ChemPubSoc Europe

DOI: 10.1002/ejoc.201900569

EurJOC European Journal of Organic Chemistry

Full Paper

Heterocycles

Synthesis and Evaluations of "1,4-Triazolyl Combretacoumarins" and Desmethoxy Analogs

Tashrique A. Khandaker,[a][‡] Jessica D. Hess,[b][‡] Renato Aguilera,[b] Graciela Andrei,[c] Robert Snoeck,[c] Dominique Schols,[c] Padmanava Pradhan,[a] and Mahesh K. Lakshman*[a,d]

Abstract: 1,4-Triazolyl combretacoumarins have been prepared by linking the trimethoxyarene unit of combretastatin A4 with coumarins, via a 1,2,3-triazole. For this, 4-azidocoumarins were accessed by a sequential two-step, one-pot reaction of 4-hydroxycoumarins with (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP), followed by reaction with NaN_3. In the reaction with BOP, a coumarin-derived phosphonium ion intermediate seems to form, leading to an O^4-(benzotriazolyl)coumarin derivative. For the CuAAC reaction of azidocoumarins with 5-ethynyl-1,2,3-trimethoxybenzene, catalytic $[(MeCN)_4Cu]PF_6$ in CH_2Cl_2/MeOH with 2,6-lutidine, at 50 °C, was suitable. The 4-azidocoumarins were less reactive as compared to PhN_3 and the NBO coefficients of the azido groups were compared by DFT analysis. Compound solubility was a problem in biological assays. On the basis of the biological and solubility data of one 1,4-triazolyl combretacoumarin, four analogs lacking one or two methoxy groups were synthesized. Reactivity differences among the phenylacetylenes were noted and the NBO coefficients of the alkynes were compared by DFT analysis. In cytotoxicity assays, 1-phenyl-4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole showed activity in CEM and MDA-MB-231 cell lines by apoptosis. The desmethoxy 6-bromo-4-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one also showed cytotoxicity against the two cell lines, but this did not appear to be consistent with apoptosis. The antiviral activity of the compounds was unremarkable.

Introduction

Coumarins (Figure 1), a family of benzopyrones, are known to have diverse bioactivities.[1–7] They have attracted attention as a potential structural motif for the development of new anticancer agents.[8–11] Derivatives of α-pyrones and coumarins have also been subjects for the development of antiviral agents, for example, against HIV1 and 2, HCV, RSV, HSV1, and A/PR8/H1N1.[12–18] Combretastatin A4 (CA4, Figure 1) is a microtubule-binding natural product that targets the colchicine-binding site and is a vascular-disrupting agent.[19–24] In a recent mechanistic study,[25] CA4 was shown to suppress VEGF signaling and cell proliferation, which may be related to its anti-angiogenic potency. Further, the crystal structure of CA4-tubulin complex has been determined and this indicates CA4 to have common as well as different interactions to colchicine within the binding site.[26] In this analysis,[26] the trimethoxy aryl ring was found to reside in a hydrophobic region containing Ala250, Cys241, Leu255, Asp354, and Ala316, and the hydroxyl group was hydrogen-bonded to Val181 and Thr179. By comparison to colchicine, CA4 was deeper in the hydrophobic pocket and the hydroxyl group of colchicine was bonded to only Val181. Further, binding of CA4 did not significantly alter the structure of tubulin.

On the basis of the biological values of coumarins and CA4, both structural motifs have been scaffolds for the development of new biologically active entities (see the examples in Figure 1[27–32]). Spurred by the importance of both coumarins and CA4, we decided to explore the synthesis and bioactivities of a class of compounds we dub *1,4-triazolyl combretacoumarins*. There are, of course, isomeric 1,4- and 1,5-combretacoumarins (Figure 2). Among the two, the latter class, which would be equivalent to (*Z*)-CA4, could be quite interesting due to the proximal disposition of two aryl rings and, in this context, 1,5-triazolyl combretastatin analogs have been prepared.[33] In the present case, synthesis of this regioisomer would require reaction of a magnesiated alkyne with an azide,[34] but the organometallic reagent would be incompatible with the coumarin. Use of Ru-catalysis is known to yield 1,5-disubstituted 1,2,3-triazoles,[35] but this was unproductive in the present study (see the Supporting Information). On the basis of these considerations, we describe the synthesis and evaluations of 1,4-combretacoumarins, which are structurally closer to (*E*)-CA4.

[a] *Department of Chemistry and Biochemistry, The City College of New York, 160 Convent Avenue, New York, NY 10031, USA*
E-mail: mlakshman@ccny.cuny.edu
Homepages: https://www.ccny.cuny.edu/profiles/mahesh-lakshman
http://www.sci.ccny.cuny.edu/~mkl/
[b] *Department of Biological Sciences, Border Biomedical Research Center, The University of Texas at El Paso, El Paso, TX 79968, USA*
[c] *Department of Microbiology and Immunology, Rega Institute of Medical Research, Herestraat 49 – Box 1043, 3000 Leuven, Leuven, Belgium*
[d] *The Ph.D. Program in Chemistry, The Graduate Center of the City University of New York, New York, NY 10016, USA*
[‡] Equal contributions (TAK: synthesis and JDH: antiproliferation assays)
Supporting information and ORCID(s) from the author(s) for this article are available on the WWW under https://doi.org/10.1002/ejoc.201900569.

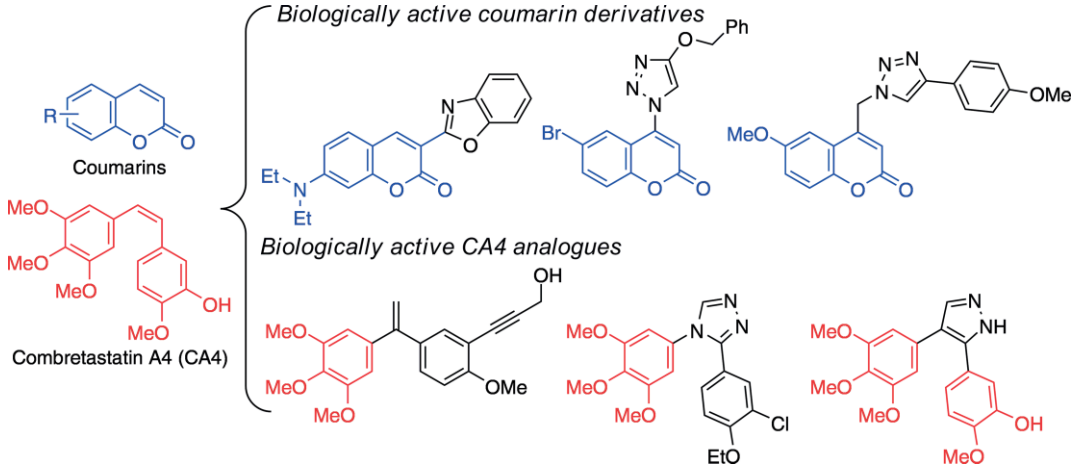


Figure 1. Structures of coumarin, CA4, and several biologically active coumarin derivatives and CA4 analogs.

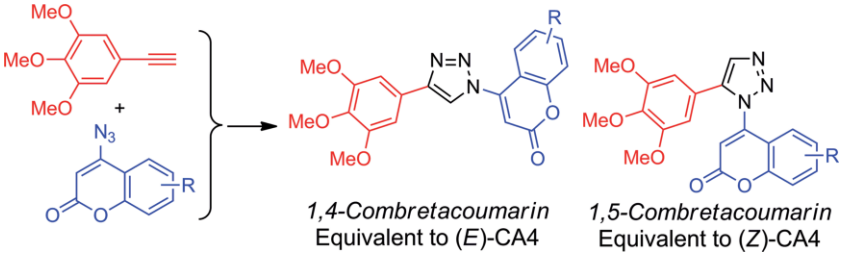
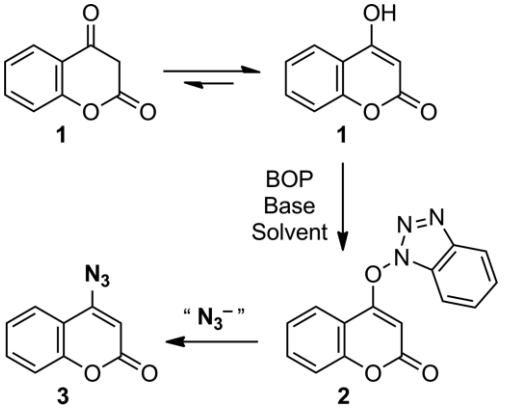


Figure 2. Structures of precursors to two isomeric *combretacoumarins*.

Results and Discussion

Our studies commenced with consideration of developing a rapid method for the synthesis of 4-azidocoumarins, one of the components needed to access the triazolyl combretacoumarins. Typically, 4-azidocoumarins are prepared from 4-hydroxycoumarins, by conversion to either the 4-chloro or 4-tosylate derivatives, followed by displacement with azide ion.[36,37] In our research on uncatalyzed nucleoside modification methods, we have developed facile approaches for C6 and C4 modification of purine and pyrimidine nucleosides.[38–47] That chemistry involved the reaction of the oxygen atoms of the amide linkages in the nucleobases with (benzotriazol-1yloxy)tris-(dimethylamino)phosphonium hexafluorophosphate (BOP), a cheap and commercially available peptide-coupling agent. On the basis of those results, we were curious to assess whether 4-hydroxycoumarin, essentially the tautomeric form of a β-keto lactone, will react with BOP under appropriate conditions. Also in the current context, we have previously demonstrated a general synthesis of 6-azidopurine nucleosides, where O^6-(benzotriazol-1-yl)inosine derivatives were isolated and then converted into the azido analogs.[43] On account of these combined observations, we were also interested in evaluating a one-pot synthesis of 4-azidocoumarins via reactions with BOP (Scheme 1).

With these considerations and on the basis of previous experience, we elected to study the reaction of 4-hydroxycoumarin (**1**) with BOP, in MeCN as a solvent, and 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) as a base. The reaction leading to the formation of the C4 benzotriazolyl derivative **2** was rapid, reaching completion within 1 h at room temperature (other solvents tested were THF and DME[48]). From the entire set of optimizations (see Table 1 in the Supporting Information), the use of MeCN and DBU for the formation of **2**, and 18-crown-6 and NaN_3 for the displacement step, proved optimal for a *two-step, one-pot* conversion of 4-hydroxycoumarin (**1**) to 4-azidocoumarin (**3**).



Scheme 1. A potential one-pot C4 hydroxyl group activation and replacement with azide.

Scale-up of reactions to 1 mmol was considered for the synthesis of various 4-azidocoumarins. However, in the scale-up, the second step of the conversion did not reach completion under the initially optimized conditions. A slight modification of the reaction conditions for this step, by increasing the

amount of 18-crown-6 to 20 mol-% and NaN_3 to 3 equiv., led to full conversion within 2 h. Using these further modified conditions, seven 4-azidocoumarins were synthesized on the 1 mmol scale (Figure 3).

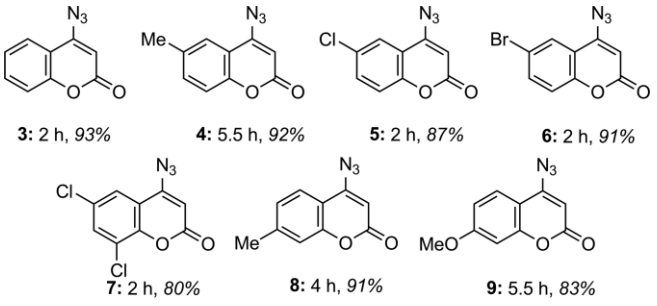
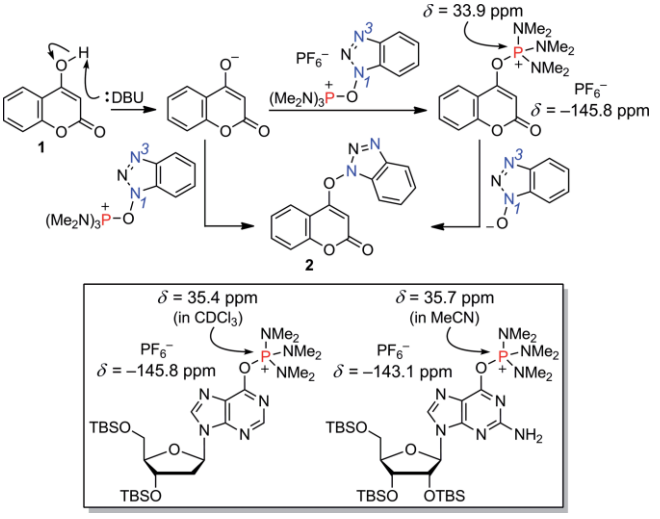


Figure 3. Structures of seven 4-azidocoumarins that were synthesized (total reaction times for the two steps are shown).

