Standard Procedure **2** was followed by use of **11** (50.1 mg, 0.352 mmol, 1.0 equiv) in ethanol (4.0 mL), K_2CO_3 (73.0 mg, 0.528 mmol, 1.5 equiv), and 4-(chloromethyl)coumarin-7-yl 4-fluorobenzenesulfonate (**6c**, 0.156 g, 0.423 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **12c** (0.129 g, 0.271 mmol) in 83% yield as white solids: mp (recrystallized from methanol/CH_2Cl_2) 249.3–250.1 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.84–7.80 (m, 2 H, 2 × ArH), 7.62–7.60 (m, 2 H, H-6 + H-5′), 7.20–7.16 (m, 2 H, 2 × ArH),

6.99 (dd, J = 8.8, 2.2 Hz, 1 H, H-6′), 6.89 (d, J = 2.2 Hz, 1 H, H-8′), 6.53 (s, 1 H, H-3′), 4.42 (s, 2 H, SCH_2), 1.91 (s, 3 H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 167.03 (C−F), 164.49 (C=O), 163.57 (N=C−S), 159.03, 157.93, 153.77, 150.71, 150.53, 131.75, 131.65, 130.28, 126.86, 118.98, 118.27, 117.38, 117.32, 117.23, 115.41, 110.72, 29.44 (SCH_2), 12.36 (CH_3); IR (neat) 3004 (w), 1717 (s, C=O), 1540 (w), 1367 (w, S=O), 1276 (m, C−F), 988 (w), 764 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{21}H_{15}FN_2O_6S_2$): 474.0356; found 474.0350.

4-([(5-Methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]methyl)-2-oxo-2*H*-chromen-7-yl 4-Nitrobenzenesulfonate (12d). The Standard Procedure **2** was followed by use of **11** (30.2 mg, 0.212 mmol, 1.0 equiv) in ethanol (4.0 mL), K_2CO_3 (44.0 mg, 0.319 mmol, 1.5 equiv), and 4-(chloromethyl)coumarin-7-yl 4-nitrobenzenesulfonate (**6d**, 0.101 g, 0.255 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **12d** (81.9 mg, 0.164 mmol) in 81% yield as light yellow solids: mp (recrystallized from methanol/CH_2Cl_2) 251.5−252.4 °C; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.40 (d, J = 8.8 Hz, 2 H, 2 × ArH), 8.08 (d, J = 8.8 Hz, 2 H, 2 × ArH), 7.73 (s, 1 H, H-6), 7.66 (d, J = 8.8 Hz, 1 H, H-5′), 7.04 (dd, J = 8.8, 2.2 Hz, 1 H, H-6′), 6.98 (d, J = 2.2 Hz, 1 H, H-8′), 6.61 (s, 1 H, H-3′), 4.48 (s, 2 H, SCH_2), 2.00 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.02 (C=O), 159.34 (N=C−S), 155.87, 154.34, 151.69, 151.23, 150.98, 149.04, 140.44, 129.85, 125.86, 124.66, 121.07, 118.42, 117.55, 116.67, 111.12, 29.95 (SCH_2), 12.68 (CH_3); IR (neat) 3006 (w), 1717 (s, C=O), 1540 (w, N=O), 1472 (w), 1260 (m, S=O), 1183 (w), 988 (w), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{21}H_{15}N_3O_8S_2$): 501.0301; found 501.0305.

4-([(5-Methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio]methyl)-2-oxo-2*H*-chromen-7-yl 2-Nitrobenzenesulfonate (12e). The Standard Procedure **2** was followed by use of **11** (25.1 mg,

