Journal of Medicinal Chemistry

pubs.acs.org/jmc Article

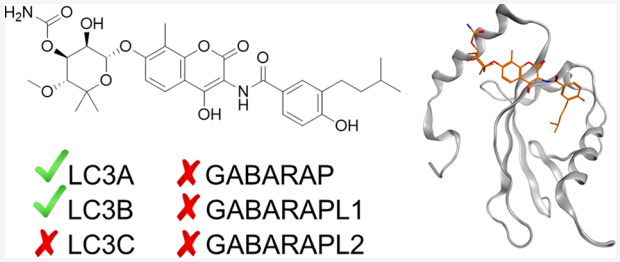
Demonstrating Ligandability of the LC3A and LC3B Adapter Interface

Markus Hartmann, Jessica Huber, Jan S. Kramer, Jan Heering, Larissa Pietsch, Holger Stark, Dalibor Odadzic, Iris Bischoff, Robert Fürst, Martin Schröder, Masato Akutsu, Apirat Chaikuad, Volker Dötsch, Stefan Knapp, Ricardo M. Biondi, Vladimir V. Rogov, and Ewgenij Proschak*

Cite This: J. Med. Chem. 2021, 64, 3720−3746 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Autophagy is the common name for a number of lysosome-based degradation pathways of cytosolic cargos. The key components of autophagy are members of Atg8 family proteins involved in almost all steps of the process, from autophagosome formation to their selective fusion with lysosomes. In this study, we show that the homologous members of the human Atg8 family proteins, LC3A and LC3B, are druggable by a small molecule inhibitor novobiocin. Structure−activity relationship (SAR) studies of the 4-hydroxy coumarin core scaffold were performed, supported by a crystal structure of the LC3A dihydronovobiocin complex. The study reports the first nonpeptide inhibitors for these protein interaction targets and will lay the foundation for the development of more potent chemical probes for the Atg8 protein family which may also find applications for the development of autophagy-mediated degraders (AUTACs).



INTRODUCTION

Macroautophagy, hereafter referred as autophagy, is a highly conserved catabolic process that together with proteasomal degradation serves in the removal and reuse of unwanted cellular components, such as damaged organelles, protein aggregates, intracellular pathogens, and other macromolecular structures (reviewed in refs 1−4). Autophagy is characterized by the formation of double-membrane vesicles, autophagosomes, which are fused with the vacuole (in fungi and plants) or lysosomes (in higher organisms) and which serve to expose engulfed cargo to the resident degradative enzymes. Among ∼40 autophagy related (Atg) proteins (reviewed in refs 2 and 5), ubiquitin-like (UBL) proteins of the Atg8 family (autophagy modifiers) play a key role. Atg8 is expressed as a precursor protein which undergoes processing by the cysteine protease Atg4 exposing a C-terminal glycine residue. Subsequently, the Atg8 C-terminus is covalently linked to phosphatidyl-ethanolamine (PE) by a ubiquitin-like conjugation cascade, resulting in incorporation into the inner and outer membrane of the double-membrane autophagosome.[2,6] The PE conjugates tether Atg8−PE to lipid bilayers;[7] and they recruit additional components of the autophagic machinery[8−10] and, via interaction with selective autophagy receptors, sequester specific cargo to autophagosomes.[11,12]

Humans encode six Atg8 homologues: LC3A, LC3B, LC3C (LC3 subfamily) and GABARAP, GABARAPL1, GABARAPL2/GATE-16 (GABARAP subfamily). LC3/GABARAPs display a similar topology as observed in ubiquitin; however, they have two additional, characteristic α-helices located N-terminally to their UBL core. This N-terminal subdomain varies significantly among the different members of the Atg8 family and packs onto the UBL core, forming a deep hydrophobic pocket (HP1). Another hydrophobic pocket (HP2) is formed by hydrophobic residues of the central α-helix and β-strand 2 of the UBL core. HP1, HP2, and β-strand 2 of Atg8/LC3/GABARAP engage in interaction networks associated with autophagy and membrane trafficking (reviewed in refs 4 and 13). The majority of selective autophagy receptors interact with Atg8/LC3/GABARAP through a so-called LIR (LC3-interacting region), also known as AIM (Atg8-interacting motif)). AIMs are short linear motifs with a central consensus sequence of Θ−X−X−Γ, where Θ is an aromatic amino acid (W/F/Y), Γ is a hydrophobic one (L/I/V), and X can be any amino acid. The aromatic residue usually interacts with the HP1 pocket, while Γ is placed into HP2 pockets. Negatively charged residues located N-terminally to Θ (but also within and after the core LIR) increase affinity of LIR-containing proteins for Atg8/LC3/GABARAP. Amino-acid variations of LIR affect specificity for Atg8-family members.[14]

Received: September 23, 2020
Published: March 26, 2021

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

One of the prototypical and most studied mammalian selective autophagy receptors is p62/SQSTM1 (p62), a modular protein involved in autophagy-related degradation of misfolded proteins and aggregates.[15] Despite the fact that a defective autophagy pathway has been linked to the development of many human diseases,[5] the efforts to generate selective autophagy small molecule modulators are in the very beginning stages. Selective targeting of the LIR interaction with LC3/GABARAP subfamily members would allow studies to understand the individual role of each family member in recruiting cargo to the autophagosome, and it would facilitate the development of small molecule degraders that would selectively recruit target proteins to the autophagosome (AUTACs).[16] In this study, we present data based on a screening approach for small molecules identifying ligands for LC3/GABARAPs that inhibit interaction with p62. We demonstrate that LC3/GABARAPs represents a druggable interface, and we characterized the aminocoumarin antibiotic novobiocin as a lead structure inhibiting the interaction of LC3A and LC3B with p62.

RESULTS AND DISCUSSION

We first established a robust AlphaScreen interaction assay between biotin-LIRtide (polypeptide including the LIR sequence DDDWTHL from p62) and GST-LC3B. The assay is based on detecting the proximity of donor and acceptor beads that attach to biotin and GST. Using this homogeneous assay, we conducted a medium-throughput screening of the Prestwick Chemicals library of approved drugs which comprises 1280 compounds (see the Screening of Libraries of Compounds That Affect the Interaction between LC3b and LIRtide section). The most promising hit was the natural product antibiotic novobiocin (**1**, Figure 1A). The direct binding of **1** to LC3B was confirmed using differential scanning fluorimetry (DSF). **1** markedly shifted the melting point of recombinant LC3B by 4.4 °C (Figure 1B).