Having shown the generality of this scalable, two-step, one-pot synthesis of 4-azidocoumarins, we then assessed the mechanism of this conversion. In our previous work, we have observed the appearance of phosphonium ion intermediates in the reactions of the amide groups of hypoxanthine[38] and guanine nucleosides,[42] with BOP. In marked contrast to this, a corresponding phosphonium ion species was not observable in the reaction of a pyrimidine nucleoside.[46] Thus, we wanted to assess how the reaction of 4-hydroxycoumarin would compare to these previous results. Plausible mechanistic pathways are shown in Scheme 2.

Deprotonation of the C4 hydroxyl in **1** can be followed by: (*a*) reaction at the phosphorus atom of BOP (in red), or (*b*) reaction at the *N*1 atom of BOP (in blue), or (*c*) an S_N2'-like reaction at the *N*3 atom of BOP (in blue). The reaction at the phosphorus atom could lead to a phosphonium intermediate from 4-hydroxycoumarin. In this case, the BtO^- that is expelled in the process can then displace HMPA from the C4 position of the coumarin, resulting in 4-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)oxy)-2*H*-chromen-2-one (**2**). On the other hand, reaction at the *N*1 or *N*3 atom would directly result in **2** with the expulsion of HMPA.

To evaluate this question, a reaction of **1** with BOP (1.2 equiv.) and DBU (1.2 equiv.), in MeCN (0.5 mL) was monitored by $^{31}P\{^1H\}$ NMR (Figure 4). Initially, BOP and MeCN were placed in an NMR tube and a ^{31}P NMR spectrum was obtained. This showed resonances at δ = 42.5 ppm (phosphonium) and δ = –145.8 ppm (PF_6^-). Then compound **1** was added and a spectrum was acquired, but it showed no new resonance. Finally, DBU was added and spectra were acquired over 99 minutes. At 5 minutes, two additional resonances were observed, one at δ = 23.9 ppm corresponding to HMPA and another at δ = 33.9 ppm. The resonance at δ = 33.9 ppm is similar in the chemical shift to those observed for nucleoside phosphonium salts shown in Scheme 2. As the reaction proceeded, a gradual decrease of phosphonium ion and BOP occurred while the formation of HMPA increased. The NMR experiment also indicated that the phosphonium ion was almost completely consumed within 24 min.



Scheme 2. Possible pathways for the formation of intermediate **2**.

Next, the effort was directed to the conversion of 3,4,5-trimethoxybenzaldehyde (**10**) to 5-ethynyl-1,2,3-trimethoxybenzene (**12**). Direct conversion of **10** by the Ohira-Bestmann protocol[49,50] returned a product that remained impure after purification. *Gem*-dibromide **11** was then prepared by reaction of **10** with CBr_4/PPh_3 in CH_2Cl_2.[51] In our hands, a DBU-based

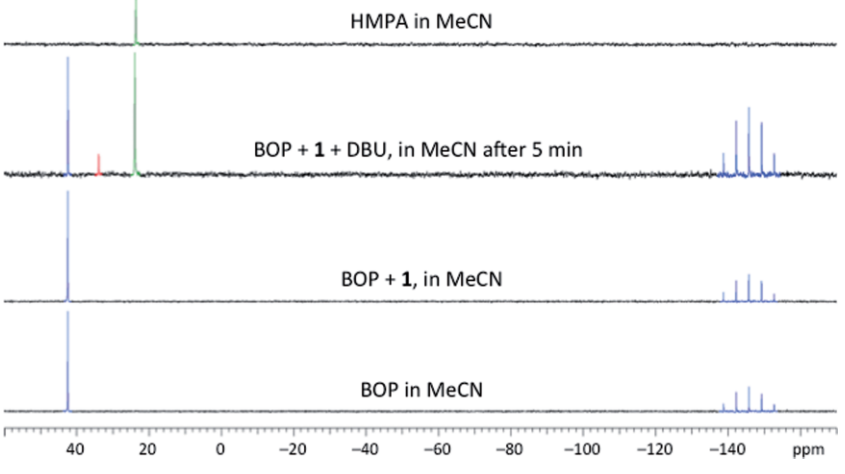
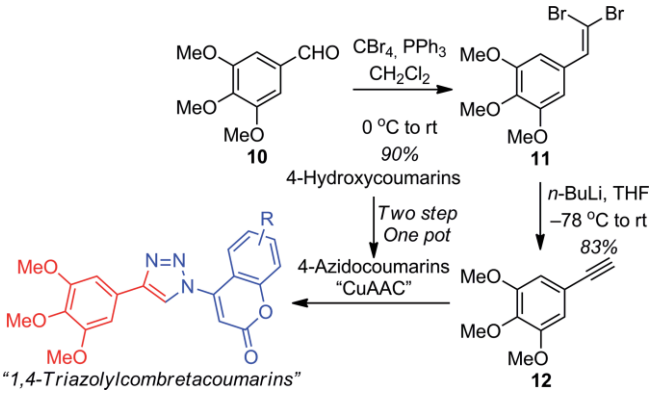


Figure 4. Monitoring the reaction of 4-hydroxycoumarin (**1**) with BOP and DBU in MeCN by $^{31}P\{^1H\}$ NMR.

conversion of **11** to **12**[52] did not result in an adequately pure product (**12**, a while solid, has been reported as a colorless oil in the multiple procedures in the original report[52]). By contrast, use of *n*BuLi,[53] led to the desired alkyne **12** (Scheme 3), isolated as a white solid.[51]

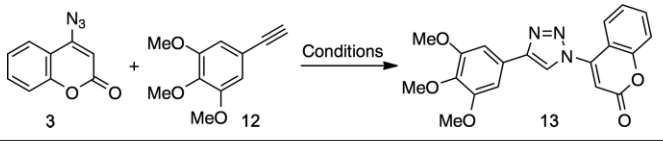


Scheme 3. Synthesis of 5-ethynyl-1,2,3-trimethoxybenzene (**12**) and the CuAAC reaction.

With the coupling partners at hand, we then proceeded to assess conditions for the copper-catalyzed azide-alkyne cycloaddition (CuAAC).[54,55] We selected the following copper-based catalytic systems: **A** = 10 mol-% of $CuSO_4 \cdot 5H_2O$/20 mol-% of Na-ascorbate, **B** = 1.4 equiv. of $CuSO_4 \cdot 5H_2O$/0.8 equiv. of Cu, **C** = 5 mol-% of $CuSO_4 \cdot 5H_2O$/20 mol-% of Na-ascorbate, **D** = 10 mol-% of Cu-2-thiophene carboxylate, and **E** = 10 mol-% of $[(MeCN)_4Cu]PF_6$. The ligation reaction proved to be nontrivial and several experiments were necessary to find appropriate conditions. Results from the screening are shown in Table 1.

In both *t*BuOH/H_2O and in CH_2Cl_2/H_2O, reactions using $CuSO_4 \cdot 5H_2O$/Na-ascorbate showed low productivity at room and elevated temperatures (entries 1–5). Previously reported conditions for CuAAC reactions of azido coumarins were also tested,[28,56] but these gave very little conversion in the present case, as did Cu-2-thiophene carboxylate (entries 6–9). Use of $[(MeCN)_4Cu]PF_6$ in CH_2Cl_2/MeOH proved to be more promising (entries 10 and 11), and a reaction at 50 °C was successful although the product contained a contaminant. The contaminant could be removed by washing the product but this diminished the yield. In CH_2Cl_2 and in the absence of MeOH, there was no reaction at room temperature (entry 12), but at 50 and 80 °C reaction occurred (entries 13 and 14) with results similar to that in entry 11. Outcomes from reactions in $ClCH_2CH_2Cl$ and $ClCH_2CH_2Cl$/MeOH were inferior to those in CH_2Cl_2 (compare entries 15 to 13 as well as 16 to 11). Because amine bases can be employed in reactions involving CuI and $[(MeCN)_4Cu]PF_6$,[54,55] we considered the addition of 2,6-lutidine to the reaction mixtures. This base minimizes by-product formation when Cu^I catalysts are used for CuAAC reactions and could augment proton transfer to the cuprated triazolide.[55,57] With the modified conditions, incomplete reactions were observed in CH_2Cl_2/MeOH at room temperature and in neat CH_2Cl_2 at 50 °C (entries

Table 1. Conditions evaluated for the reaction of 4-azidocoumarin (**3**) with 5-ethynyl-1,2,3-trimethoxybenzene (**12**).[a]



Entry	Catalyst	Solvent and additives	Time [h], *T* °C	Result
1	**A**	1:1 *t*BuOH/H_2O[b] (4 mL)	15 h, r.t., then 24 h, 50 °C	Inc[c]
2	**A**	1:1 *t*BuOH/H_2O[b] (4 mL)	16 h, 50 °C	Inc[c]
3	**A**	1:1 *t*BuOH/H_2O[b] (4 mL)	24 h, 50 °C	39 %[d]
4	**A**	1:1 CH_2Cl_2/H_2O[b] (4 mL)	17 h, r.t.	Inc[c]
5	**A**	1:1 CH_2Cl_2/H_2O[b] (4 mL)	24 h, 50 °C	Inc[e]
6	**B**	*t*BuOH (2 mL)	24 h, 65 °C	Inc[c]
7	**C**	1:1 EtOH/H_2O (5.8 mL)	48 h, r.t.	Inc[c]
8	**D**	3:1 CH_2Cl_2/MeOH (2 mL)	24 h, r.t.	Inc[c]
9	**D**	3:1 CH_2Cl_2/MeOH (2 mL)	24 h, 50 °C	25 %[f]
10	**E**	3:1 CH_2Cl_2/MeOH (2 mL)	2 h, r.t., then 24 h, 50 °C	Inc[c]
11	**E**	3:1 CH_2Cl_2/MeOH (2 mL)	24 h 50 °C	72 %, 42 %[g]
12	**E**	CH_2Cl_2 (2 mL)	48 h, r.t.	No conversion
13	**E**	CH_2Cl_2 (2 mL)	24 h, 50 °C	70 %, 53 %[g]
14	**E**	CH_2Cl_2 (2 mL)	24 h, 80 °C	77 %, 49 %[g]
15	**E**	$ClCH_2CH_2Cl$ (2 mL)	24 h, 65 °C	Inc[e]
16	**E**	3:1 $ClCH_2CH_2Cl$/MeOH (2 mL)	24 h, 65 °C	39 %[f]
17	**E**	3:1 CH_2Cl_2/MeOH (2 mL) + 1 equiv. of 2,6-lutidine	48 h, r.t.	Inc[e]
18	**E**	CH_2Cl_2 (2 mL) + 1 equiv. of 2,6-lutidine	24 h, 50 °C	Inc[e]
19	**E**	3:1 CH_2Cl_2/MeOH (2 mL) + 1 equiv. of 2,6-lutidine	8 h, 50 °C	89 %[h]

[a] Reactions were conducted with 0.20 mmol of 4-azidocoumarin (**3**) and 0.24 mmol of 5-ethynyl-1,2,3-trimethoxybenzene (**12**). [b] Deionized H_2O was used. [c] Inc = incomplete reaction, a trace to very little product formation was observed by tlc. [d] Reaction was incomplete and yield is of isolated and purified product. [e] Inc = incomplete reaction, by tlc significant conversion was observed but residual **3** was observed. [f] Reaction was incomplete and yield is of isolated and purified product containing a slight inseparable contaminant. [g] First value = yield of isolated and purified product containing a contaminant (reaction was incomplete). Second value = yield after sonication with 25 % CH_2Cl_2 in hexanes (8 mL) to remove the impurity. [h] Yield is of isolated and purified product.

17 and 18), but when both MeOH and 2,6-lutidine were present, a complete reaction was attained at 50 °C within 8 h, and a good product yield, as well as purity, was attained (entry 19).

Therefore, conditions in entry 19 were used to prepare the seven combretacoumarins shown in Figure 5. In order to confirm the 1,4-disubstitution pattern, the NOESY spectrum of compound **13** (in [D_6]DMSO) was analyzed. This showed a distinct correlation between the C5 triazolyl proton (δ = 9.35 ppm, singlet) and the chemically equivalent aryl protons (δ = 7.31 ppm, singlet) as well as the vinyl proton of the coumarin (δ = 7.00 ppm, singlet).