0.176 mmol, 1.0 equiv) in ethanol (4.0 mL), K_2CO_3 (36.6 mg, 0.265 mmol, 1.5 equiv), and 4-(chloromethyl)coumarin-7-yl 2-nitrobenzenesulfonate (**6e**, 83.8 mg, 0.212 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **12e** (70.0 mg, 0.138 mmol) in 84% yield as light yellow solids: mp (recrystallized from methanol/CH_2Cl_2) 259.4–260.1 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (d, J = 8.0 Hz, 1 H, ArH), 7.87–7.86 (m, 2 H, 2 × ArH), 7.74–7.68 (m, 3 H, H-6 + H-5′ + ArH), 7.25–7.22 (m, 1 H, H-6′), 7.14 (d, J = 2.0 Hz, 1 H, H-8′), 6.60 (s, 1 H, H-3′), 4.49 (s, 2 H, SCH_2), 1.99 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.05 (C=O), 159.81, 156.02, 154.14, 150.81, 150.19, 149.59, 135.87, 132.14, 131.94, 127.76, 125.78, 125.02, 120.83, 118.67, 117.50, 116.20, 111.16, 31.76 (SCH_2), 12.53 (CH_3); IR (neat) 3006 (w), 1717 (s, C=O), 1653 (s, C=O), 1559 (w, N=O), 1276 (s, S=O), 850 (w), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{21}H_{15}N_3O_8S_2$): 501.0301; found 501.0300.

2-Oxo-4-([(6-oxo-1,6-dihydropyrimidin-2-yl)thio]methyl)-2*H*-chromen-7-yl Benzenesulfonate (14a). The Standard Procedure **2** was followed by use of **13** (30.1 mg, 0.235 mmol, 1.0 equiv) in ethanol (4.0 mL), K_2CO_3 (48.7 mg, 0.352 mmol, 1.5 equiv), and 4-(chloromethyl)coumarin-7-yl benzenesulfonate (**6a**, 98.8 mg, 0.282 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 3.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **14a** (88.2 mg, 0.199 mmol) in 85% yield as white solids: mp (recrystallized from dichloromethane/methanol) 187.4–188.3 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.85–7.82 (m, 3 H, H-6 + 2 × ArH), 7.70–7.66 (m, 1 H, ArH), 7.60 (d, J = 8.0 Hz, 1 H, H-5′), 7.59-7.51 (m, 2 H, 2 × ArH), 7.04 (d, J = 8.0 Hz, 1 H, H-6′), 6.86 (s, 1 H, H-8′), 6.58 (s, 1 H,

H-3′), 6.23–6.21 (m, 1 H, H-5), 4.48 (s, 2 H, SCH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.91 (C=O), 159.61 (N=C–S), 154.88 (C=O), 154.14, 151.59, 148.93, 134.78, 129.45, 129.19, 128.34, 128.24, 125.44, 118.94, 116.99, 116.28, 111.55, 111.23, 29.99 (SCH_2); IR (neat), 3006 (w), 1718 (s, C=O), 1653 (s, C=O), 1558 (m), 1353 (m, S=O), 1276 (s), 836 (m), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{20}H_{14}N_2O_6S_2$): 442.0293; found 442.0290**.**

2-Oxo-4-([(6-oxo-1,6-dihydropyrimidin-2-yl)thio]methyl)-2*H*-chromen-7-yl 4-Methylbenzenesulfonate (14b). The Standard Procedure **2** was followed by use of **13** (30.2 mg, 0.236 mmol, 1.0 equiv) in ethanol (4.0 mL), K_2CO_3 (48.8 mg, 0.353 mmol, 1.5 equiv), and 4-(chloromethyl)coumarin-7-yl 4-methylbenzenesulfonate (**6b**, 0.103 g, 0.283 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 3.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **14b** (91.4 mg, 0.200 mmol) in 85% yield as white solids: mp (recrystallized from methanol/CH_2Cl_2) 191.4–192.5 °C; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.85 (d, J = 6.8 Hz, 1 H, H-6), 7.71 (d, J = 8.2 Hz, 2 H, 2 × ArH), 7.60 (d, J =8.8 Hz, 1 H, H-5′), 7.31 (d, J = 8.2 Hz, 2 H, 2 × ArH), 7.06 (dd, J = 8.8, 2.0 Hz, 1 H, H-6′), 6.85 (d, J = 2.0 Hz, 1 H, H-8′), 6.58, (s, 1 H, H-3′), 6.23 (d, J = 6.8 Hz, 1 H, H-5), 4.49 (s, 2 H, SCH_2), 2.43 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.80 (C=O), 159.68 (C=O), 159.61 (N=C−S), 154.76, 154.15, 151.75, 148.98, 146.08, 131.84, 130.05, 128.36, 125.41, 118.96, 116.92, 116.22, 111.52, 111.18, 30.03 (SCH_2), 21.70 (CH_3); IR (neat) 3007 (w), 1717 (s, C=O), 1653 (s, C=O), 1558 (m), 1353 (m, S=O), 1276 (s), 1183 (w), 996 (m), 764 (s) cm^{-1} ; HRMS (FAB) calcd for ($C_{21}H_{16}N_2O_6S_2$): 456.0450; found 456.0454.