To investigate the interaction further, we performed a NMR titration experiment, stepwise adding **1** to ^{15}N-labeled LC3B up to 8-fold molar excess. Upon titration with **1**, LC3B resonances displayed significant chemical shift perturbations (CSPs), exclusively in the fast exchange mode (Figures 1C and S1A). These CSPs are similar in direction and magnitude to the CSPs induced by titration of canonical p62 LIR-containing peptide (Figure 1D), suggesting similar interaction site for both **1** and p62 LIR with the LC3B surface. However, p62 LIR induced CSPs were in the slow to intermediate exchange modes, indicating higher affinity of LC3B to p62 LIR in comparison to **1**. Mapping of the **1**-induced CSPs on the sequence and surface of LC3B (Figure S1) resulted in characteristic shifts of residues involved in binding of LIR peptides, with the largest CSP allocated in the LC3B HP2 pocket. In contrast, only small CSPs were observed for the LC3B resonances in HP1 area, indicating that, in contrast to typical LIRs, only one of the two hydrophobic pockets was occupied by **1** (Figure 2).

In order to elucidate the preliminary structure–activity relationships (SARs) of novobiocin derivatives, we developed a robust orthogonal assay system based on homogeneous time-resolved FRET (HTRF) technology. Therefore, LC3A and LC3B were expressed as fusion proteins with a SNAP tag, which was subsequently used for labeling with terbium cryptate acting as the FRET donor. LIR motif coupled to superfolder GFP acted as the FRET acceptor (Figure 3A). Titration of 10

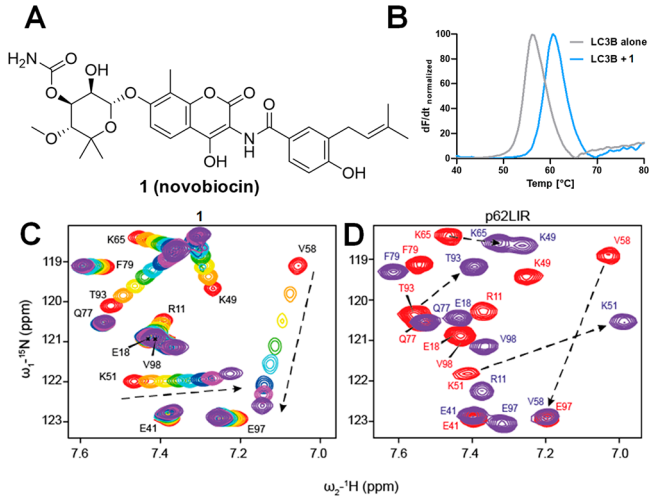


Figure 1. (A) Structure of novobiocin (**1**) (B) **1** causes a shift of the melting temperature of recombinant LC3B in the DSF assay. (C) Representative (fingerprint) areas of LC3B ^{1}H–^{15}N HSQC spectra upon titration with **1** (overlaid). The rainbow color code represents increasing molar ratios from free LC3B (red) upon 8-fold molar excess of **1** (purple). (D) Representative (fingerprint) areas of LC3B ^{1}H–^{15}N HSQC spectra upon interaction with the p62 LIR peptide. Spectra of the free (red) and p62 bound form (purple) of LC3B are shown.

nM of LC3s with increasing concentration of sGFP-LIR chimera led to determination of the K_d of the LIR–LC3 interaction (0.258 μM for LC3A and 0.126 μM for LC3B, Figure 3B). Additionally, using this assay, we were able to show that **2** was able to sufficiently displace the p62 LIR on LC3A and LC3B, potentially by competing its interaction with HP2 (Figure 3C).

Using the aforementioned assay, we investigated the preliminary SARs of novobiocin (**1**) by subsequent structure simplification. In the first step, we reduced the double bond of the 3-hydroxybenzamide residue. Then, the novobiose carbohydrate moiety was removed. Subsequent removal of the methyl moiety in the 8-position of the aminocoumarin core and the hydroxyl moiety of the benzamide substituent led to the simplified compound **6** (Table 1).

We investigated the SARs of the novobiocin derivatives using three orthogonal techniques: HTRF displacement assay, ITC, and DSF. The hydrogenation of the allyl double bond (compound **2**) led to improved K_d values and an increase in ΔT_m. The aglycon of **2**, compound **3**, exhibited decreased affinity toward both LC3A and LC3B. A very pronounced decrease in ΔT_m underlined the important role of the novobiose part for protein stabilization. In contrast, the removal of neither the methyl group in the 8-position nor the hydroxy group of the benzamide part had a pronounced effect on affinity. The removal of both groups resulted in compound **6** and even led to an improved potency in displacement of the LIR peptide from LC3A and LC3B in comparison to the aglycon **3**.

In order to rationalize the SARs of novobiocin derivatives, we crystallized LC3A with the most potent inhibitor **2**. In the X-ray structure, **2** bound to the interface between LC3A and p62, or more precisely to the LIR docking site (LDS). The 4-hydroxy-coumarin core was enclosed by a positively charged clamp formed by two lysine residues K30 and K49 (Figure 4A, cyan). The 3-hydroxybenzamide occupied a lipophilic binding