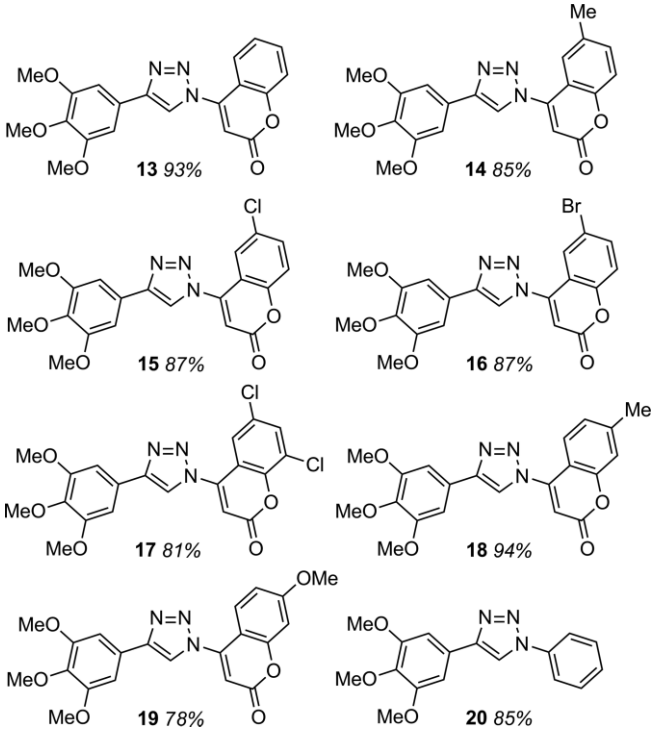
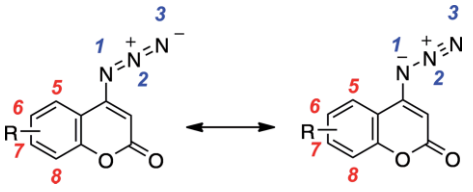


Figure 5. Products prepared via CuAAC reactions of azidocoumarins and PhN_3 with 5-ethynyl-1,2,3-trimethoxybenzene.

Despite the reported exothermicity and extremely rapid rates of many CuAAC reactions,[57] the azido coumarins used herein appeared to be slow reacting. On account of this unanticipated reactivity, we assessed the reactivity of **12** with phenylazide (PhN_3) using [$(MeCN)_4Cu$]PF_6 and 2,6-lutidine, in 3:1 CH_2Cl_2/MeOH. In contrast to the reactions of the azido coumarins, the reaction of PhN_3 was complete *within 3 h at room temperature*, giving product **20** in 85 % yield. This not just indicated a reactivity difference between azido coumarins and PhN_3 but it also indicated that the potentially turnover-limiting alkyne cupration was likely not the cause.

Therefore, after initial geometry optimization, the natural bond order (NBO) analyzed natural charges on the nitrogen atoms of the azido functionality in the azido coumarins and PhN_3 were evaluated by B3LYP Density Functional Theory (DFT) computations at the 6-311++g (2d, 2p) level. These data are shown in Table 2. In CuAAC reactions the internal nitrogen atom of the azide moiety (*N*1 in the structures above Table 2) is nucleophilic and interacts with the copper ion, whereas the terminal nitrogen atom (*N*3 in the structures) is the electrophile.

Table 2. Computed NBO coefficients of the azide nitrogen atoms in the coumarins and PhN_3.



Compound	*N*1	*N*2	*N*3
R = H (**3**)	–0.346	+0.257	+0.003
R = 6-Me (**4**)	–0.344	+0.252	+0.005
R = 6-Cl (**5**)	–0.345	+0.256	+0.009
R = 6-Br (**6**)	–0.347	+0.257	+0.010
R = 6,8-diCl (**7**)	–0.348	+0.257	+0.015
R = 7-Me (**8**)	–0.345	+0.257	+0.000
R = 7-OMe (**9**)	–0.345	+0.257	–0.002
Ph-N^1=N^2=N^3	–0.356	+0.251	–0.069

From the data in Table 2, the NBO charges on the *N*1 atom of the coumarins did not show marked variations and are generally similar to that of PhN_3. No major variations were seen with the NBO charges on the *N*2 atom as well in all cases. With the exception of the 7-OMe derivative that showed some negative charge on the *N*3 atom, all other coumarin derivatives show electron deficiency at this site. This is in marked contrast to the *N*3 atom of PhN_3 that by comparison, shows a greater negative charge at the *N*3 atom. In the coumarins, the negative charge can be delocalized onto the carbonyl groups. This could influence the electrophilicity of the *N*3 atom, but PhN_3 reacted more readily than the coumarins. The mesomeric effect of a 7-OMe group on the coumarin in **9** leads to restoration of some negative charge at the *N*3 center. Notably, **9** was very slow reacting in the CuAAC even as compared to the other azido coumarins, with the reaction remaining incomplete at 8 h (the reaction was complete within 24 h). These results seem to indicate that reasons beyond electron density considerations at the reacting atoms may be responsible for the reactivities of the azido coumarins.

After these analyses on the initial 1,4-combretacoumarin series were completed, we undertook the preparation of additional analogs to assess if compounds with better solubility properties could be prepared. From the initial biological data (vide infra), we selected the relatively soluble bromo analog **16**, which also showed activity, for additional modifications. We decided to remove one or two methoxy groups on the combretastatin portion of the analogs and therefore, CuAAC reactions of mono and dimethoxyphenyl acetylenes were conducted with 4-azido-6-bromo-2*H*-chromen-2-one (**6**). These desmethoxy products are shown in Figure 6.

Some additional reactivity factors became evident in these CuAAC reactions where the structure of the alkyne appeared to have an influence. Reactions leading to **21** and **24** were complete within 8 h, that leading to **22** was complete within 24 h, but the reaction towards **23** was the most difficult, remaining incomplete even at 24 h. In the reaction with *p*-methoxyphenylacetylene two modifications were attempted to assess whether the reaction could be improved: (*a*) addition of

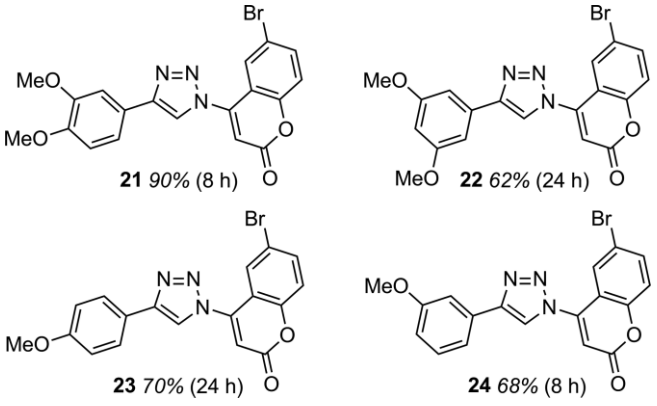
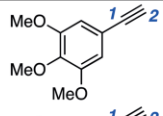
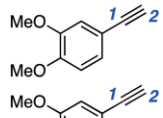
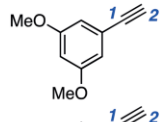
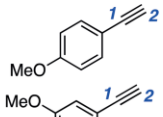
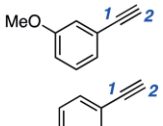
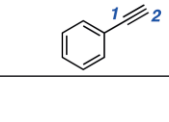


Figure 6. Desmethoxy "*1,4-combretacoumarin*" analogs prepared from 4-azido-6-bromo-2*H*-chromen-2-one (**6**).

1.2 equiv. of the alkyne at the start of the reaction followed by addition of another 1.2 equiv. after 8 h, and a total reaction time of 24 h, and (*b*) addition of $[(MeCN)_4Cu]PF_6$ in two aliquots (0.1 equiv. at the start of reaction and 0.1 equiv. after 8 h) and a total reaction time was 24 h. However, neither modification led to any improvement. The strong mesomeric effect of a *p*-methoxy group leading to increased electron density at the terminal alkynyl carbon atom could be a factor for this outcome. This effect could also influence the cycloaddition itself. Therefore, we assessed the natural charges on the alkynyl carbon atoms of the five alkynes used in this study, as well as phenylacetylene, by B3LYP Density Functional Theory (DFT) computations at the 6-311++g (2d, 2p) level (Table 3). In comparing the coefficients of the alkynyl C1 carbon atoms, the coefficient of *p*-methoxyphenylacetylene is the lowest in the set, and reactions with this alkyne remained incomplete under a variety of conditions. The NBO coefficients of the C2 alkynyl carbon atoms in the *m*-methoxy and *m*,*m*-dimethoxyphenylacetylene are similar, lower than those of the remaining alkynes, and comparable to that of phenylacetylene. As would be anticipated, the presence of a *p*-methoxy group increases electron density at the alkynyl C2 carbon atoms. On the basis of these data, one must surmise that these electronic differences contribute to the subtle reactivity differences observed.

Table 3. Computed NBO coefficients of the alkynyl carbon and hydrogen atoms in the methoxy-substituted phenylacetylenes.

Alkyne	C1	C2	≡C-**H**
	−0.024	−0.212	0.223
	−0.024	−0.209	0.223
	−0.025	−0.197	0.224
	−0.019	−0.210	0.230
	−0.026	−0.195	0.224
	−0.027	−0.192	0.225

Quantification of Cytotoxicity Against Human Cancer Cell Lines

To evaluate the cytotoxic potential of compounds **13**–**20**, a live cell imaging-based differential nuclear staining (DNS) assay was performed against two human cancer cell lines. The DNS assay utilizes two fluorescent nuclear stains, Hoechst 33342 and propidium iodide (PI), to selectively label living and dead cells.[58] An initial screen for cytotoxicity was conducted using the CEM leukemia cell line. Stock solutions of each compound were prepared at their highest soluble concentration in DMSO (Table 4). Stock and serially diluted solutions of the compounds were directly added to wells containing cells suspended in complete media at a final concentration of 1 % v/v DMSO and assessed in quadruplicate after 48 hours. The CC_{50} is defined as the concentration of compound that causes loss of membrane integrity to 50% of the cell population as compared to untreated cells. CC_{50} values were determined by linear interpolation of the two concentrations with cell death nearest the 50% mark.

Table 4. CC_{50} values of the 1,4-combretacoumarins (**13**–**19**) and the phenyl derivative (**20**) that were synthesized.

Compound	Cell line	Incubation [h]	CC_{50} [μM]	Std. Dev. [μM]
13	CEM	48		NA[a]
14	CEM	48		NA[a]
15	CEM	48	37.2	0.6114
16	CEM	48		NA[a]
17	CEM	48		NA[a]
18	CEM	48		NA[a]
19	CEM	48		NA[a]
20	CEM	48	34.6	2.5939
	MB-231	72	55.4	5.0951

[a] NA = Not available, unable to obtain at highest soluble concentration.

Analysis of **13**, **18**, and **19** treated cells revealed significant compound crystallization and did not induce quantifiable CEM cell death. Similarly, treatment with **14**, **16**, and **17** did not induce significant CEM cell death at the prepared concentrations. Compound **15**, one of the least soluble compounds in the series, demonstrated cytotoxicity at its highest soluble concentration. A CC_{50} value of 37.2 ± 0.6114 μM was calculated for **15** (Table 4). Phenyl derivative **20**, the most readily soluble of the compounds, induced cell death at a CC_{50} of 34.6 ± 2.5939 μM (Table 4). Accordingly, compound **20** was isolated for use in a secondary DNS assay conducted on the MDA-MB-231 (MB-231) triple-negative breast cancer cell line.

In this DNS assay, MB-231 cells were treated with serial dilutions of **20** starting at 100 μM. Treatments were directly added to cell-containing wells (as indicated previously) and assessed in quadruplicate after 72 hours. Treatment with **20** revealed a CC_{50} value of 55.4 ± 5.0951 μM against this cell line (Table 4).

The four desmethoxy compounds (**21**–**24**) were also assessed for cytotoxicity against CEM and MB-231 cell lines. In

addition, combretastatin A4 (CA4), a known tubulin-depolymerizing agent, was analyzed as a standard for apoptosis.[59] CEM and MB-231 cells were treated with compounds **21**–**24** at concentrations ranging from 100 μM to 0.5 μM, using the protocols previously indicated. Compounds **22**, **23**, and **24** all demonstrated similar cytotoxic activity and induced significant death in CEM cells at approximately 10 μM. This pattern was not observed upon subsequent analysis of MB-231 cells, which appeared selective to compound **23**. These data are shown in Table 5. Figure 7 shows the dose-response curves for compounds **20** and **23** against CEM and MB-231 cell lines.

Table 5. CC_{50} values of the desmethoxy 1,4-combretacoumarins (**21**–**24**) that were synthesized and CA4.

Compound	Cell line	Incubation [h]	CC_{50} [μM]	Std. Dev. [μM]
21	CEM	48	–	–
22			10.5	0.2696
23			9.8	0.0755
24			10.3	0.4143
CA4			0.036	0.0012
21	MB-231	72	–	–
22			–	–
23			10.7	0.8585
24			–	–
CA4			0.0037	0.0003

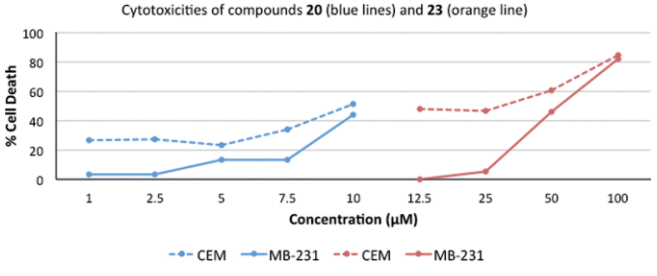


Figure 7. Dose-response curves for CEM and MB-231 cells.