2-Oxo-4-([(6-oxo-1,6-dihydropyrimidin-2-yl)thio]methyl)-2*H*-chromen-7-yl 4-Fluorobenzenesulfonate (14c). The Standard Procedure **2** was followed by use of **13** (40.2 mg,

0.314 mmol, 1.0 equiv) in ethanol (5.0 mL), K_2CO_3 (65.0 mg, 0.471 mmol, 1.5 equiv), and 4-(chloromethyl)coumarin-7-yl 4-fluorobenzenesulfonate (**6c**, 0.139 g, 0.376 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 3.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **14c** (125.4 mg, 0.272 mmol) in 88% yield as white solids: mp (recrystallized from methanol/CH_2Cl_2) 243.3–244.1 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.02–7.94 (m, 4 H, H-6 + H-5′ + 2 × ArH), 7.55–7.51 (m, 2 H, 2 × ArH), 7.20 (d, J = 2.0 Hz, 1 H, H-8′), 7.11 (dd, J = 8.8, 2.0 Hz, 1 H, H-6′), 6.61 (s, 1 H, H-3′), 6.18 (d, J = 5.6 Hz, 1 H, H-5), 4.58 (s, 2 H, SCH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 167.49 (C–F), 164.92 (C=O), 163.86, 159.86 (N=C−S), 154.29, 154.07, 151.34, 149.36, 141.07, 131.31, 131.21, 130.63, 125.55, 118.73, 117.14, 116.98, 116.76, 116.12, 111.16, 29.90 (SCH_2); IR (neat) 3007 (w), 1717 (s, C=O), 1367 (w, S=O), 1276 (m, C−F), 1183 (w), 989 (m), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{20}H_{13}FN_2O_6S_2$): 460.0200; found 460.0200

2-Oxo-4-([(6-oxo-1,6-dihydropyrimidin-2-yl)thio]methyl)-2*H*-chromen-7-yl 4-Nitrobenzenesulfonate (14d). The Standard Procedure **2** was followed by use of **13** (40.1 mg, 0.313 mmol, 1.0 equiv) in ethanol (6.0 mL), K_2CO_3 (64.9 mg, 0.469 mmol, 1.5 equiv), and 4-(chloromethyl)coumarin-7-yl 4-nitrobenzenesulfonate (**6d**, 0.149 g, 0.375 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 3.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **14d** (0.130 g, 0.266 mmol) in 85% yield as pale yellow solids: mp (recrystallized from methanol/CH_2Cl_2) 246.5–247.4 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.45 (d, J = 8.6 Hz, 2 H, 2 × ArH), 8.21 (d, J = 8.6 Hz, 2 H, 2 × ArH), 7.98–7.95 (m, 2 H, H-6 + H-5′), 7.27 (s, 1 H, H-8′), 7.17 (d, J = 8.8 Hz, 1 H, H-6′), 6.62 (s, 1 H, H-3′), 6.18 (br,

1 H, H-5), 4.58 (s, 2 H, SCH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.04 (C=O), 162.42 (C=O), 158.99 (N=C−S), 154.50, 153.81, 150.50, 150.30, 147.93, 137.34, 131.89, 127.21, 125.70, 118.17, 117.73, 115.63, 110.71, 109.23, 29.62 (SCH_2); IR (neat) 3007 (w), 1717 (s, C=O), 1559 (w, N=O), 1378 (w, S=O), 1276 (m), 849 (m), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{20}H_{13}N_3O_8S_2$): 487.0144; found 487.0140**.**