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720–3746

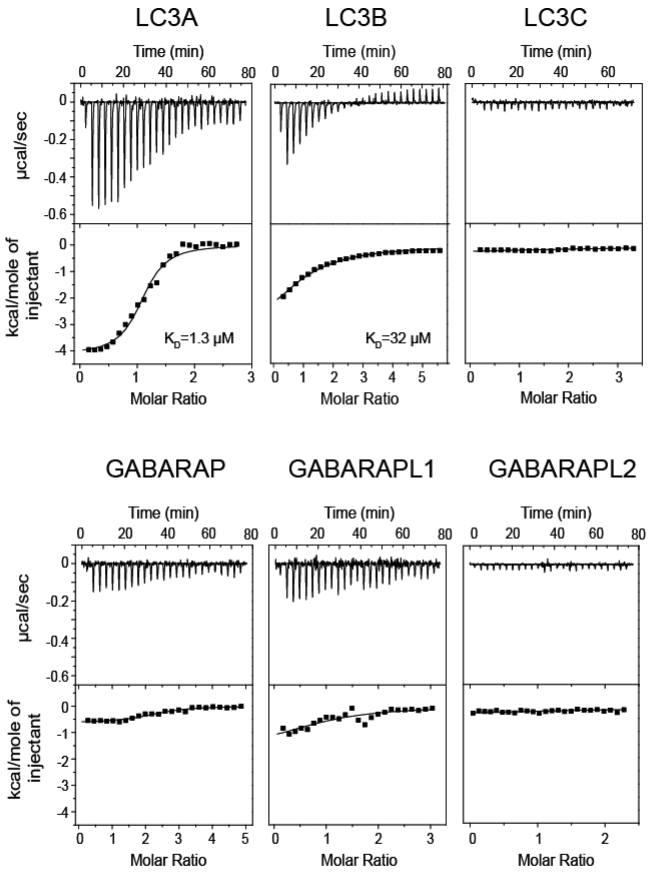


Figure 2. ITC analysis of compound **1** binding to all human Atg8-family proteins. The top panels represent the raw injection heats while the bottom panels show the integrated and normalized heat per titration step. Calculated K_d values (solid line representing a nonlinear least-squares fit) to a single site binding model for LC3A and LC3B are shown. To elucidate the selectivity of **1**, we performed isothermal titration calorimetry (ITC) experiments, titrating **1** into all six human Atg8-proteins. While we observed pronounced binding of **1** to LC3A and LC3B, no significant interactions were detected for LC3C as well as to the three GABARAP subfamily proteins. LC3A displayed an affinity that was in the micromolar range (K_d = 1.3 μM), typical for Atg8:LIR interactions. The determined K_d value for LC3B (32 μM) was higher which was in agreement with the NMR titration data.

pocket formed by residues F52, I35, I67, I66, and L63 (Figure 4A, green). The two methyl groups of the novobiose moiety displayed superficial interactions with the lipophilic pocket formed by residues I23 and L53 (yellow), while the urethane group directly interacted with H27 via a directed hydrogen bond. Superposition with the crystal structure of LC3B in complex with the LIR peptide revealed that **2** mimics the peptide interaction (Figure 4B). The branched alkyl chain of the 3-hydroxybenzamide part occupies the hydrophobic pocket HP2, which is responsible for recognition of Γ (Figure 4C and D). The hydrophobic pocket HP1, which is occupied by an aromatic residue in the peptide complex, is not completely filled with the two methyl groups of the novobiose moiety. Finally, the β sheet interactions with the backbone of V54 and F52 are patterned by the amide NH in the 3-position and the hydroxy group in the 4-position of the coumarin, respectively (Figure 4E and F).

We have showed experimentally that novobiocin derivatives are potent to disrupt interaction between the p62 LIR motif and LC3A/LC3B proteins. However, our results allowed us to extend this potency to all LIR motifs, recognizing the LDS on Atg8/LC3/GABARAPs surface. A vast majority of all functionally active selective autophagy receptors and adaptors possess such LIRs,[4] allowing one to target a broad set of autophagy-related interactions by the derivatives of **1**.

Guided by the X-ray structure, which showed that the benzamide substituent occupied the hydrophobic pocket HP2, thereby mimicking the Leu residue of the LIR motif, we explored the SAR of this moiety (Table 2). We varied the linker between the hydrophobic residue and the lipophilic part, choosing between methylene, thioether, sulfonamide, and ether isosters. In most cases, the methylene linker was preferred, followed by the thioether, sulfonamide, and ether. The X-ray structure of 2 in complex with LC3A (Figure 4) clarifies this preference. The dihedral angle at the methylene is 119°. A comparable angle can be accommodated by the thioether, but not by sulfonamide and ether bioisosters.[17] Furthermore, the nonpolar methylene and thioether linkers are more suitable for the hydrophobic environment enclosed by valine, leucine, and isoleucine side chains. However, when using unsaturated or aromatic lipophilic substituents, the ether linker (**9h**−**9j**) outperformed the methylene bearing compound **6i**. Considering the size of the lipophilic residue, potency increases with the size of the chain. The *n*-butyl series (**6e**−**9e**) delivers the most potent compounds with all linkers. However, in the case of the optimal methylene linker, propyl is already sufficient to reach the same K_d values. Branched series (**6a**−**8a**, **6g**−**9g**) did not outperform the *n*-alkyl counterparts. Cyclopropyl isoster (compounds **6f**, **7f**, and **9f**) of series **a** were well-tolerated.

In the next step, we varied the position of the isopentyl side chain and the substituent in the para-position of the benzamide (Table 3). Although the X-ray structure suggests that the 4-hydroxy group is involved in hydrogen bond interactions to a water molecule (Figure 4A), it can be replaced by a fluoro substituent with a marginal loss in potency (compound **10**). Methylation of the hydroxy group resulted in equipotent compound **11**. As expected, the transition of the isopentyl group to the ortho-position resulted in loss of potency (compound **12**).

As mentioned before, the 4-isopentyl benzamide moiety occupies the HP2 pocket like the leucine residue of the LIR motif. Subsequently, we investigated whether amino acids can be attached to the coumarin scaffold, and prepared the series **13a**−**g** (Table 4). The SAR of the amino acid series appears to be very restrictive when compared to the benzamide series. Small substituents (glycine **13a**, alanine **13b**, and valine **13c**) are not tolerated. Leucine **13e**, but not isoleucine **13f**, yielded some potency. A clear preference of the *S*-configuration (compound **13e**) over the enantiomer **13d** could be observed. The introduction of the non-natural *n*-butyl substituent (compound **13g**) yielded only a very weak inhibitor.

Inspection of the X-ray structure of **2** in complex with LC3A suggested, that the novobiose moiety does not fully occupy the hydrophobic pocket HP1, which recognizes the aromatic residue Trp or Phe of the LIR motif. Furthermore, the LIR motif is flanked by acidic residues which exhibit ionic interactions with LC3s. Therefore, we hypothesized that a gain in binding affinity can be reached by exploiting one of these interactions. HP1 is located next to the 7-position of the coumarin scaffold. First, we introduced several aromatic residues via Suzuki coupling to the 7-bromo-substituted coumarin precursor. 1-Naphthyl substituent **14a** was well-