In all the aforementioned DNS assays, three experimental controls were included in each 96-well plate and assessed in quadruplicate. Untreated and DMSO-treated (1 % v/v) cells were used as negative controls to establish basal levels of cell death due to cell manipulation or culture conditions and to account for solvent-induced cell death. As a positive control for cytotoxicity, cells were treated with 1.62 mM H_2O_2.

Evaluation of Phosphatidylserine Translocation in CEM Cells with Compounds 20 and 23

Externalization of phosphatidylserine (PS) to the cell surface is a well-established marker of cells undergoing early apoptosis. Annexin V is an intracellular protein with high affinity for PS which is routinely fluorochrome-labeled with FITC and used as a probe for PS translocation via flow cytometry.[60] In early-stage apoptosis, the cell membrane is still intact and is not yet permeable to propidium iodide (PI). To determine whether cell death was occurring via apoptosis or necrosis, CEM cells were treated using 100 μM, 30 μM, and 60 μM concentrations of **20** for 24 h, followed by annexin-FITC and PI staining. Non-treated and DMSO-treated cells (1 % v/v) served as negative controls and H_2O_2 (1.62 mM) as a positive control for apoptosis/necrosis. Apoptotic cell populations are expressed as the sum of annexin-FITC positive stained cells, whereas the necrotic populations are cells that stained with PI but negative for annexin-FITC.

In this assay, compound **20** was found to induce significant PS externalization in CEM cells at its highest soluble concentration. These data demonstrate a concentration-dependent increase in apoptosis/necrosis. However, they suggest that compound **20** preferentially induces cell death via apoptosis (Figure 8, Panel A). In a subsequent assay, CEM cells treated with compound **23** were analyzed using concentrations ranging from 9.8 μM to 49 μM using the method previously described. Compound **23** did not demonstrate appreciable PS externalization when compared to the vehicle control (Figure 8, Panel B). Thus, the modes of action of these two compounds are possibly quite different.

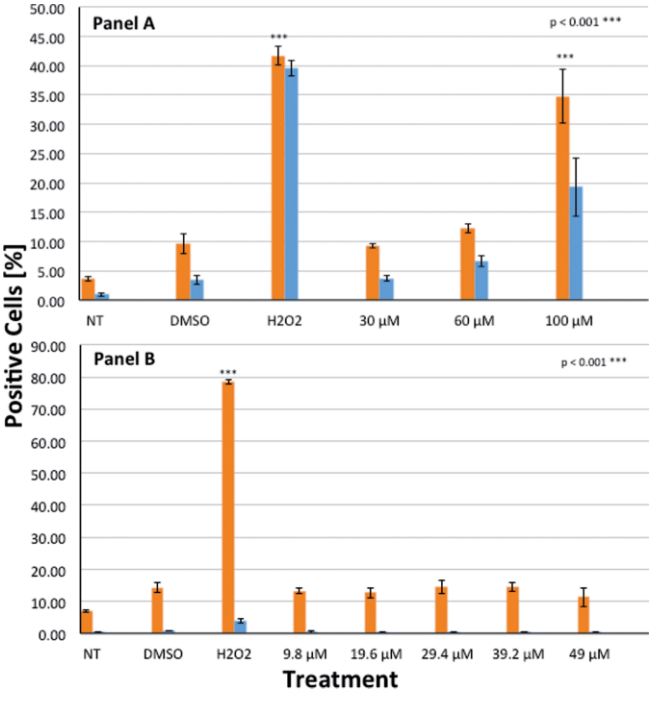


Figure 8. Apoptosis assays on compounds **20** and **23**-treated CEM cells (panels A and B, respectively) using annexin V-FITC and propidium iodide. Orange bars: apoptosis and blue bars: necrosis.

Antiviral Assays

The compounds were evaluated against different herpesviruses, including herpes simplex virus type 1 (HSV-1) strain KOS, thymidine kinase-deficient (TK^-) HSV-1 KOS strain resistant to acyclovir (ACV^r), herpes simplex virus type 2 (HSV-2) strain G, varicella-zoster virus (VZV) strain Oka, TK^- VZV strain 07–1, human cytomegalovirus (HCMV) strains AD-169 and Davis as well as vaccinia virus, adenovirus-2, vesicular stomatitis virus, para-influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus, respiratory syncytial virus (RSV), feline coronavirus (FIPV) and influenza A virus subtypes H1N1 (A/PR/8), H3N2 (A/HK/7/87), and influenza B virus (B/HK/5/72). Unfortu-

nately, none of the compounds showed particularly interesting antiviral activity. In human embryonic lung cells, compound **21** showed an EC_{50} of 72.48 μM against Davis strain CMV, compounds **17**, **20**, and **22** showed EC_{50} values of 66.87, 39.86, and 58.48 μM, respectively, against TK^+ VZV, and compound **20** also showed an EC_{50} of 39.11 against TK^- VZV.

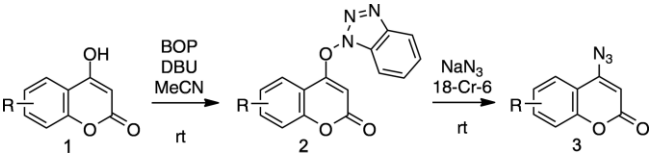
Conclusions

In summary, in this work, we have demonstrated a simple and facile two-step, one-pot conversion of 4-hydroxycoumarins to the corresponding 4-azido derivatives. This involves activation of the 4-hydroxyl group with BOP and DBU in MeCN. The intermediate O^4-(benzotriazolyl)coumarin derivatives formed in situ, possibly via the intermediacy of a phosphonium ion, can be reacted with NaN_3 and 18-crown-6 to give the 4-azidocoumarins. These products were subjected to CuAAC reactions with 5-ethynyl-1,2,3-trimethoxybenzene using $[(MeCN)_4Cu]PF_6$ in CH_2Cl_2/MeOH, and 2,6-lutidine as additive, at 50 °C. Critical to the success of these reactions was the need for both MeOH and 2,6-lutidine. Using these conditions, seven 1,4-combretacoumarins and one phenyl derivative, 1-phenyl-4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole, were synthesized. The latter was prepared in order to compare the reactivities of the 4-azidocoumarins with PhN_3. Interestingly, although the azidocoumarins required an elevated temperature to attain complete reactions, PhN_3 reacted at room temperature and within a shorter reaction time. To understand possible reasons for this difference in reactivities, DFT computations were utilized to evaluate the NBO coefficients of the azido groups in the 4-azidocoumarins but a link between the NBO coefficients on the nitrogen atoms of the azido groups and reactivity was not forthcoming. However, a difference was observed between the azido coumarins and PhN_3. Four desmethoxy 1,4-combretacoumarins were prepared from reactions of 4-azido-6-bromo-2*H*-chromen-2-one (**6**) with two di- and two mono-methoxy phenylacetylenes. In these reactions, we found that the structures of the phenylacetylenes also influence the CuAAC reactions, and DFT computations were used to assess the NBO coefficients of the alkynyl carbon atoms. As anticipated, the terminal alkynyl carbon atom has a greater negative charge when a *p*-methoxy group is present. The varying electronic properties of the alkynes are likely also linked to the subtle reactivity differences observed. Antiproliferative and antiviral assays were undertaken. Unfortunately, the 1,4-combretacoumarins suffered from solubility problems. Among the various compounds, 1-phenyl-4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (**20**) was relatively soluble and showed antiproliferative effect, appearing to operate via an apoptotic pathway due to significant phosphotidylserine (PS) externalization. Three of the four desmethoxy compounds showed comparable cytotoxicity against CEM cells. Of these, 6-bromo-4-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (**23**) was also selective for MB-231 cells. However, this compound did not demonstrate appreciable PS externalization, in contrast to 1-phenyl-4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (**20**). None of the compounds synthesized showed significant antiviral activity against a broad spectrum of DNA and RNA viruses.

Experimental Section

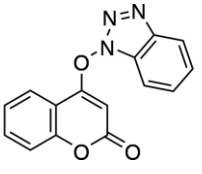
General Experimental Considerations: Analytical thin layer chromatography (TLC) was performed on 200 μm-aluminum-backed silica plates and visualized under ultraviolet light. CH_2Cl_2 and MeCN used for reactions were freshly distilled from calcium hydride prior to use. EtOAc, CH_2Cl_2, and hexanes used for compound purifications were distilled prior to use. Compounds were purified by column chromatography on 200–300 silica gel mesh. All other reagents were obtained from commercial sources and used without further purification. ^1H NMR spectra were obtained at 500 MHz in $CDCl_3$ and are referenced to the residual $CHCl_3$. ^{13}C NMR spectra were obtained at 125 MHz in $[D_6]$DMSO, $CDCl_3$, or CD_2Cl_2 (see details under specific compound headings), and are referenced to the resonance of the solvent (δ = 39.51, 77.23, and 54.00 ppm, respectively). Chemical shifts (δ) are in ppm and coupling constants (*J*) are in Hertz.

Representative Procedure for the Synthesis of 4-Azidocoumarins



In an oven-dried 25 mL round bottomed flask equipped with a stir bar was placed 4-hydroxycoumarin (**1**, 162 mg, 1.00 mmol) in dry MeCN (10 mL). DBU (179 μL, 1.20 mmol, 1.20 equiv.) was added and the resulting homogeneous, light-yellow solution was stirred at room temperature for 10 min. BOP (531 mg, 1.20 mmol, 1.20 equiv.) was added and the mixture was stirred for 1 h at r.t. To the white mixture, NaN_3 (195 mg, 3.00 mmol, 3.00 equiv.) and 18-crown-6 (52.0 mg, 0.20 mmol, 0.20 equiv.) were added sequentially. The mixture was stirred at room temperature for 1 h at which time TLC (SiO_2/25 % EtOAc in hexanes) indicated the reaction to be complete. The mixture was transferred to a separatory funnel and partitioned between EtOAc and brine. The aqueous layer was separated and back extracted with EtOAc (3 ×). The combined organic layer was dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification was performed by chromatography on a silica gel column packed in hexanes, and eluted with hexanes followed by 25 % EtOAc in hexanes. All azidocoumarins were synthesized and purified using this procedure (see individual compound headings for details).

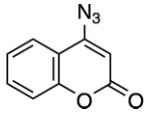
4-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)oxy)-2*H*-chromen-2-one (2)



In an oven-dried 25 mL round bottomed flask equipped with a stir bar, was placed 4-hydroxycoumarin (**1**, 81 mg, 0.50 mmol) in dry MeCN (5 mL). DBU (90.0 μL, 0.60 mmol, 1.20 equiv.) was added and the resulting homogeneous, light-yellow solution was stirred at room temperature for 10 min. BOP (265 mg, 0.60 mmol, 1.20 equiv.) was added and the mixture was stirred for 1 h at r.t. TLC (SiO_2/25 % EtOAc in hexanes) indicated the reaction to be complete. The mixture was transferred to a separatory funnel and partitioned between EtOAc and brine. The aqueous layer was separated

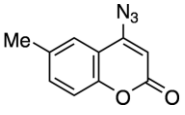
and back extracted with EtOAc (3 ×). The combined organic layer was dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. Chromatography of the crude material on a silica column packed in hexanes and eluted with hexanes followed by 25 % EtOAc in hexanes gave 135 mg (97 % yield) of **2** as a white solid. R_f (SiO_2/25 % EtOAc in hexanes) = 0.20. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.18 (d, J = 8.4 Hz, 1H, Ar-H), 8.09 (dd, J = 8.1, 1.3 Hz, 1H, Ar-H), 7.73 (t, J = 7.9 Hz, 1H, Ar-H), 7.63 (t, J = 7.6 Hz, 1H, Ar-H), 7.55–7.51 (m, 2H, Ar-H), 7.48–7.46 (m, 2H, Ar-H), 5.25 (s, 1H, =CH). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 165.4, 160.8, 153.7, 143.6, 134.1, 130.0, 127.7, 126.0, 125.0, 122.6, 121.3, 117.5, 112.4, 108.3, 94.0. HRMS (ESI/TOF) m/z calculated for $C_{15}H_9N_3O_3$ [M + Na]$^+$: 302.0542, found 302.0557.