2-Oxo-4-([(6-oxo-1,6-dihydropyrimidin-2-yl)thio]methyl)-2*H*-chromen-7-yl 2-Nitrobenzenesulfonate (14e). The Standard Procedure **2** was followed by use of **13** (30.1 mg, 0.235 mmol, 1.0 equiv) in ethanol (4.0 mL), K_2CO_3 (48.7 mg, 0.352 mmol, 1.5 equiv), and 4-(chloromethyl)coumarin-7-yl 2-nitrobenzenesulfonate (**6e**, 0.112 g, 0.282 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 3.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **14e** (99.5 mg, 0.204 mmol) in 87% yield as pale yellow solids: mp (recrystallized from methanol/CH_2Cl_2) 256.4–257.1 °C; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.23 (d, J = 8.0 Hz, 1 H, ArH), 8.11–8.07 (m, 2 H, 2 × ArH), 8.00 (d, J = 8.8 Hz, 1 H, H-5′), 7.93–7.89 (m, 2 H, H-6 + ArH), 7.30 (d, J = 2.4 Hz, 1 H, H-8′), 7.21 (dd, J = 8.8, 2.4 Hz, 1 H, H-6′), 6.63 (s, 1 H, H-3′), 6.17 (d, J = 5.2 Hz, 1 H, H-5), 4.58 (s, 2 H, SCH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.73 (C=O), 163.10 (C=O), 159.68, 155.21, 154.49, 151.18, 150.98, 148.61, 138.03, 134.00, 132.57, 127.90, 126.60, 126.38, 118.86, 118.41, 116.31, 111.39, 109.92, 30.30 (SCH_2); IR (neat) 3008 (w), 1717 (s, C=O), 1653 (s, C=O), 1559 (m, N=O), 1276 (s, S=O), 841 (w), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{20}H_{13}N_3O_8S_2$): 487.0144; found 487.0140.

2-([(7-(Benzyloxy)-2-oxo-2*H*-chromen-4-yl)methyl]thio)quinazolin-4(3*H*)-one (15a). The Standard Procedure **2** was followed by use of **9** (30.1 mg, 0.169 mmol, 1.0 equiv) in THF (4.0

mL), K_2CO_3 (35.0 mg, 0.253 mmol, 1.5 equiv), and 7-benzyloxy-4-(chloromethyl)coumarin (**8a**, 60.9 mg, 0.203 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **15a** (59.0 mg, 0.133 mmol) in 82% yield as white solids: mp (recrystallized from methanol/CH_2Cl_2) 235.5–236.3 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.92 (d, J = 8.8 Hz, 1 H, H-5), 7.86 (d, J = 7.2 Hz, 1 H, H-5′), 7.46–7.45 (m, 2 H, H-7 + H-8), 7.41–7.37 (m, 3 H, H-6 + 2 × ArH), 7.35–7.33 (m, 1 H, ArH), 7.21 (d, J = 8.4 Hz, 1 H, ArH), 7.06–7.00 (m, 3 H, H-6′ + H-8′ + ArH), 6.45 (s, 1 H, H–3′), 5.74 (s, 1 H, NH), 5.21 (s, 2 H, OCH_2), 4.52 (s, 2 H, SCH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.98 (C=O), 165.63 (C=O), 161.41 (N=C–S), 160.38, 155.14, 154.37, 151.56, 136.36, 131.14, 128.60, 128.16, 127.91, 126.80, 126.16, 124.33, 122.03, 121.23, 112.72, 112.14, 111.32, 102.02, 69.94 (OCH_2), 29.66 (SCH_2); IR (neat) 3005 (w), 1734 (s, C=O), 1684 (s, C=O), 1558 (m), 1276 (s), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{25}H_{18}N_2O_4S$): 442.0990; found 442.0997.