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

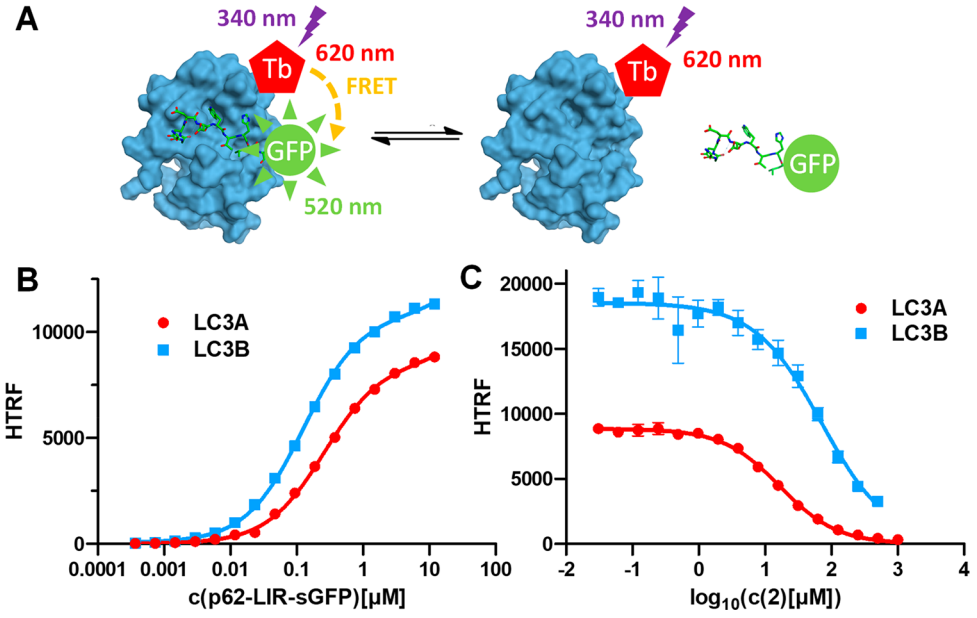
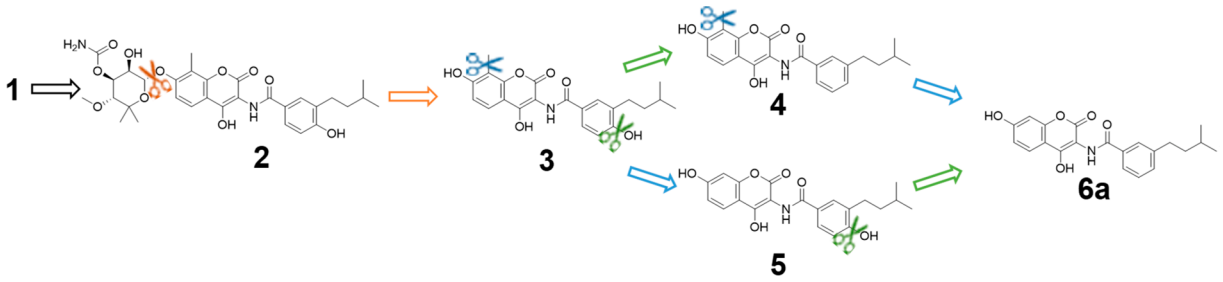


Figure 3. (A) Principle of the HTRF displacement assay. The p62-LIR peptide is expressed as sGFP fusion, while LC3s are coupled to Tb-cryptate. (B) Association curves of the p62-LIR-sGFP to LC3s. (C) Displacement of 1 μM p62-LIR-sGFP from 10 nM LC3s by **2**.

Table 1. Investigation of Preliminary Structure–Activity Relationships of Novobiocin Derivatives



	LC3A				LC3B			
compd	IC_{50} [μM][a]	K_i^{calc} [μM][b]	K_d [μM][c]	ΔT_m[d]	IC_{50} [μM][a]	K_i^{calc} [μM][b]	K_d [μM][c]	ΔT_m[d]
1	42.9 ± 7.1	8.8	7.5	4.4	172.0 ± 17.4	19.3	17.4	4.4
2	16.0 ± 1.0	3.3	2.8	5.6	72.3 ± 7.4	8.1	11.4	5.6
3	118.6 ± 16.7	24.3	14.2	0.7	250.8 ± 10.9	28.1	21.6	1.9
4	159.8 ± 10.6	32.7	15.7	0.0	260.9 ± 5.8	29.3	23.5	1.8
5	127.7 ± 4.0	26.2	27.5	0.8	227.6 ± 11.1	25.5	20.5	2.5
6a	64.8 ± 3.7	13.3	17.2	1.4	139.6 ± 10.4	15.7	25.8	2.0

[a]IC_{50} ± SD value is determined by the HRTF displacement assay. [b]K_i calculated from HTRF displacement. [c]K_d determined by ITC. [d]ΔT_m determined by DSF.

tolerated, while 2-benzofurane isoster **14e** led to full inactivity (Table 5). Introduction of the 2-biphenyl moiety resulted in almost inactive compound **14b**. The introduction of *meta*- and *para*-benzoic acids (compounds **14c** and **14d**) was also tolerated by LC3s. A series of 5-substituted *N*-alkyl pyrazoles **14f–14h** also did not lead to more potent inhibitory values.

We checked the tolerability of amide and sulfonamide isosters of the phenolic –OH group in the 7-position which exhibit potential H-bond donor and acceptor interactions and would allow further extension. Sulfonamide substituents in the 7-position of the coumarin scaffold could be introduced and were intended to combine the narrow angle to fit the HP1 with a polar group pointing toward the solvent. Almost all compounds of the series **14i–14o** were well tolerated, especially compound **14o**, which incorporates a sulfonamide, and a well-tolerated 2-naphthyl moiety from **14a** showed best inhibitory values in this study. Notably, selectivity of **14o** toward the other members of the Atg8 family remained comparable to **1** and **2** (Supporting Information, Table S3). Finally, amide derivatives **14p** and **14q**, which should reach out for ionic interactions, were prepared, without observation of a potency increase. The acyl amide **14r** was slightly more active than the extended derivatives **14p** and **14q**, which could probably be an indication of the importance of the potential H-bond donor and acceptor interactions mentioned above.

Chemistry. Derivatives **2** and **3** could be prepared directly from **1** (Scheme 1). Thereby, Pd-catalyzed hydrogenation was used to reduce the double bond of **1**, yielding **2** (dihydronovobiocin). The aglycon **3** was prepared by cleaving the glycoside bond using acetyl chloride in EtOH.