4-Azido-2*H*-chromen-2-one (3)[36d]



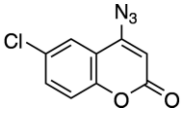
Pale-yellow solid (175 mg, 93 % yield). R_f (SiO_2/25 % EtOAc in hexanes) = 0.58. Mp: 157–159 °C (associated with a color change to light brown). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.72 (dd, J = 7.9, 1.2 Hz, 1H, Ar-H), 7.59 (td, J = 7.8, 1.2 Hz, 1H, Ar-H), 7.35 (dd, J = 8.4, 0.9 Hz, 1H, Ar-H), 7.29 (td, J = 7.6, 0.9 Hz, 1H, Ar-H), 6.13 (s, 1H, =CH). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 160.7, 153.8, 153.6, 133.4, 124.5, 123.6, 117.2, 115.1, 100.5. HRMS (ESI/TOF) m/z calculated for $C_9H_5N_3O_2$ [M + H]$^+$: 188.0455, found 188.0461.

4-Azido-6-methyl-2*H*-chromen-2-one (4)[36d]



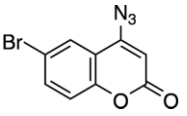
Reaction time for displacement with azide was 4.5 h. Pale-yellow solid (185 mg, 92 % yield). R_f (SiO_2/25 % EtOAc in hexanes) = 0.45. Mp: 141–143 °C (associated with a color change to sandy brown). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.49 (s, 1H, Ar-H), 7.39 (d, J = 8.4 Hz, 1H, Ar-H), 7.24 (d, J = 8.4 Hz, 1H, Ar-H), 6.11 (s, 1H, =CH), 2.41 (s, 3H, Me). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 160.9, 153.5, 151.9, 134.4, 134.3, 123.2, 116.9, 114.7, 100.3, 21.0. HRMS (ESI/TOF) m/z calculated for $C_{10}H_7N_3O_2$ [M + Na]$^+$: 224.0430, found 224.0437.

4-Azido-6-chloro-2*H*-chromen-2-one (5)



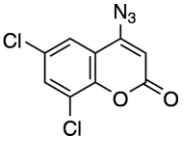
Pale-yellow solid (193 mg, 87 % yield). R_f (SiO_2/25 % EtOAc in hexanes) = 0.53. Mp: 168–170 °C (associated with a color change to dark brown). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.70 (d, J = 2.2 Hz, 1H, Ar-H), 7.54 (dd, J = 8.8, 2.3 Hz, 1H, Ar-H), 7.29 (d, J = 8.8 Hz, 1H, Ar-H), 6.15 (s, 1H, =CH). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 160.0, 152.5, 152.2, 133.3, 130.1, 123.2, 118.6, 116.1, 101.2. HRMS (ESI/TOF) m/z calculated for $C_9H_4ClN_3O_2$ [M + Na]$^+$: 243.9884, found 243.9888.

4-Azido-6-bromo-2*H*-chromen-2-one (6)[28]



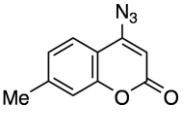
Pale-yellow solid (243 mg, 91 % yield). R_f (SiO_2/25 % EtOAc in hexanes) = 0.49. Mp: 169–171 °C (associated with a color change to dark brown). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.85 (d, J = 2.2 Hz, 1H, Ar-H), 7.67 (dd, J = 8.8, 2.2 Hz, 1H, Ar-H), 7.23 (d, J = 8.8 Hz, 1H, Ar-H), 6.15 (s, 1H, =CH). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 160.0, 152.7, 152.5, 136.2, 126.3, 118.9, 117.3, 116.6, 101.2. HRMS (ESI/TOF) m/z calculated for $C_9H_4BrN_3O_2$ [M + Na]$^+$: 287.9379, found 287.9377.

4-Azido-6,8-dichloro-2*H*-chromen-2-one (7)



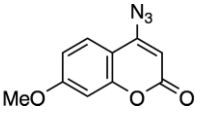
Pale-yellow solid (205 mg, 80 % yield). R_f (SiO_2/25 % EtOAc in hexanes) = 0.58. Mp: 170–172 °C (associated with a color change to brown). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.64 (d, J = 2.2 Hz, 1H, Ar-H), 7.62 (d, J = 2.3 Hz, 1H, Ar-H), 6.18 (s, 1H, =CH). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 158.7, 152.3, 148.3, 133.3, 129.8, 123.2, 121.8, 117.1, 101.6. HRMS (ESI/TOF) m/z calculated for $C_9H_3Cl_2N_3O_2$ [M + Na]$^+$: 277.9495, found 277.9498.

4-Azido-7-methyl-2*H*-chromen-2-one (8)[36d]



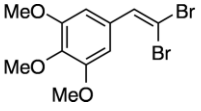
Reaction time for displacement with azide was 3 h. Pale-yellow solid (183 mg, 91 % yield). R_f (SiO_2/25 % EtOAc in hexanes) = 0.39. Mp: 162–164 °C (associated with a color change to sandy brown). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.58 (d, J = 8.1 Hz, 1H, Ar-H), 7.15 (s, 1H Ar-H), 7.10 (d, J = 8.1 Hz, 1H, Ar-H), 6.06 (s, 1H, =CH), 2.46 (s, 3H, Me). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 161.0, 153.9, 153.7, 144.9, 125.7, 123.3, 117.2, 112.6, 99.3, 22.0. HRMS (ESI/TOF) m/z calculated for $C_{10}H_7N_3O_2$ [M + Na]$^+$: 224.0430, found 224.0443.

4-Azido-7-methoxy-2*H*-chromen-2-one (9)[36d]



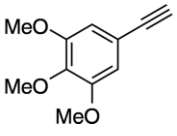
Reaction time for displacement with azide was 4.5 h. Pale-orange solid (180 mg, 83 % yield). R_f (SiO_2/25 % EtOAc in hexanes) = 0.58. Mp: 171–173 °C (associated with a color change to brown). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.60 (d, J = 8.8 Hz, 1H, Ar-H), 6.84 (dd, J = 8.8, 2.3 Hz, 1H, Ar-H), 6.82 (d, J = 2.2 Hz, 1H, Ar-H), 5.97 (s, 1H, =CH), 3.88 (s, 3H, Me). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 164.1, 161.2, 155.7, 153.8, 124.7, 112.8, 108.4, 100.9, 97.3, 56.0. HRMS (ESI/TOF) m/z calculated for $C_{10}H_7N_3O_3$ [M + Na]$^+$: 240.0380, found 240.0381.

5-(2,2-Dibromovinyl)-1,2,3-trimethoxybenzene (11)[51]



3,4,5-Trimethoxybenzaldehyde (**10**, 1.96 g, 10.0 mmol) was placed in an oven-dried 100 mL round bottomed flask, equipped with a stir bar. CH_2Cl_2 (50 mL) and CBr_4 (3.98 g, 12.0 mmol, 1.20 equiv.) were added sequentially. The solution was cooled to 0 °C with stirring, and PPh_3 (5.25 g, 20.0 mmol, 2.00 equiv.) was added in portions. The mixture was stirred at 0 °C for 0.5 h, warmed to room temperature, and stirred for an additional 3 h. TLC (SiO_2/10 % EtOAc in hexanes) indicated the reaction to be complete. The mixture was evaporated under reduced pressure and EtOAc was added. The crude mixture was filtered through a short pad of silica gel and the filtrate was concentrated under reduced pressure. Chromatography of the crude material on a silica gel column packed in hexanes and eluted with 5 % followed by 10 % EtOAc in hexanes, gave 3.16 g (90 % yield) of **11** as a pale yellow solid. R_f (SiO_2/10 % EtOAc in hexanes) = 0.30. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.41 (s, 1H, =CH), 6.80 (s, 2H, Ar-H), 3.87 (s, 3H, Me), 3.86 (s, 6H, Me). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 153.3, 138.7, 136.8, 130.8, 106.1, 89.0, 61.1, 56.4.

5-Ethynyl-1,2,3-trimethoxybenzene (12)[51]

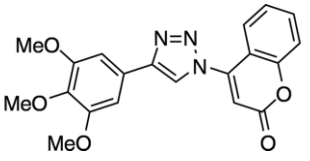


In an oven-dried, 3-neck 25 mL round-bottomed flask equipped a stir bar, a solution of 5-(2,2-dibromovinyl)-1,2,3-trimethoxybenzene (**11**, 1.00 g, 2.84 mmol) in dry THF (7.1 mL) was cooled to –78 °C, under a nitrogen atmosphere. To the stirring mixture 1.6 M *n*BuLi in hexanes (4.44 mL, 2.50 equiv.) was added dropwise. The black mixture was stirred for 4 h at –78 °C and for 1 h at room temperature. TLC (SiO_2/10 % EtOAc in hexanes) indicated the reaction to be complete. The reaction was quenched with saturated aq. NH_4Cl and transferred to a separatory funnel using EtOAc. The mixture was partitioned between EtOAc and water. The aqueous layer was separated and back extracted with EtOAc (3 ×). The combined organic layer was washed with brine, dried with anhydrous Na_2SO_4, and evaporated under reduced pressure. Chromatography on a silica gel column packed in hexanes and eluted with hexanes followed by 10 % EtOAc in hexanes, gave 455 mg (83 % yield) of **12** as a white solid. R_f (SiO_2/10 % EtOAc in hexanes) = 0.28. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.73 (s, 2H, Ar-H), 3.86 (s, 6H, Me), 3.85 (s, 3H, Me), 3.03 (s, 1H, ≡CH). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 153.3, 139.6, 117.2, 109.6, 83.9, 76.4, 61.1, 56.4.

Representative procedure for the synthesis of 4-(4-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-1-yl)-coumarins

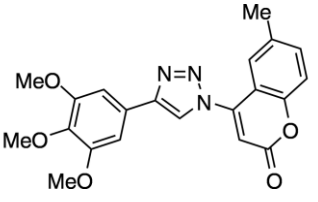
In an oven-dried 12 mL vial equipped with a stir bar was placed 4-azidocoumarin (**3**, 94.0 mg, 0.50 mmol) in dry CH_2Cl_2 (3.75 mL), and 5-ethynyl-1,2,3-trimethoxybenzene (**12**, 115 mg, 0.60 mmol, 1.20 equiv.) was added. $[(MeCN)_4Cu]PF_6$ (19.0 mg, 0.05 mmol, 0.10 equiv.) and dry MeOH (1.25 mL) were added to the resulting homogeneous, light-yellow solution. Next, 2,6-lutidine (58.0 µL, 0.50 mmol, 1.00 equiv.) was added, the mixture was flushed with nitrogen gas, and the resulting mixture was stirred for 8 h at 50 °C. TLC (SiO_2/25 % EtOAc in hexanes) indicated the reaction to be complete. The yellow mixture was transferred to a separatory funnel using CH_2Cl_2, and partitioned between CH_2Cl_2 and brine. The aqueous layer was separated and back extracted with CH_2Cl_2 (3 ×). The combined organic layer was dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. Purification was performed by column chromatography on a silica gel column (see individual compound headings for details).

4-(4-(3,4,5-Trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (13)

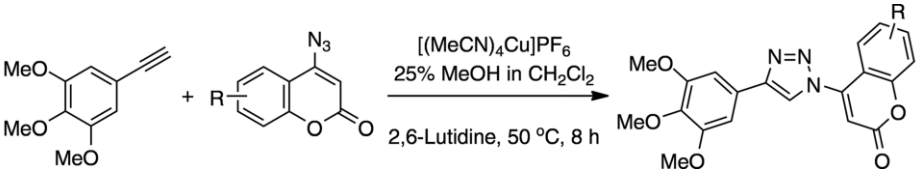


Synthesized from azidocoumarin **3** (94 mg) and 5-ethynyl-1,2,3-trimethoxybenzene (**12**, 115 mg). Chromatography on a silica gel column packed in hexanes and sequentially eluted with hexanes, 25 % EtOAc in hexanes, 40 % EtOAc in hexanes, and EtOAc gave 176 mg (93 % yield) of **13** as a pale-yellow solid. R_f (SiO_2/25 % EtOAc in hexanes) = 0.13. Mp: 234–236 °C (dec., and associated with a color change to dark brown with no phase change). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.17 (s, 1H, Ar-H), 7.99 (d, J = 8.1 Hz, 1H, Ar-H), 7.70 (t, J = 7.8 Hz, 1H, Ar-H), 7.50 (d, J = 8.3 Hz, 1H, Ar-H), 7.40 (t, J = 7.7 Hz, 1H, Ar-H), 7.16 (s, 2H, Ar-H), 6.63 (s, 1H, =CH), 3.97 (s, 6H, Me), 3.92 (s, 3H, Me). ^{13}C NMR (125 MHz, [D_6]DMSO): δ = 159.5, 153.8, 153.4, 147.1, 145.8, 137.8, 133.5, 125.8, 125.0, 123.0, 117.2, 114.1, 109.8, 103.0, 60.1, 56.0. HRMS (ESI/TOF) m/z calculated for $C_{20}H_{17}N_3O_5$ $[M + Na]^+$: 402.1060, found 402.1069.