2-([(7-[(4-Methylbenzyl)oxy]-2-oxo-2*H*-chromen-4-yl)methyl]thio)quinazolin-4(3*H*)-one (15b). The Standard Procedure **2** was followed by use of **9** (40.1 mg, 0.225 mmol, 1.0 equiv) in THF (4.0 mL), K_2CO_3 (46.6 mg, 0.337 mmol, 1.5 equiv), and 4-(chloromethyl)-7-[(4-methylbenzyl)oxy]coumarin (**8b**, 85.0 mg, 0.270 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **15b** (83.0 mg, 0.182 mmol) in 86% yield as white solids: mp (recrystallized from methanol/CH_2Cl_2) 260.5–261.4 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.02 (d, J = 8.0 Hz, 1 H, H-5), 7.90 (d, J = 8.8 Hz, 1 H, H-5′), 7.78–7.74 (m, 1 H, H-7), 7.54 (d, J = 8.4 Hz, 1 H, H-8), 7.42 (t, J = 8.0 Hz, 1 H, H-6), 7.34 (d, J = 8.0 Hz, 2 H, 2 × ArH), 7.19 (d, J = 8.0 Hz, 2 H,

2 × ArH), 7.07−7.04 (m, 2 H, H-6′ + H-8′), 6.51 (s, 1 H, H–3′), 5.17 (s, 2 H, OCH_2), 4.67 (s, 2 H, SCH_2), 2.29 (s, 3 H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.98, 165.63, 161.56 (C=O), 160.05 (N=C–S), 155.13, 154.37, 151.67, 136.36, 131.14, 128.60, 128.16, 127.99, 126.80, 126.14, 124.40, 122.03, 121.23, 112.92, 112.14, 111.32, 102.05, 69.85 (OCH_2), 29.34 (SCH_2), 20.78 (CH_3); IR (neat) 3001 (w), 2989 (w), 1717 (s, C=O), 1674 (s, C=O), 1559 (m), 1457 (m), 1260 (s), 764 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{26}H_{20}N_2O_4S$): 456.1144; found 456.1140**.**

2-([(7-(Benzyloxy)-2-oxo-2*H*-chromen-4-yl)methyl]thio)-5-methylpyrimidin-4(3*H*)-one (16a). The Standard Procedure **2** was followed by use of **11** (40.4 mg, 0.284 mmol, 1.0 equiv) in ethanol (5.0 mL), K_2CO_3 (58.9 mg, 0.426 mmol, 1.5 equiv), and 7-benzyloxy-4-(chloromethyl)coumarin (**8a**, 0.102 g, 0.341 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **16a** (95.7 mg, 0.236 mmol) in 83% yield as white solids: mp (recrystallized from methanol/CH_2Cl_2) 230.5–231.3 °C; ^1H NMR (DMSO-d_6, 400 MHz) δ 7.82–7.80 (m, 2 H, H-6 + H-5′), 7.47–7.31 (m, 5 H, 5 × ArH), 7.09 (d, J = 2.8 Hz, 1 H, H-8′), 7.05 (d, J = 8.4 Hz, 1 H, H-6′), 6.39 (s, 1 H, H-3′), 5.22 (s, 2 H, OCH_2), 4.56 (s, 2 H, SCH_2), 1.86 (s, 3 H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.25, 161.54 (C=O), 160.00, 158.68, 155.04, 151.60, 150.66, 136.24, 128.55, 128.13, 127.87, 127.36, 119.72, 112.91, 111.78, 111.61, 102.06, 69.93 (OCH_2), 29.46 (SCH_2), 12.47 (CH_3); IR (neat) 3005 (w), 1702 (s, C=O), 1609 (s, C=O), 1456 (m), 1331 (m), 1275 (s), 1138 (m), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{22}H_{18}N_2O_4S$): 406.0990; found 406.0990.

5-Methyl-2-([(7-[(4-methylbenzyl)oxy]-2-oxo-2*H*-chromen-4-yl)methyl]thio) pyrimidin-