The synthesis of all other derivatives was not possible from **1**, and it is therefore split into the preparation of different

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720–3746

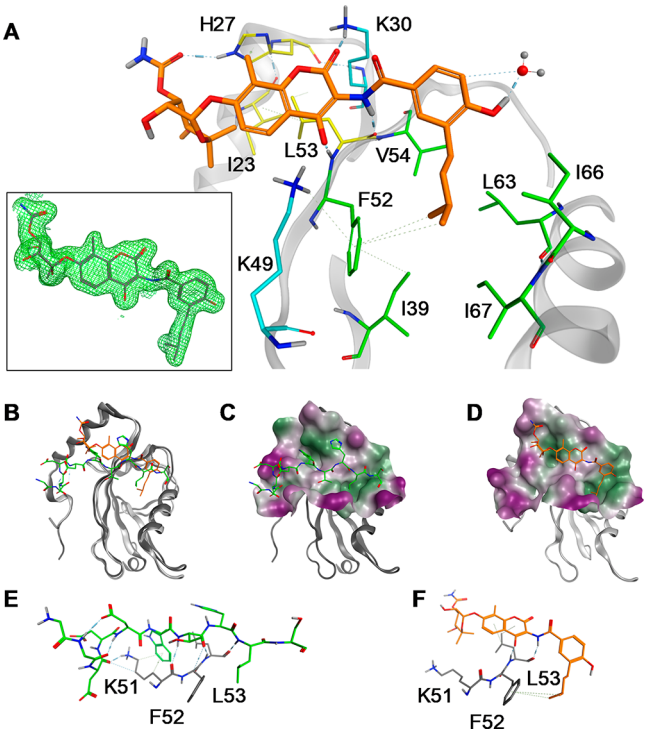


Figure 4. Structural analysis of **2** in complex with LC3A. (A) Directed interactions of **2** to LC3A. (B) Superposition of the LC3A in complex with 2 and with the p62 LIR motif. (C, D) The p62 LIR motif occupies two hydrophobic binding pockets, while only the leucine binding pocket is occupied by **2**. (E, F) **2** mimics the β-strand-like backbone interactions of p62-LIR peptide.

coumarin cores and various benzoic acids. The synthesis of the two 7-hydroxy coumarin cores **18a,b** could be accomplished in an adapted procedure published by Gammon et al.[18] (Scheme 2). In the first step, **16a,b** was chemoselectively benzylated at the 4-OH group with potassium carbonate as the base and potassium iodide as the catalyst to get **17a,b**. The cyclization was performed through a Claisen condensation between **17a,b** and diethyl carbonate with the use of NaH as the base. The following nitration was accomplished in a similar way as published by Manjappa et al. with the use of sodium nitrite and nitric acid in acetic acid.[19] The reduction and debenzylation of **19a,b** over palladium over charcoal and in hydrogen atmosphere provided **15a,b** in a quantitative yield.

For the 7-NO_2 and 7-Br coumarin core **15c,d**, the synthesis procedure according to Stecher et al. was adapted.[20] Therefore, the 2-hydroxy group of 7-nitro and 7-bromo salicylic acid (**20a,b**) was protected by acetylation using acetic anhydride, triethylamine as the base and DMAP as the catalyst. The resulting intermediates **21a,b** were converted in the corresponding acyl chloride by refluxing with thionyl chloride. The acyl chloride was coupled under decarboxylative conditions with the malonate derivative **22b**, which was previously synthesized by *N*-Boc protection and selective monoester hydrolysis of diethyl 2-aminomalonate **22a**. The cyclization of the resulting crude product was accomplished by an intramolecular transesterification under alkaline conditions and afforded **23a,b** in adequate yields. The cleavage of the *N*-Boc group with hydrogen chloride in dioxane leads to the 3-amino-4-hydroxy-coumarins **15c,d** as the hydrochloride-dioxane adducts.

The different isosteric benzoic acids were accessible through different routes (Scheme 3). Alkoxybenzoic acids **24a–j** could be synthesized through classical alkylation of **25**, followed by alkaline ester hydrolysis of the methyl 3-alkoxybenzoates **26a–j**. The 3-thioalkyl benzoic acids **28a–g** could be synthesized through a procedure published by Migliore et al. by reacting 3-thiobenzoic acid (**27**) with the corresponding alkyl halides in EtOH and potassium hydroxide.[21] The 3-alkylsulfonamido-benzoic acids **32a–g** were synthesized by an one-pot reaction of methyl 3-aminobenzoate **29** with alkylsulfonyl chloride, followed by ester hydrolysis and cleavage of disulfonamides. The starting point for the 3-alkylbenzoic acids **36a–i** was methyl 3-formylbenzoate (**33**), which was converted into the corresponding methyl 3-alkenylbenzoates (**34a–i**) by Wittig reaction with alkyltriphenyl bromides and LiHMDS as the base. These alkenes could be converted into methyl 3-alkylbenzoates **35a–i** by reduction with hydrogen over palladium on charcoal. The following ester hydrolysis was performed as before and yielded the 3-alkylbenzoic acids **36a–i**.

The syntheses of 2-isopentylbenzoic acid (**40**) and 4-fluoro-3-isopentylbenzoic acid (**44**) started with protection of the corresponding formylbenzoic acids **37** and **41** by benzylation of the carboxyl group, followed by Wittig reaction with isobutyl triphenyl phosphonium bromide under the same conditions as previously described (Scheme 4). The hydrogenation over palladium on charcoal resulted in simultaneous reduction of the double bond and cleavage of the benzyl group. Starting point for the benzoic acids **51** and **53** was methyl 4-hydroxybenzoate **45** which was *ortho*-formylated to **46** using paraformaldehyde in the presence of triethylamine and magnesium dichloride. Then the 4-hydroxy group was benzylated, and the protected methyl 4-(benzyloxy)-3-formylbenzoate **47** was converted to methyl 4-(benzyloxy)-3-(3-methylbut-1-en-1-yl)benzoate **48** by Wittig reaction as described before. The following hydrogenation over palladium on charcoal lead to methyl 4-hydroxy-3-isopentylbenzoate (**49**). While alkaline ester hydrolysis followed by acetylation with acetic anhydride and pyridine yielded **51**, methylation with dimethyl sulfate, followed by ester hydrolysis leads to 4-methoxy-3-isopentylbenzoic acid **53**.

The amide coupling between the amino coumarin cores **15a–d** and the benzoic acids **24a–j**, **28a–g**, **32a–g**, **36b–i**, **40**, **44**, **51**, and **53** or the commercially available *N*-Boc amino acids **56a–g** was accomplished under standard conditions by using HOBt and EDC × HCl (Scheme 5).

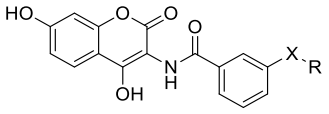
The reduction of **54** over palladium on charcoal in a hydrogen atmosphere delivered **57** in quantitative yield. The sulfonamido derivatives **14i–o** were synthesized through the reaction of **57** and the corresponding aryl- and alky sulfonyl chlorides, while the reaction between **57** and the respective acid anhydrides led to the *N*-acyl derivatives **14p–r** (Scheme 6). The aryl and pyrazole derivatives **14a–h** could be synthesized in moderate yields through a Suzuki coupling between **55** and the corresponding boronic acids or pinacol boronic esters.