6-Methyl-4-(4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (14)

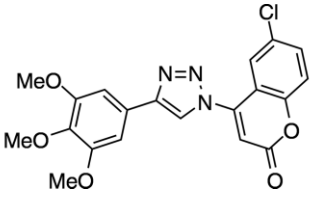


Synthesized from azidocoumarin **4** (101 mg) and 5-ethynyl-1,2,3-trimethoxybenzene (**12**, 115 mg). Chromatography on a silica gel column packed in hexanes and sequentially eluted with hexanes, 25 % EtOAc in hexanes, 40 % EtOAc in hexanes, EtOAc, 5 % MeOH in EtOAC, 10 % MeOH in EtOAc, and 20 % MeOH in EtOAc gave 168 mg (85 % yield) of **14** as a pale-yellow solid. R_f (SiO_2/25 % EtOAc in hexanes) = 0.19. Mp: 228–230 °C (dec., and associated with a color change to dark brown with no phase change). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.16 (s, 1H, Ar-H), 7.74 (s, 1H, Ar-H), 7.50 (d,



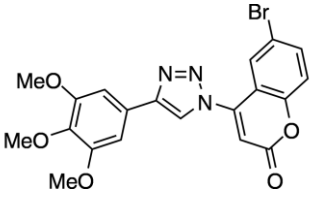
J = 8.4 Hz, 1H, Ar-H), 7.39 (d, J = 8.5 Hz, 1H, Ar-H), 7.17 (s, 2H, Ar-H), 6.58 (s, 1H, =CH), 3.97 (s, 6H, Me), 3.92 (s, 3H, Me), 2.42 (s, 3H, Me). ^{13}C NMR (125 MHz, CD_2Cl_2): δ = 160.4, 154.5, 153.1, 148.8, 147.3, 139.5, 135.7, 135.2, 125.6, 125.3, 121.0, 117.8, 114.8, 110.6, 103.8, 61.1, 56.8, 21.3. HRMS (ESI/TOF) m/z calculated for $C_{21}H_{19}N_3O_5$ [M + Na]$^+$: 416.1217, found 416.1230.

6-Chloro-4-(4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (15)



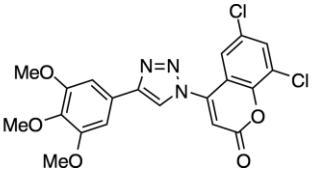
Synthesized from azidocoumarin **5** (111 mg) and 5-ethynyl-1,2,3-trimethoxybenzene (**12**, 115 mg). Chromatography on a silica gel column packed in hexanes and sequentially eluted with hexanes, 25 % EtOAc in hexanes, 40 % EtOAc in hexanes, EtOAc, 5 % MeOH in EtOAC, and 10 % MeOH in EtOAc gave 198 mg (97 % yield) of **15**. Because minor impurities were detectable in the product by ^{1}H NMR, the product was suspended in 8 mL of 25 % CH_2Cl_2 in hexanes, sonicated, and filtered to give 180 mg (87 % yield) of **15** as a yellow solid. R_f (SiO_2/25 % EtOAc in hexanes) = 0.14. Mp: 222–224 °C (dec., and associated with a color change to dark brown with no phase change). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.17 (s, 1H, Ar-H), 8.09 (d, J = 2.0 Hz, 1H, Ar-H), 7.64 (dd, J = 8.8, 2.0 Hz, 1H, Ar-H), 7.44 (d, J = 8.9 Hz, 1H, Ar-H), 7.16 (s, 2H, Ar-H), 6.64 (s, 1H, =CH), 3.97 (s, 6H, Me), 3.92 (s, 3H, Me). ^{13}C NMR (125 MHz, CD_2Cl_2): δ = 159.6, 154.5, 153.4, 149.1, 146.2, 139.5, 134.1, 131.0, 125.9, 125.1, 120.7, 119.6, 116.1, 111.0, 103.8, 61.1, 56.8. HRMS (ESI/TOF) m/z calculated for $C_{20}H_{16}ClN_3O_5$ [M + Na]$^+$: 436.0671, found 436.0693.

6-Bromo-4-(4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (16)



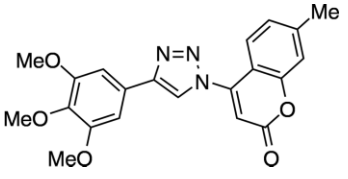
Synthesized from azidocoumarin **6** (133 mg) and 5-ethynyl-1,2,3-trimethoxybenzene (**12**, 115 mg). Chromatography on a silica gel column packed in hexanes and sequentially eluted with hexanes, 25 % EtOAc in hexanes, 40 % EtOAc in hexanes, EtOAc, and 10 % MeOH in EtOAc gave 218 mg (95 % yield) of **16**. Because minor impurities were detectable in the product by ^{1}H NMR, the product was suspended in 8 mL of 25 % CH_2Cl_2 in hexanes, sonicated, and filtered to give 200 mg (87 % yield) of **16** as a pale-yellow solid. R_f (SiO_2/25 % EtOAc in hexanes) = 0.12. MP: 225–227 °C (dec., and associated with a color change to dark brown with no phase change). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.24 (d, J = 2.2 Hz, 1H, Ar-H), 8.16 (s, 1H, Ar-H), 7.78 (dd, J = 8.8, 2.2 Hz, 1H, Ar-H), 7.38 (d, J = 8.8 Hz, 1H, Ar-H), 7.16 (s, 2H, Ar-H), 6.62 (s, 1H, =CH), 3.97 (s, 6H, Me), 3.92 (s, 3H, Me). ^{13}C NMR (125 MHz, CD_2Cl_2): δ = 159.5, 154.6, 153.9, 149.1, 146.1, 139.6, 137.0, 128.9, 125.1, 120.8, 119.8, 118.3, 116.5, 111.0, 103.8, 61.1, 56.8. HRMS (ESI/TOF) m/z calculated for $C_{20}H_{16}BrN_3O_5$ [M + Na]$^+$: 480.0166, found 480.0187.

6,8-Dichloro-4-(4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (17)



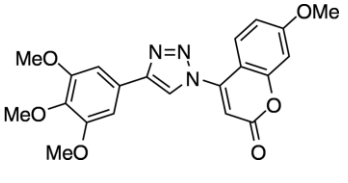
Synthesized from azidocoumarin **7** (128 mg) and 5-ethynyl-1,2,3-trimethoxybenzene (**12**, 115 mg). Chromatography on a silica gel column packed in hexanes and sequentially eluted with hexanes, 25 % EtOAc in hexanes, 40 % EtOAc in hexanes, EtOAc, 10 % MeOH in EtOAc, and 15 % MeOH in EtOAc gave 182 mg (81 % yield) of **17** as a yellow solid. R_f (SiO_2/25 % EtOAc in hexanes) = 0.13. Mp: 224–226 °C (dec., and associated with a color change to dark brown with no phase change). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.16 (s, 1H, Ar-H), 8.02 (d, J = 2.3 Hz, 1H, Ar-H), 7.75 (d, J = 2.3 Hz, 1H, Ar-H), 7.16 (s, 2H, Ar-H), 6.67 (s, 1H, =CH), 3.97 (s, 6H, Me), 3.92 (s, 3H, Me). ^{13}C NMR (125 MHz, 0.5 mL of CD_2Cl_2 + 20 µL of TFA, 305.15 K): δ = 159.9, 154.4, 149.3, 146.4, 139.1, 134.9, 131.7, 124.5, 122.2, 117.0, 112.6, 104.7, 61.7, 57.0. Two quaternary resonances were not observable, possibly due to overlap with other resonances. ^{1}H NMR showed minimal degradation of the product after an overnight ^{13}C NMR experiment at 305.15 K in the NMR solvent containing TFA. HRMS (ESI/TOF) m/z calculated for $C_{20}H_{15}Cl_2N_3O_5$ [M + H]$^+$: 448.0462, found 448.0461.

7-Methyl-4-(4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (18)



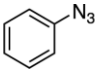
Synthesized from azidocoumarin **8** (101 mg) and 5-ethynyl-1,2,3-trimethoxybenzene (**12**, 115 mg). Chromatography on a silica gel column packed in hexanes and sequentially eluted with hexanes, 25 % EtOAc in hexanes, 40 % EtOAc in hexanes, EtOAc, 5 % MeOH in EtOAc, 10 % MeOH in EtOAc, and 20 % MeOH in EtOAc gave 184 mg (94 % yield) of **18** as a pale-yellow solid. R_f (SiO_2/25 % EtOAc in hexanes) = 0.19. Mp: 242–244 °C (dec., and associated with a color change to dark brown with no phase change). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.16 (s, 1H, Ar-H), 7.87 (d, J = 8.2 Hz, 1H, Ar-H), 7.30 (s 1H, Ar-H), 7.20 (d, J = 8.3 Hz, 1H), 7.16 (s, 2H, Ar-H), 6.55 (s, 1H, =CH), 3.97 (s, 6H, Me), 3.91 (s, 3H, Me), 2.51 (s, 3H, Me). ^{13}C NMR (125 MHz, 0.6 mL of CD_2Cl_2 + 30 µL of TFA, 305.15 K): δ = 163.2, 154.9, 154.4, 148.9, 148.3, 147.6, 139.1, 128.0, 125.7, 124.4, 122.5, 118.6, 112.3, 109.5, 104.8, 61.8, 57.0, 22.2. ^{1}H NMR showed minimal degradation of the product after an overnight ^{13}C NMR experiment at 305.15 K in the NMR solvent containing TFA. HRMS (ESI/TOF) m/z calculated for $C_{21}H_{19}N_3O_5$ [M + Na]$^+$: 416.1217, found 416.1234.

7-Methoxy-4-(4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (19)



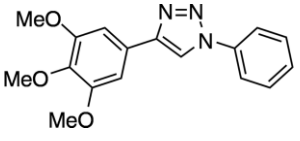
Synthesized from azidocoumarin **9** (109 mg) and 5-ethynyl-1,2,3-trimethoxybenzene (**12**, 115 mg). This reaction took 24 h to reach completion. Chromatography on a silica gel column packed in hexanes and sequentially eluted with hexanes, 25 % EtOAc in hexanes, 40 % EtOAc in hexanes, EtOAc, and 20 % MeOH in EtOAc gave 159 mg (78 % yield) of **19** as a yellow solid. R_f (SiO_2/25 % EtOAc in hexanes) = 0.06. Mp: 236–238 °C (dec., and associated with a color change to dark brown with no phase change). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.15 (s, 1H, Ar-H), 7.93 (d, J = 8.8 Hz, 1H, Ar-H), 7.15 (s, 2H, Ar-H), 6.95–6.93 (m, 2H, Ar-H), 6.43 (s, 1H, =CH), 3.97 (s, 6H, Me), 3.93 (s, 3H, Me), 3.91 (s, 3H, Me). ^{13}C NMR (125 MHz, $CDCl_3$ + 1 drop of TFA): δ = 164.9, 162.3, 156.5, 154.0, 147.6, 138.8, 127.3, 124.0, 121.3, 114.5, 107.4, 105.8, 103.8, 101.7, 61.4, 56.5, 56.4. ^{1}H NMR showed minimal degradation of the product after an overnight ^{13}C NMR experiment at 298.1 K in the NMR solvent containing TFA. HRMS (ESI/TOF) *m/z* calculated for $C_{21}H_{19}N_3O_6$ $[M + H]^+$: 410.1347, found 410.1350.

Azidobenzene[61]



In an oven-dried 25 mL round-bottomed flask equipped with a stir bar was placed $PhB(OH)_2$ (488 mg, 4.00 mmol) in MeOH (12 mL). To this NaN_3 (312 mg, 4.80 mmol, 1.20 equiv.) and $CuSO_4 \cdot 5H_2O$ (100 mg, 0.40 mmol, 0.10 equiv.) were added sequentially. The reaction mixture was stirred overnight, open to air. TLC (SiO_2/hexanes) indicated the reaction to be complete. The mixture was evaporated under reduced pressure and the mixture was partitioned between EtOAc and water. The aqueous layer was separated and back-extracted with EtOAc (3 ×). The combined organic layer was washed with brine, dried with anhydrous Na_2SO_4, and evaporated under reduced pressure. Chromatography of the crude material on a silica gel column packed in hexanes and eluted with hexanes gave 82.0 mg (17 % yield) of PhN_3 as a pale-yellow liquid. R_f (SiO_2/hexanes) = 0.68. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.35 (t, J = 7.8 Hz, 2H, Ar-H), 7.14 (t, J = 7.4 Hz, 1H, Ar-H), 7.03 (d, J = 8.1 Hz, 2H, Ar-H).