4(3*H*)-one (16b). The Standard Procedure **2** was followed by use of **11** (40.2 mg, 0.283 mmol, 1.0 equiv) in ethanol (5.0 mL), K_2CO_3 (58.6 mg, 0.424 mmol, 1.5 equiv), and 4-(chloromethyl)-7-[(4-methylbenzyl)oxy]coumarin (**8b**, 0.107 g, 0.339 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **16b** (0.104 g, 0.229 mmol) in 81% yield as white solids: mp (recrystallized from methanol/CH_2Cl_2) 230.1–231.3 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.82–7.80 (m, 2 H, H-6 + H-5′), 7.34 (d, J = 7.6 Hz, 2 H, 2 × ArH), 7.20 (d, J = 7.6 Hz, 2 H, 2 × ArH), 7.07 (s, 1 H, H-8′), 7.04 (d, J = 8.8 Hz, 1 H, H-6′), 6.39 (s, 1 H, H-3′), 5.17 (s, 2 H, OCH_2), 4.55 (s, 2 H, SCH_2), 2.29 (s, 3 H, CH_3), 1.86 (s, 3 H, CH_3); ^{13}C NMR (Pyridine-d_5, 100 MHz) δ 165.12 (C=O), 162.32 (N=C−S), 160.76 (C=O), 159.62, 156.13, 152.05, 151.49, 138.20, 133.76, 129.66, 128.30, 126.32, 119.32, 113.26, 113.06, 112.51, 102.47, 70.63 (OCH_2), 30.49 (SCH_2), 21.04 (CH_3), 13.01 (CH_3); IR (neat) 3006 (w), 1717 (m, C=O), 1671 (m, C=O), 1558 (m), 1275 (s), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{23}H_{20}N_2O_4S$): 456.1144; found 456.1140.

2-([(7-[(4-Chlorobenzyl)oxy]-2-oxo-2*H*-chromen-4-yl)methyl]thio)-5-methyl pyrimidin-4(3*H*)-one (16c). The Standard Procedure **2** was followed by use of **11** (30.3 mg, 0.213 mmol, 1.0 equiv) in ethanol (4.0 mL), K_2CO_3 (44.2 mg, 0.320 mmol, 1.5 equiv), and 7-[(4-chlorobenzyl)oxy]-4-(chloromethyl)coumarin **8c**, 85.7 mg, 0.256 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **16c** (76.9 mg, 0.175 mmol) in 82% yield as white solids: mp (recrystallized from methanol/CH_2Cl_2) 252.1–253.5 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.82–7.80 (m, 2 H, H-6 + H-5′), 7.50–7.44 (m, 4 H, 4 × ArH), 7.08 (d, J = 2.6 Hz, 1 H, H-8′),

7.04 (dd, J = 9.2, 2.6 Hz, 1 H, H-6′), 6.39 (s, 1 H, H-3′), 5.22 (s, 2 H, OCH_2), 4.56 (s, 2 H, SCH_2), 1.86 (s, 3 H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.40 (C=O), 161.29, 159.94, 157.94, 155.02, 151.49, 150.89, 135.27, 132.71, 129.64, 128.52, 126.35, 119.06, 112.82, 111.88, 111.69, 102.04, 69.01 (OCH_2), 29.43 (SCH_2), 12.44 (CH_3); IR (neat) 3007 (w), 1700 (s, C=O), 1653 (s, C=O), 1559 (m), 1457 (m), 1275 (s), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{22}H_{17}ClN_2O_4S$): 440.0600; found 440.0601.

2-([(7-(Benzyloxy)-2-oxo-2*H*-chromen-4-yl)methyl]thio)pyrimidin-4(3*H*)-one (17a). The Standard Procedure **2** was followed by use of **13** (20.2 mg, 0.158 mmol, 1.0 equiv) in ethanol (4.0 mL), K_2CO_3 (32.7 mg, 0.236 mmol, 1.5 equiv), and 7-benzyloxy-4-(chloromethyl)coumarin (**8a**, 56.9 mg, 0.189 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **17a** (54.4 mg, 0.139 mmol) in 88% yield as light yellow solids: mp (recrystallized from methanol/CH_2Cl_2) 220–225 °C; ^1H NMR (DMSO-d_6, 400 MHz) δ 7.95 (br, 1 H, H-6), 7.82 (d, J = 8.8 Hz, 1 H, H-5′), 7.47–7.32 (m, 5 H, 5 × ArH), 7.10–7.09 (m, 1 H, H-8′), 7.07–7.04 (m, 1 H, H-6′), 6.42, (s, 1 H, H-3′), 6.19 (br, 1 H, H-5), 5.23 (s, 2 H, OCH_2), 4.58 (s, 2 H, SCH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.92 (C=O), 162.46 (C=O), 161.49, 159.95 (N=C–S), 155.03, 154.43, 151.43, 136.21, 128.50, 128.08, 127.84, 126.30, 112.83, 111.82, 111.59, 109.32, 101.99, 69.89 (OCH_2), 29.62 (SCH_2); IR (neat) 3008 (m), 1699 (s, C=O), 1684 (s, C=O), 1559 (m), 1276 (s), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{21}H_{16}N_2O_4S$): 392.0831; found 392.0836.