CONCLUSION

This study describes the discovery of the first nonpeptidic druglike inhibitor of the LC3A and LC3B adapter proteins demonstrating druggability with druglike small molecule inhibitors. Furthermore, selectivity toward other members of the Atg8 family was achieved. We provide first insights into the

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720–3746

Table 2. SAR Exploration of the Phenyl Substituent in the 3-Position of the Benzamide Group[a]



	X = -CH_2-		X = -S-		X = -NH-SO_2-		X = -O-	
R	LC3A	LC3B	LC3A	LC3B	LC3A	LC3B	LC3A	LC3B
	**6a**		**7a**		**8a**			
	13	16	52	66	53	59		
					**8b**		**9b**	
					130	108	i.a.	i.a.
	**6c**		**7c**		**8c**		**9c**	
	20	23	42	48	43	43	129	i.a.
	**6d**		**7d**		**8d**		**9d**	
	12	14	32	40	144	90	143	108
	**6e**		**7e**		**8e**		**9e**	
	12	14	18	23	26	25	69	71
	**6f**		**7f**				**9f**	
	13	17	13	17			137	i.a.
	**6g**		**7g**		**8g**		**9g**	
	20	25	47	63	39	43	103	108
							**9h**	
							20	18
	**6i**						**9i**	
	75	67					44	53
							**9j**	
							44	26

[a] K_i values in μM are calculated from HTRF displacement. i.a.: inactive.

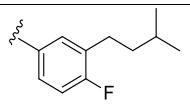
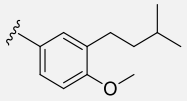
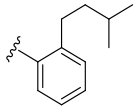
interaction of compound **2** with LC3A, thereby paving the way to structure-based design of more potent inhibitors of this key protein−protein interaction of the autophagy pathway. We further explored the structure−activity relationships of the benzamide part which mimics leucine of the LIR motif and points into the hydrophobic pocket HP2. Although the interaction with HP2 can be considered as essential, the SAR investigated in this study seems rather flat and diverse substituents were identified which were able to occupy this lipophilic area and displace the p62 LIR from LC3A and LC3B. Previous studies suggest that interactions with HP1 are more important; however, novobiocin derivatives insufficiently occupy this interaction area. Therefore, future efforts should aim at occupying HP1 either by extending a different position of the coumarin core or by scaffold hopping to a central moiety which allows for efficient extension of hydrophobic interactions in HP1. Our synthetic efforts to extend the SAR to the hydrophobic pocket HP1 led to the identification of substitution patterns which were tolerated by LC3A and LC3B, which, however, were not sufficient to get a substantial increase in affinity. Still, the insights in the SAR provide a basis for further development of LC3 inhibitors, by targeting conserved surfaces that are involved in the binding of all typical LIR motifs, including p62, and have potential applications in context of AUTACs.

EXPERIMENTAL SECTION

General. All starting materials, solvents, and reagents were purchased from commercial suppliers, (e.g., Sigma-Aldrich, Apollo Scientific, Acros Organics) and used without further purification. NMR spectra were recorded on a Bruker AMX 250, Bruker AV 300, Bruker AV 400, or Bruker AV500 spectrometer (Bruker Corporation, Billerica, MA, USA). Chemical shifts (δ) are reported in ppm relative to tetramethylsilane (TMS) as reference. Multiplicity is reported as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m,

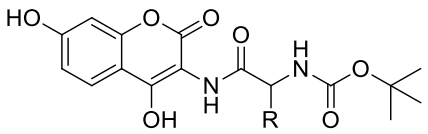
https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

Table 3. Further Variations of the Benzamide Moiety[a]

		LC3A		LC3B	
		IC_{50} [μM]	K_i^{calc} [μM]	IC_{50} [μM]	K_i^{calc} [μM]
10		106.5 ± 4.2	21.8	172.0 ± 7.4	19.3
11		64.4 ± 1.6	13.2	143.0 ± 5.7	16.0
12		215.0 ± 5.9	44.0	347.6 ± 25.6	39.0

[a]IC_{50} ± SD value is determined by the HRTF displacement assay, and K_i is calculated.

Table 4. Replacement of the Benzamide Moiety by Boc-Substituted Amino Acids[a]



	R	LC3A		LC3B	
		IC_{50} [μM]	K_i^{calc} [μM]	IC_{50} [μM]	K_i^{calc} [μM]
13a		i.a.	-	i.a.	-
13b		921.9 ± 26.0	188.8	i.a.	-
13c		i.a.	-	i.a.	-
13d		i.a.	-	i.a.	-
13e		410.6 ± 11.3	84.1	700.5 ± 79.9	78.6
13f		i.a.	-	i.a.	-
13g		667.7 ± 62.7	136.8	>1000	-

[a]IC_{50} ± SD value is determined by the HRTF displacement assay, and K_i is calculated. i.a.: inactive.

multiplet. Approximate coupling constants (J) are given in hertz (Hz). Mass spectra were obtained on a VG Platform II system (Thermo Fischer Scientific, Inc., Waltham, MA, USA) using electrospray ionization (ESI). High-resolution mass spectra were recorded on a MALDI LTQ ORBITRAP XL instrument (Thermo Fisher Scientific). Compounds were purified by preparative HPLC using a Shimadzu Preparative Prominence Modular HPLC (Shimadzu, Kyoto, Japan) with the following conditions: column, Luna (10μ C18(2) 100 Å; 250 × 21.2 mm; Phenomenex, Torrance, CA, USA); mobile phase, different gradients of acetonitrile/H_2O + 0.1% formic acid at a flow rate of 21 mL/min and UV-detection at 254 and 280 nm. Compound purity was analyzed on a LC2020 system (Shimadzu, Kyoto, Japan) using UV detection at 254 and 280 nm equipped with a Luna column (10μ C18(2) 100 Å; 250 × 21.2 mm; Phenomenex, Torrance, CA, USA) using different gradients (solvent A, acetonitrile; solvent B, H_2O + 0.1% formic acid) at a flow rate of 1 mL/min and was if not otherwise considered >95%. Method A: solvent mixture A/B 5:95 isocratic for 2 min to 90:10 after additional 12 min and holding at 90:10 for additional 6 min, back to 5:95 in 1 min and holding for additional 4 min. Method B: solvent mixture A/B 50:50 isocratic for 2 min to 90:10 after additional 12 min and holding at 90:10 for additional 6 min, back to 50:50 in 1 min and holding for additional 4 min. Method C: solvent A/B 60:40 to 95:5 in 7 min, holding for additional 8 min at 95:5 and back to 60:40 in 3 min and holding for additional 2 min. Thin layer chromatography for reaction control was performed on silica gel 60 F_{254} aluminum sheets (Merck, Germany). Preparative column chromatography was performed on silica gel 63–200 μM (Merck, Germany).