1-Phenyl-4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (20)[62]



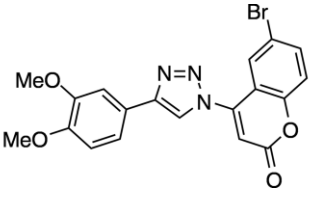
In an oven-dried 12 mL vial equipped with a stir bar was placed PhN_3 (59.0 mg, 0.50 mmol) in dry CH_2Cl_2 (3.75 mL), and 5-ethynyl-1,2,3-trimethoxybenzene (**12**, 115 mg, 0.60 mmol, 1.20 equiv.) was added. $[(MeCN)_4Cu]PF_6$ (19.0 mg, 0.05 mmol, 0.10 equiv.) and dry MeOH (1.25 mL) were added to the resulting solution. Next, 2,6-lutidine (58.0 µL, 0.50 mmol, 1.00 equiv.) was added, the resulting mixture was flushed with nitrogen gas, and stirred for 3 h at room temperature. TLC (SiO_2/25 % EtOAc in hexanes) indicated the reaction to be complete. The light-yellow mixture was transferred to a separatory funnel using CH_2Cl_2 and partitioned between CH_2Cl_2 and brine. The aqueous layer was separated and back-extracted with CH_2Cl_2 (3 ×). The combined organic layer was dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. Chromatography on a silica gel column packed in hexanes and sequentially eluted with hexanes, 25 % EtOAc in hexanes, 40 % EtOAc in hexanes, and EtOAc gave 133 mg (85 % yield) of **20** as a pale-yellow solid. R_f (SiO_2/25 % EtOAc in hexanes) = 0.15. Mp: 142–144 °C (associated with a color change to pale yellow). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.16 (s, 1H, Ar-H), 7.80 (d, J = 7.9 Hz, 2H, Ar-H), 7.56 (t, J = 7.7 Hz, 2H, Ar-H), 7.47 (t, J = 7.4 Hz, 1H, Ar-H), 7.15 (s, 2H, Ar-H), 3.96 (s, 6H, Me), 3.90 (s, 3H, Me). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 153.9, 148.5, 138.6, 137.2, 130.0, 129.0, 126.0, 120.8, 117.6, 103.2, 61.2, 56.5. HRMS (ESI/TOF) *m/z* calculated for $C_{17}H_{17}N_3O_3$ $[M + H]^+$: 312.1348, found 312.1335.

General procedure for the synthesis of compounds 21–24

In an oven-dried 12 mL vial equipped with a stir bar was placed 4-azido-6-bromocoumarin (**6**, 133 mg, 0.5 mmol) in dry CH_2Cl_2 (3.75 mL), and 1-ethynyl-3,5-dimethoxybenzene (0.60 mmol, 1.20 equiv.) was added. $[(MeCN)_4Cu]PF_6$ (19.0 mg, 0.05 mmol, 0.10 equiv.) and dry MeOH (1.25 mL) were added to the resulting solution. Next, 2,6-lutidine (58.0 µL, 0.50 mmol, 1.00 equiv.) was added, the mixture was flushed with nitrogen gas, and the resulting mixture was stirred for 8–24 h at 50 °C. After an appropriate period of time, the reaction was checked by TLC for completion. The mixture was transferred to a separatory funnel using CH_2Cl_2 and partitioned between CH_2Cl_2 and brine. The aqueous layer was separated and back-extracted with CH_2Cl_2 (3 ×). The combined organic layer was dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. See the individual compound headings for specific reaction times and purification details.

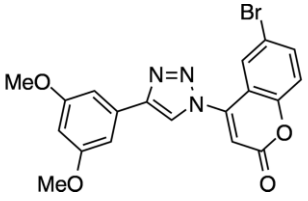
Note: the synthesis of compound **23** was modified (see Results and Discussion for details). In this case, at the start of the reaction, one aliquot of 1-ethynyl-4-methoxybenzene (79 mg, 0.60 mmol, 1.20 equiv.) was added and the volume of CH_2Cl_2 was 3 mL. Quantities of all other reagents were as stated above and the reaction temperature was 50 °C. After 8 h, a second aliquot of 1-ethynyl-4-methoxybenzene (79 mg, 0.60 mmol, 1.20 equiv.) was added along with 0.75 mL of CH_2Cl_2, and the reaction was allowed to proceed for an additional 16 h at 50 °C.

6-Bromo-4-(4-(3,4-dimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (21)



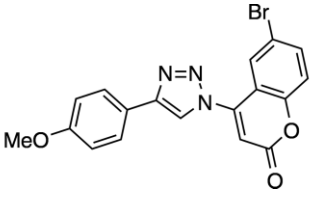
Synthesized from azidocoumarin **6** and 4-ethynyl-1,2-dimethoxybenzene (97 mg, 0.60 mmol, 1.20 equiv.). TLC (SiO_2/10 % EtOAc in hexanes) after 8 h indicated the reaction to be complete. Chromatography was precluded by the low solubility of the product. Therefore, the product was initially sonicated in 25 % CH_2Cl_2 in hexanes (4 mL) and the supernatant was removed. Next, the precipitate was heated in PhH (4 mL), cooled, and the supernatant was removed. The precipitate was dried under vacuum to give 192 mg (90 % yield) of **21** as a pale-yellow solid. R_f (SiO_2/25 % EtOAc in hexanes) = 0.13. Mp: 217–220 °C (dec., and associated with a color change to dark brown with no phase change). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.25 (s, 1H, Ar-H), 8.18 (s, 1H, Ar-H), 7.78 (d, J = 8.8 Hz, 1H, Ar-H), 7.57 (s, 1H, Ar-H), 7.42 (d, J = 7.8 Hz, 1H, Ar-H), 7.37 (d, J = 8.8 Hz, 1H, Ar-H), 6.98 (d, J = 7.9 Hz, 1H, Ar-H), 6.62 (s, 1H, =CH), 4.01 (s, 3H, Me), 3.96 (s, 3H, Me). ^{13}C NMR (150 MHz, triple resonance cryoprobe with inverse detection, CD_2Cl_2): δ = 159.6, 153.9, 150.7, 150.3, 149.1, 146.1, 136.9, 128.9, 122.4, 120.2, 119.8, 119.2, 118.3, 116.6, 112.3, 110.9, 109.9, 56.5, 56.4. HRMS (ESI/TOF) *m/z* calculated for $C_{19}H_{14}BrN_3O_4$ $[M + H]^+$: 428.0240, found 428.0249.

6-Bromo-4-(4-(3,5-dimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (22)



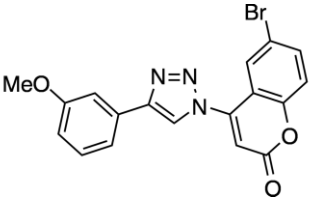
Synthesized from azidocoumarin **6** and 1-ethynyl-3,5-dimethoxybenzene (97 mg, 0.60 mmol, 1.20 equiv.). TLC (SiO_2/25 % EtOAc in hexanes) after 24 h indicated the reaction to be complete. Chromatography was precluded by the low solubility of the product. Therefore, the product was initially sonicated in 25 % CH_2Cl_2 in hexanes (8 mL) and filtered. Next, the precipitate was heated in PhH (3 mL), cooled, and the supernatant was removed. The precipitate was dried under vacuum to give 132 mg (62 % yield) of **22** as a pale-yellow solid. R_f (SiO_2/25 % EtOAc in hexanes) = 0.22. Mp: 241–243 °C (dec., and associated with a color change to dark brown with no phase change). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.21 (s, 1H, Ar-H), 8.18 (s, 1H, Ar-H), 7.78 (d, J = 9.0 Hz, 1H, Ar-H), 7.38 (d, J = 8.8 Hz, 1H, Ar-H), 7.10 (s, 2H, Ar-H), 6.63 (s, 1H, =CH), 6.54 (s, 1H, Ar-H), 3.89 (s, 6H, Me). ^{13}C NMR (150 MHz, triple resonance cryoprobe with inverse detection, CD_2Cl_2): δ = 162.1, 159.5, 153.8, 149.0, 146.1, 137.0, 131.4, 128.8, 121.3, 119.8, 118.3, 116.5, 111.1, 104.6, 101.6, 56.1. HRMS (ESI/TOF) m/z calculated for $C_{19}H_{14}BrN_3O_4$ $[M + H]^+$: 428.0240, found 428.0261.

6-Bromo-4-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (23)



Synthesized from azidocoumarin **6** with two aliquot additions of 1-ethynyl-4-methoxybenzene (79 mg, 0.60 mmol, 1.20 equiv.), see the note above. TLC (SiO_2/25 % EtOAc in hexanes) after 24 h indicated the reaction was incomplete. Chromatography was precluded by the low solubility of the product. Therefore, the product was initially washed with 25 % CH_2Cl_2 in hexanes (2 × 4 mL) and the supernatant was removed. Next, the precipitate was heated in PhH (4 mL), cooled, and the supernatant was removed. The precipitate was dried under vacuum to give 140 mg (70 % yield) of **23** as a pale-yellow solid. R_f (SiO_2/25 % EtOAc in hexanes) = 0.26. Mp: 219–220 °C (dec., and associated with a color change to dark brown with no phase change). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.26 (s, 1H, Ar-H), 8.10 (s, 1H, Ar-H), 7.87 (d, J = 8.5 Hz, 2H, Ar-H), 7.78 (d, J = 8.9 Hz, 1H, Ar-H), 7.37 (d, J = 8.9 Hz, 1H, Ar-H), 7.04 (d, J = 8.5 Hz, 2H, Ar-H), 6.61 (s, 1H, =CH), 3.89 (s, 3H, Me). ^{13}C NMR (150 MHz, triple resonance cryoprobe with inverse detection, CD_2Cl_2): δ = 161.0, 159.6, 153.9, 149.0, 146.1, 136.9, 128.9, 128.0, 122.2, 120.0, 119.8, 118.3, 116.6, 115.1, 110.8, 55.9. HRMS (ESI/TOF) m/z calculated for $C_{18}H_{12}BrN_3O_3$ $[M + H]^+$: 398.0135, found 398.0159.

6-Bromo-4-(4-(3-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (24)



Synthesized from azidocoumarin **6** and 1-ethynyl-3-methoxybenzene (76 µL, 0.60 mmol, 1.20 equiv.). TLC (SiO_2/20 % EtOAc in hexanes) indicated the reaction to be complete in 8 h (although ^{1}H NMR analysis of the reaction mixture indicated a trace amount of residual azide). Chromatography was precluded by the low solubility of the product. Therefore, the product was initially heated in PhH (4 mL), cooled, and the supernatant was removed. Next, the precipitate was sonicated in 25 % CH_2Cl_2 in hexanes (4 mL) and the supernatant was removed. The precipitate was dried under vacuum to give 135 mg (68 % yield) of **24** as an off-white solid. R_f (SiO_2/25 % EtOAc in hexanes) = 0.32. Mp: 240–242 °C (dec., and associated with a color change to dark brown with no phase change). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.22 (s, 1H, Ar-H), 8.19 (s, 1H, Ar-H), 7.78 (d, J = 9.0 Hz, 1H, Ar-H), 7.54 (s, 1H, Ar-H), 7.47 (d, J = 7.0 Hz, 1H, Ar-H), 7.42 (t, J = 7.7 Hz, 1H, Ar-H), 7.38 (d, J = 8.7 Hz, 1H, Ar-H), 6.99 (d, J = 7.8 Hz, 1H, Ar-H), 6.63 (s, 1H, =CH), 3.92 (s, 3H, Me). ^{13}C NMR (150 MHz, triple resonance cryoprobe with inverse detection, CD_2Cl_2): δ = 160.9, 159.5, 153.9, 149.0, 146.1, 137.0, 131.0, 130.8, 128.8, 121.2, 119.8, 118.9, 118.3, 116.6, 115.4, 111.9, 111.1, 56.0. HRMS (ESI/TOF) m/z calculated for $C_{18}H_{12}BrN_3O_3$ $[M + H]^+$: 398.0135, found 398.0137.

Acknowledgments

This work was supported by grant CHE-1265687 from the National Science Foundation to MKL. Infrastructural support at CCNY was provided by National Institutes of Health grant G12MD007603 from the National Institute of Minority Health and Health Disparities (NIMHD). The authors thank the personnel of the Cytometry, Screening and Imaging Core Facilities, which is supported by a Research Centers in Minority Institutions NIMHD grant 5G12MD007592 to the Border Biomedical Research Center at the University of Texas at El Paso. The authors express their gratitude to Mrs. Leentje Persoons, Mrs. Ellen De Waegenaere, Mrs. Bianca Stals, Mrs. Kirsten Lepaige, and Mrs. Nathalie Van Winkel for excellent technical assistance in the antiviral assays. Indofine Chemical Company is thanked for samples of some of the 4-hydroxycoumarins that have been utilized in this work.