2-([(7-[(4-Bromobenzyl)oxy]-2-oxo-2*H*-chromen-4-yl)methyl]thio)pyrimidin-4(3*H*)-one (17d). The Standard Procedure **2** was followed by use of **13** (30.5 mg, 0.238 mmol, 1.0 equiv) in ethanol (4.0 mL), K_2CO_3 (49.3 mg, 0.357 mmol, 1.5 equiv), and 7-[(4-bromobenzyl)oxy]-4-

(chloromethyl)coumarin (**8d**, 0.108 g, 0.286 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the desired conjugated compound **17d** (99.5 mg, 0.212 mmol) in 89% yield as white solids: mp (recrystallized from methanol/CH_2Cl_2) 250.4–251.3 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.84 (d, J = 7.2 Hz, 1 H, H-6), 7.58 (d, J = 8.4 Hz, 2 H, 2 × ArH), 7.45–7.41 (m, 3 H, H-5′ + 2 × ArH), 7.05 (d, J = 2.4 Hz, 1 H, H-8′), 7.01 (dd, J = 8.8, 2.4 Hz, 1 H, H-6′), 6.39 (s, 1 H, H-3′), 5.50 (d, J = 7.2 Hz, 1 H, H-5), 5.20 (s, 2 H, OCH_2), 4.40 (s, 2 H, SCH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.10 (C=O), 166.45 (N=C–S), 161.10 (C=O), 160.24, 155.01, 154.19, 152.88, 135.80, 131.47, 129.99, 126.67, 121.23, 112.63, 112.16, 111.09, 108.44, 101.97, 69.00 (OCH_2), 29.48 (SCH_2); IR (neat) 3006 (w), 1702 (s, C=O), 1609 (s, C=O), 1331 (m), 1275 (s), 1138 (m), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{21}H_{15}BrN_2O_4S$): 469.9930; found 469.9930.

2-([(7-Hydroxy-2-oxo-2*H*-chromen-4-yl)methyl]thio)quinazolin-4(3*H*)-one (18). The Standard Procedure **2** was followed by use of **9** (50.1 mg, 0.281 mmol, 1.0 equiv) in THF (4.0 mL), K_2CO_3 (58.3 mg, 0.423 mmol, 1.5 equiv), and 4-(chloromethyl)-7-hydroxycoumarin (**4**, 71.0 mg, 0.337 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h and then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the conjugated compound **18** (88.2 mg, 0.250 mmol) in 89% yield as white solids: mp (recrystallized from methanol/CH_2Cl) 257.2–259.6 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.01 (d, J = 7.6 Hz, 1 H, H-5), 7.80–7.73 (m, 2 H, H-5′ + H-7), 7.53 (d, J = 8.4 Hz, 1 H, H-8), 7.41 (t, J = 7.2 Hz, 1 H, H-6), 6.82 (d, J = 8.8 Hz, 1 H, H-6′), 6.72 (s, 1 H, H-8′), 6.43 (s, 1 H, H-3′), 4.64 (s, 2 H, SCH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.39 (C=O), 161.21 (N=C–S), 160.16 (C=O), 155.26, 154.25, 151.75, 148.09, 134.71, 126.60, 126.58,

126.11, 125.93, 120.07, 113.04, 111.32, 110.35, 102.54, 29.31 (SCH_2); IR (neat) 3008 (w), 1708 (m, C=O), 1588 (m), 1458 (m), 1260 (m), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{18}H_{12}N_2O_4S$): 352.0520; found 352.0522.