*(3R,4S,5R,6R)-5-Hydroxy-6-((4-hydroxy-3-(4-hydroxy-3-isopentylbenzamido)-8-methyl-2-oxo-2H-chromen-7-yl)oxy)-3-methoxy-2,2-dimethyltetrahydro-2H-pyran-4-yl Carbamate (**2**).* Novobiocin sodium salt (**1**) (1.0 g, 1.6 mmol) of was dissolved in MeOH (40 mL), and palladium on charcoal (w = 10%, 100 mg) was added. The suspension was stirred in an autoclave for 4 h at 50 °C under hydrogen atmosphere at 4 bar. The suspension was filtered through a Celite pad, which was rinsed with MeOH (40 mL). The solvent was removed in vacuo, and product (1.0 g, 1.6 mmol, 99%) was obtained as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.73 (s, 1H), 8.47 (s, 1H), 7.72 (s, 1H), 7.64–7.60 (m, 2H), 6.91 (d, J = 8.9 Hz, 1H), 6.77 (d, J = 8.3 Hz, 1H), 6.66 (bs, 2H), 5.52 (d, J = 4.9 Hz, 1H), 5.44 (d, J = 2.0 Hz, 1H), 5.16 (dd, J = 3.0/9.8 Hz, 1H), 4.05 (m, 1H), 3.48 (s, 4H), 2.54 (m, 2H), 2.17 (s, 3H), 1.58–1.41 (m, 3H), 1.28 (s, 3H), 1.10 (s, 3H), 0.92 (d, J = 6.5 Hz, 6H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 170.2, 165.6, 162.9, 157.2, 156.3, 155.2, 152.0, 129.3, 127.7, 126.4, 122.9, 122.0, 118.1, 114.0, 111.5, 107.5, 98.5, 96.6, 80.9, 77.7, 70.4, 68.9, 60.9, 28.4, 27.4, 22.8, 22.5, 8.5 ppm; MS (ESI+) m/z: 615.30 $[M + H]^+$; HRMS (MALDI+) m/z: calcd for $[C_{31}H_{38}N_2O_{11} + H]^+$: 615.25484, found: 615.25398, Δ = 1.4 ppm; HPLC: t_R (Method A) = 16.43 min.

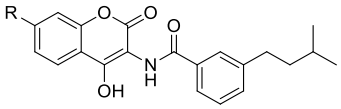
*N-(4,7-Dihydroxy-8-methyl-2-oxo-2H-chromen-3-yl)-4-hydroxy-3-isopentylbenzamide (**3**).* **2** (0.30 g, 0.49 mmol, 1.0 equiv) was dissolved in EtOH (5 mL) and heated up to 80 °C. Acetyl chloride (0.10, 0.98 mmol, 2.0 equiv) was added, and the reaction mixture was stirred for additional 3 h at 80 °C. The mixture was poured on ice water, and the formed precipitate was separated through filtration to obtain the product (0.15 mg, 0.38 mmol, 78%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.78 (bs, 1H), 10.33 (s, 1H), 9.88 (s, 1H), 9.10 (s, 1H), 7.75 (m, 1H), 7.63 (m, 1H), 7.51 (m, 1H), 6.83 (m, 2H), 2.65 (m, 2H), 2.10 (s, 3H), 1.51–1.39 (m, 3H,), 0.85 (s, 6H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 166.6, 160.9, 159.7, 159.0, 158.4, 151.3, 130.0, 128.3, 127.4, 124.9, 121.5, 114.3, 111.8, 108.1, 100.5, 100.4, 27.5, 27.4, 22.5, 8.1 ppm; MS (ESI−) m/z: 396.80 $[M - H]^+$; HRMS (MALDI+) m/z: calcd for $[C_{22}H_{23}NO_6 + H]^+$: 398.15981, found: 398.15960, Δ = 0.5 ppm; HPLC t_R (Method A) = 16.23 min.

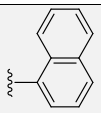
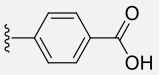
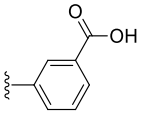
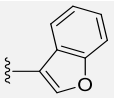
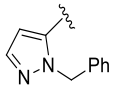
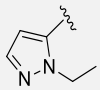
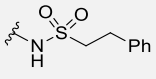
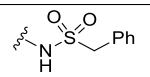
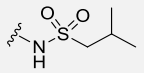
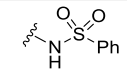
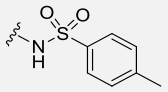
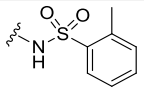
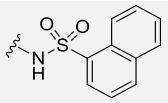
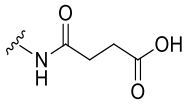
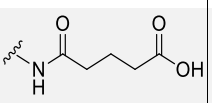
General Procedure for Amide Coupling (Procedure A). The respective benzoic acid or Boc-protected amino acid (0.9–1.1 equiv) was dissolved in dry DMF, and HOBt (0.9–1.1 equiv) and EDC × HCl (0.9–1.1 equiv) were added. After the solution clarified, the corresponding 3-aminocoumarin (1.0 equiv) was added together with triethylamine (2.5 equiv) and the mixture was stirred at room temperature overnight. The solution was treated with 1 M aqueous NaOH (2.0 equiv) and stirred for additional 5 h to cleave formed esters. The reaction mixtures were acidified with aqueous citric acid (w = 10%), and the aqueous phase was extracted with EtOAc (3×). The combined organic phases were washed with brine, dried over $MgSO_4$, and filtered, and the solvent was removed in vacuo. Further purification of the products was realized by preparative HPLC.