Keywords: Alkynes · Click chemistry · Coumarin · Copper · Coumarin · Triazole

[1] *Advances in Structure and Activity Relationship of Coumarin Derivatives* (Ed.: S. Penta), Academic Press, Amsterdam, **2016**.
[2] *Coumarins: Biology, Applications and Mode of Action* (Eds.: R. O'Kennedy, R. D. Thornes), John Wiley & Sons, Chichester, **1997**.
[3] J. R. S. Hoult, M. Payá, *Gen. Pharmacol.* **1996**, *27*, 713–722.
[4] F. Borges, F. Roleira, N. Milhazes, L. Santana, E. Uriarte, *Curr. Med. Chem.* **2005**, *12*, 887–916.

[5] S. H. Bairagi, P. P. Salaskar, S. D. Loke, N. N. Surve, D. V. Tandel, M. D. Dusara, *Int. J. Pharm. Res.* **2012**, *4*, 16–19.
[6] E. Jameel, T. Umar, J. Kumar, N. Hoda, *Chem. Biol. Drug Des.* **2016**, *87*, 21–38, and cited references .
[7] T. Kubrak, R. Podgórski, M. Stompor, *Eur. J. Clin. Exp. Med.* **2017**, *15*, 169–175.
[8] S. K. Venkata, B. M. Gurupadayya, R. S. Chandan, D. K. Nagesha, B. Vishwanathan, *Curr. Drug Delivery* **2016**, *13*, 186–201.
[9] A. Thakur, R. Singla, V. Jaitak, *Eur. J. Med. Chem.* **2015**, *101*, 476–495.
[10] S. Emami, S. Dadashpour, *Eur. J. Med. Chem.* **2015**, *102*, 611–630.
[11] M. Kaur, S. Kohli, S. Sandhu, Y. Bansal, G. Bansal, *Anti-Cancer Agents Med. Chem.* **2015**, *15*, 1032–1048.
[12] S. Thaisvivongs, D. L. Romero, R. A. Tommasi, M. N. Janakiraman, J. W. Strohbach, S. R. Turner, C. Biles, R. R. Morge, P. D. Johnson, P. A. Aristoff, P. K. Tomich, J. C. Lynn, M.-M. Horng, K.-T. Chong, R. R. Hinshaw, W. J. Howe, B. C. Finzel, K. D. Watenpaugh, *J. Med. Chem.* **1996**, *39*, 4630–4642.
[13] S. R. Turner, J. W. Strohbach, R. A. Tommasi, P. A. Aristoff, P. D. Johnson, H. I. Skulnick, L. A. Dolak, E. P. Seest, P. K. Tomich, M. J. Bohanon, M.-M. Horng, J. C. Lynn, K.-T. Chong, R. R. Hinshaw, K. D. Watenpaugh, M. N. Janakiraman, S. Thaisrivongs, *J. Med. Chem.* **1997**, *41*, 3467–3476.
[14] S. E. Haen, J. V. N. Vara Prasad, F. E. Boyer, J. M. Domagala, E. L. Ellsworth, C. Gajda, H. W. Hamilton, L. J. Markoski, B. A. Steinbaugh, B. D. Tait, E. A. Lunney, P. J. Tummino, D. Ferguson, D. Hupe, C. Nouhan, S. J. Gracheck, J. M. Saunders, S. VanderRoest, *J. Med. Chem.* **1997**, *40*, 3707–3711.
[15] J. Neyts, E. De Clercq, R. Sinha, Y. H. Chang, A. R. Das, S. K. Chakraborty, S. C. Hong, S.-C. Tsay, M.-H. Hsu, J. R. Hwu, *J. Med. Chem.* **2009**, *52*, 1486–1490.
[16] J. R. Hwu, S.-Y. Lin, S.-C. Tsay, E. De Clercq, P. Leyssen, J. Neyts, *J. Med. Chem.* **2011**, *54*, 2114–2126.
[17] B. M. Bizzarri, L. Botta, E. Capecchi, I. Celestino, P. Checconi, A. T. Palamara, L. Nencioni, R. Saladino, *J. Nat. Prod.* **2017**, *80*, 3247–3254.
[18] M. Z. Hassan, H. Osman, M. A. Ali, M. J. Ashan, *Eur. J. Med. Chem.* **2016**, *123*, 236–255.
[19] A. Chaudhary, S. N. Pandeya, P. Kumar, P. P. Sharma, S. Gupta, N. Soni, K. K. Verma, G. Bharadwaj, *Mini-Rev. Med. Chem.* **2007**, *7*, 1186–1205.
[20] G. R. Petit, S. B. Singh, E. Hamel, C. M. Lin, D. S. Alberts, D. Garcia-Kendal, *Experientia* **1989**, *45*, 209–211.
[21] C. M. Lin, S. B. Singh, P. S. Chu, R. O. Dempcy, J. M. Schmidt, G. R. Pettit, E. Hamel, *Mol. Pharmacol.* **1988**, *34*, 200–208.
[22] C. M. Lin, H. H. Ho, G. R. Pettit, E. Hamel, *Biochemistry* **1989**, *28*, 6984–6991.
[23] E. Pasquier, S. Honoré, D. Braguer, *Drug Resist. Updates* **2006**, *9*, 74–86.
[24] C. Dumontet, M. A. Jordan, *Nat. Rev. Drug Discovery* **2010**, *9*, 790–803.
[25] M. Su, J. Huang, S. Liu, Y. Xiao, X. Qin, J. Liu, C. Pi, T. Luo, J. Li, X. Chen, Z. Luo, *Sci. Rep.* **2016**, *6*, 28139; 10.1038/srep28139.
[26] R. Gasapri, A. E. Prota, K. Bargsten, A. Cavalli, M. O. Steinmetz, *Chem* **2017**, *2*, 102–113.
[27] S. Lee, K. Sivakumar, W.-S. Shin, F. Xie, Q. Wang, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4596–4599.
[28] W. Zhang, Z. Li, M. Zhou, F. Wu, X. Hou, H. Luo, H. Liu, X. Han, G. Yan, Z. Ding, R. Li, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 799–807.
[29] A. Bistrović, N. Stipaničev, T. Opačak-Bernardi, M. Jukić, S. Martinez, Lj. Glavaš-Obrovac, S. Raić-Malić, *New J. Chem.* **2017**, *41*, 7531–7543.
[30] A. Hamze, E. Rasalofonjatovo, O. Provot, C. Mousset, D. Veau, J. Rodrigo, J. Bignon, J.-M. Liu, J. Wdzieczak-Bakala, S. Thoret, J. Dubois, J.-D. Brion, M. Alami, *ChemMedChem* **2011**, *6*, 2179–2191.
[31] R. Romagnoli, P. G. Baraldi, O. Cruz-Lopez, C. L. Cara, M. D. Carrion, A. Brancale, E. Hamel, L. Chen, R. Bortolozzi, G. Basso, G. Viola, *J. Med. Chem.* **2010**, *53*, 4248–4258.
[32] A. W. Brown, M. Fisher, G. M. Tozer, C. Kanthou, J. P. A. Harrity, *J. Med. Chem.* **2016**, *59*, 9473–9488.
[33] K. Odlo, J. Fournier-Dit-Chabert, S. Ducki, O. A. B. S. M. Gani, I. Sylte, T. V. Hansen, *Bioorg. Med. Chem.* **2010**, *18*, 6874–6885.
[34] A. Krasiński, V. V. Fokin, K. B. Sharpless, *Org. Lett.* **2004**, *6*, 1237–1240.
[35] L. Zhang, X. Chen, P. Xue, H. H. Y. Sun, I. D. William, K. B. Sharpless, V. V. Fokin, G. Jia, *J. Am. Chem. Soc.* **2005**, *127*, 15998–15999.
[36] a) C. Oh, I. Yi, K. P. Park, *J. Heterocycl. Chem.* **1994**, *31*, 841–844; b) W. Stadlbauer, *Monatsh. Chem.* **1986**, *117*, 1305–1323; c) W. Stadlbauer, A.-S. Karem, T. Kappe, *Monatsh. Chem.* **1987**, *118*, 81–89; d) K. Ito, J. Hariya, *Heterocycles* **1987**, *26*, 35–38.
[37] Y.-C. Zheng, Y.-C. Duan, J.-L. Ma, R.-M. Xu, X. Zi, W.-L. Lv, M.-M. Wang, X.-W. Ye, S. Zhu, D. Mobley, Y.-Y. Zhu, J.-W. Wang, J.-F. Li, Z. R. Wang, W. Zhao, H.-M. Liu, *J. Med. Chem.* **2013**, *56*, 8543–8560; T. Saito, T. Ohkubo, H. Kuboki, M. Maeda, K. Tsuda, T. Karakasa, S. Satsumabayashi, *J. Chem. Soc., Perkin Trans. 1* **1998**, 3065–3080.
[38] S. Bae, M. K. Lakshman, *J. Am. Chem. Soc.* **2007**, *129*, 782–789.
[39] S. Bae, M. K. Lakshman, *J. Org. Chem.* **2008**, *73*, 1311–1319.
[40] S. Bae, M. K. Lakshman, *J. Org. Chem.* **2008**, *73*, 3707–3713.
[41] S. Bae, S. Chaturvedi, M. K. Lakshman, *Curr. Protoc. Nucleic Acid Chem.* **2009**, *36*, 1.22.1–1.22.23.
[42] M. K. Lakshman, J. Frank, *Org. Biomol. Chem.* **2009**, *7*, 2933–2940.
[43] M. K. Lakshman, M. K. Singh, D. Parrish, R. Balachandran, B. W. Day, *J. Org. Chem.* **2010**, *75*, 2461–2473.
[44] S. Satishkumar, P. K. Vuram, S. S. Relangi, V. Gurram, H. Zhou, R. J. Kreitman, M. M. Montemayor, L. Yang, M. Kaliyaperumal, S. Shama, N. Pottabathini, M. K. Lakshman, *Molecules* **2015**, *20*, 18437–18463.
[45] V. Basava, L. Yang, P. Pradhan, M. K. Lakshman, *Org. Biomol. Chem.* **2016**, *14*, 7069–7083.
[46] H. K. Akula, H. Kokatla, G. Andrei, R. Snoeck, D. Schols, J. Balzarini, M. K. Lakshman, *Org. Biomol. Chem.* **2017**, *15*, 1130–1139.
[47] H. K. Akula, M. K. Lakshman, *Curr. Protoc. Nucleic Acid Chem.* **2019**, *76*, e73.
[48] A. Nesturi, *Synthesis of 4-Azidocoumarins and Their Use in Copper-Catalyzed Azide-Alkyne Cycloaddition Reactions*, MS Thesis. The City College of New York, **2013**. https://academicworks.cuny.edu/cgi/viewcontent.cgi?article=1519&context=cc_etds_theses.
[49] S. Ohira, *Synth. Commun.* **1989**, *19*, 561–564.
[50] a) G. J. Roth, B. Liepold, S. G. Müller, H. J. Bestmann, *Synthesis* **2004**, 59–62; b) S. Müller, B. Liepold, G. J. Roth, H. J. Bestmann, *Synlett* **1996**, 521–522.
[51] See for examples: a) L. Burroughs, J. Ritchie, M. Ngwenya, D. Khan, W. Lewis, S. Woodward, *Beilstein J. Org. Chem.* **2015**, *11*, 273–279; b) N. J. Lawrence, F. A. Ghani, L. A. Hepworth, J. A. Hadfield, A. T. McGown, R. G. Pritchard, *Synthesis* **1999**, 1656–1660.
[52] A. K. Morri, Y. Thummala, V. R. Doddi, *Org. Lett.* **2015**, *17*, 4640–4643.
[53] E. J. Corey, P. L. Fuchs, *Tetrahedron Lett.* **1972**, *13*, 3769–3772.
[54] C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3062.
[55] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599; *Angew. Chem.* **2002**, *114*, 2708.
[56] K. Sivakumar, F. Xie, B. M. Cash, S. Long, H. N. Barnhill, Q. Wang, *Org. Lett.* **2004**, *6*, 4603–4606.
[57] L. Zhu, C. J. Brassard, X. Zhang, P. M. Guha, R. J. Clark, *Chem. Rec.* **2016**, *16*, 1501–1517.
[58] C. Lema, A. Varela-Ramirez, R. J. Aguilera, *Curr. Cell Biochem.* **2011**, *1*, 1–14.
[59] S. L. Young, D. J. Chaplin, *Expert Opin. Invest. Drugs* **2004**, *13*, 1171–1182.
[60] I. Vermes, C. Haanen, H. Steffens-Nakken, C. A. Reutellingsperger, *J. Immunol. Methods* **1995**, *184*, 39–51.
[61] C.-Z. Tao, X. Cui, J. Li, A.-X. Liu, L. Liu, Q.-X. Guo, *Tetrahedron Lett.* **2007**, *48*, 3525–3529.
[62] G.-F. Zha, W.-Y. Fang, Y.-G. Li, J. Leng, X. Chen, H.-L. Qin, *J. Am. Chem. Soc.* **2018**, *140*, 17666–17673.

Received: April 14, 2019