2-([(7-Hydroxy-2-oxo-2*H*-chromen-4-yl)methyl]thio)-5-methylpyrimidin-4(3*H*)-one (19). The Standard Procedure **2** was followed by use of **11** (40.2 mg, 0.283 mmol, 1.0 equiv) in ethanol (5.0 mL), K_2CO_3 (58.6 mg, 0.424 mmol, 1.5 equiv), and 4-(chloromethyl)-7-hydroxycoumarin (**4**, 71.4 mg, 0.339 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the conjugated compound **19** (74.2 mg, 0.235 mmol) in 83% yield as white solids: mp (recrystallized from methanol/CH_2Cl_2) 240.4–242.2 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.47–7.45 (m, 2 H, H-6 + H-5′), 6.62 (dd, J = 8.6, 1.6 Hz, 1 H, H-6′), 6.56 (d, J = 1.6 Hz, 1 H, H-8′), 6.10 (s, 1 H, H-3′), 4.31 (s, 2 H, SCH_2), 1.76 (s, 3 H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.47 (C=O), 164.42 (N=C–S), 164.21 (C=O), 160.76, 155.38, 154.12, 151.36, 125.87, 115.14, 113.92, 109.01, 108.09, 102.47, 29.62 (SCH_2), 13.96 (CH_3); IR (neat) 3008 (w), 1708 (s, C=O), 1588 (m), 1458 (m), 1276 (m), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{15}H_{12}N_2O_4S$): 316.0518; found 316.0516.

2-([(7-Hydroxy-2-oxo-2*H*-chromen-4-yl)methyl]thio)pyrimidin-4(3*H*)-one (20). The Standard Procedure **2** was followed by use of **13** (40.1 mg, 0.313 mmol, 1.0 equiv) in ethanol (4.0 mL), K_2CO_3 (64.9 mg, 0.469 mmol, 1.5 equiv), and 4-(chloromethyl)-7-hydroxycoumarin (**4**, 79.1 mg, 0.375 mmol, 1.2 equiv). After the reaction mixture was stirred at 40 °C for 4.0 h, then workup, the residue was purified by use of gravity column chromatography on silica gel (2.0% methanol in CH_2Cl_2) to give the conjugated compound **20** (75.4 mg, 0.250 mmol) in 81% yield as yellow solids: mp (recrystallized from methanol/CH_2Cl_2) 223.3–225.2 °C; ^{1}H NMR

(DMSO-d_6, 400 MHz) δ 7.57–7.53 (m, 2 H, H-6 + H-5′), 6.71–6.67 (m, 2 H, H-6′ + H-8′), 6.21 (s, 1 H, H-3′), 5.65 (d, J = 5.6 Hz, 1 H, H-5), 4.35 (s, 2 H, SCH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.04 (C=O), 166.53 (N=C–S), 162.68 (C=O), 160.47, 155.14, 153.84, 153.74, 126.04, 113.29, 109.80, 109.10, 107.67, 102.39, 29.49 (SCH_2); IR (neat) 2989 (w), 1708 (s, C=O), 1588 (m), 1276 (m), 750 (s) cm^{-1}; HRMS (FAB) calcd for ($C_{14}H_{10}N_2O_4S$): 302.0361; found 302.0364.

ASSOCIATED CONTENT

Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org."

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Author Contributions

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ABBREVIATIONS

ADP, adenosine diphosphate; CHIK, chikungunya; CHIKV, Chikungunya virus; CC_{50}, cytotoxic concentration; DNA, deoxyribonucleic acid; EC_{50}, half maximal effective concentration; HBV, hepatitis B virus; HCV, hepatitis C virus; RNA, ribonucleic acid; HIV, human immunodeficiency virus; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium; PARP, poly ADP ribose polymerase; RT–PCR, reverse transcription polymerase chain reaction; SAR, structure–activity relationship; SI, selectivity index; TLC, thin layer chromatography.

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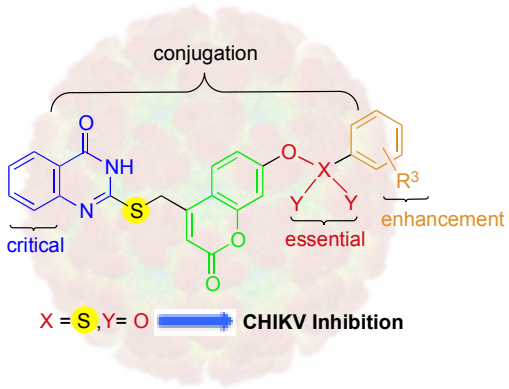
TOC Graphic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