*N-(4,7-Dihydroxy-8-methyl-2-oxo-2H-chromen-3-yl)-3-isopentylbenzamide (**4**).* According to general procedure A: 311 mg (1.50 mmol) of **15b**, 317 mg (1.65 mmol) of **36a**, 260 mg (1.65 mmol) of

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720–3746

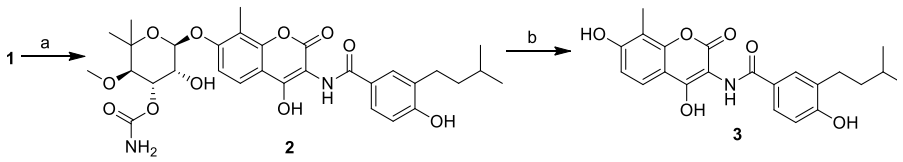
Table 5. Exploration of the Hydrophobic Pocket HP1 by Variation of the 7-Position of the Coumarin Core[a]



	R	LC3A IC$_{50}$ [μM]	LC3A K$_i^{calc}$ [μM]	LC3B IC$_{50}$ [μM]	LC3B K$_i^{calc}$ [μM]
14a		42.8 ± 1.6	8.8	225.3 ± 31.2	25.3
14b		514.5 ± 31.2	105.4	i.a.	
14c		92.1 ± 3.9	18.9	149.3 ± 4.2	16.7
14d		35.3 ± 0.6	7.2	131.7 ± 4.0	14.8
14e		i.a.		i.a.	
14f		813.1 ± 51.0	166.5	i.a.	
14g		190.1 ± 2.8	38.9	320.0 ± 2.5	35.9
14h		218.7 ± 2.3	44.8	346.3 ± 5.3	38.8
14i		36.7 ± 1.1	7.5	66.3 ± 2.9	7.4
14j		73.1 ± 6.1	15.0	119.9 ± 8.0	13.4
14k		110.3 ± 4.1	22.6	175.5 ± 10.6	19.7
14l		85.9 ± 5.0	17.6	123.0 ± 7.8	13.8
14m		59.9 ± 2.8	12.3	89.0 ± 2.5	10.0
14n		76.0 ± 3.2	15.6	97.6 ± 1.9	10.9
14o		31.2 ± 1.3	6.4	37.3 ± 1.4	4.2
14p		216.5 ± 8.0	44.3	382.3 ± 4.4	42.9
14q		228.8 ± 7.4	46.9	386.0 ± 7.1	43.3
14r		120.0 ± 2.6	24.6	223.6 ± 13.1	25.1

[a]IC$_{50}$ ± SD value is determined by the HRTF displacement assay, and K_i is calculated. i.a.: inactive.

Scheme 1. Direct Derivatization of 1[a]



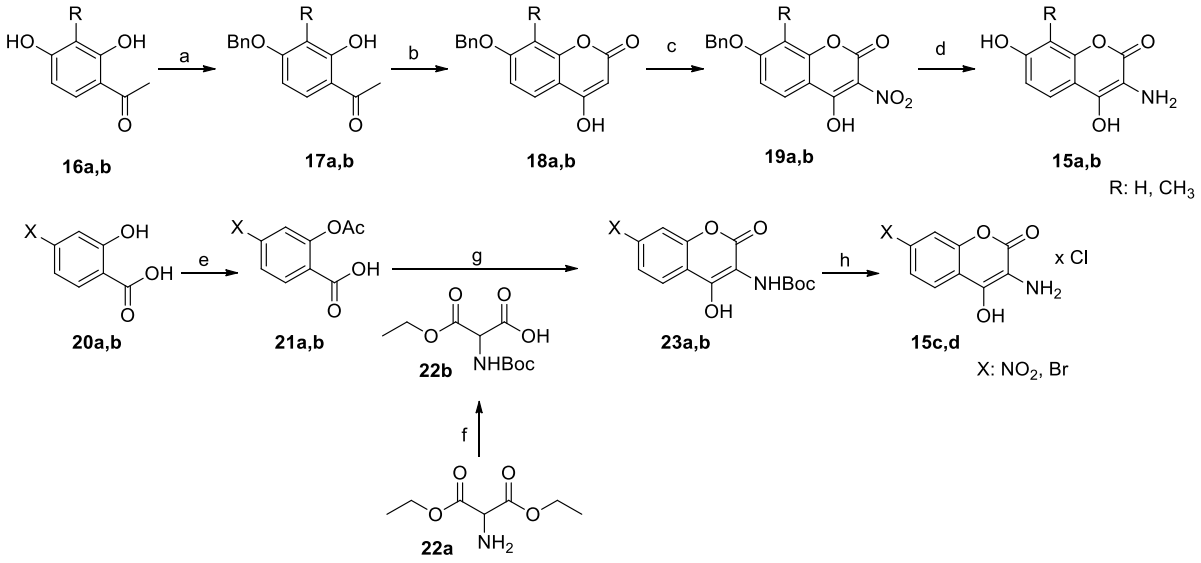
[a](a) H_2, Pd/C, MeOH, 50 °C, 4 h; (b) AcCl, EtOH, reflux, 2 h.

HOBt, 316 mg (1.65 mmol) of EDC × HCl, 15 mL of DMF and 0.4 mL of triethylamine. The desired product (185 mg, 0.49 mmol, 32%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.69 (bs, 1H), 10.43 (s, 1H), 9.38 (s, 1H), 7.85 (s, 1H), 7.82−7.80 (m, 1H), 7.58 (d, J = 8.7 Hz, 1H), 7.41 (d, J = 5.1 Hz, 2H), 6.89 (d, J = 8.7 Hz, 1H), 2.66 (t, J = 7.7 Hz, 2H), 2.18 (s, 3H), 1.64−1.48 (m, 3H), 0.93 (d, J = 6.2 Hz, 6H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6) δ = 166.7, 160.9, 160.4, 159.1, 151.5, 142.5, 133.9, 131.5, 128.1, 127.8, 125.4, 121.6, 111.8, 110.4, 108.0, 99.9, 40.3, 33.0, 27.1, 22.4 (2 × C), 8.1 ppm; MS (ESI−) m/z: 379.95 [M − H]$^-$; HRMS (MALDI+) m/z: calcd for [$C_{22}H_{23}NO_5$ + H]$^+$: 382.16490, found: 382.16459, Δ = 0.8 ppm; HPLC: t_R (Method A) = 18.61 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-4-hydroxy-3-isopentylbenzamide (**5**).* According to general procedure A: 483 mg (2.5 mmol) of **15a**, 751 mg (3.0 mmol) of **51**, 474 mg (3.0 mmol) of HOBt, 575 mg (3.0 mmol) of EDC × HCl, 15 mL of DMF, and 0.9 mL of triethylamine. The desired product (84 mg, 0.22 mmol, 9%) was obtained as a colorless solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 11.91 (bs, 1H), 10.54 (s, 1H), 9.96 (s, 1H), 9.18 (s, 1H), 7.77 (d, J = 2.0 Hz, 1H), 7.72−7.67 (m, 2H), 6.85 (d, J = 8.4 Hz, 1H), 6.82 (dd, J

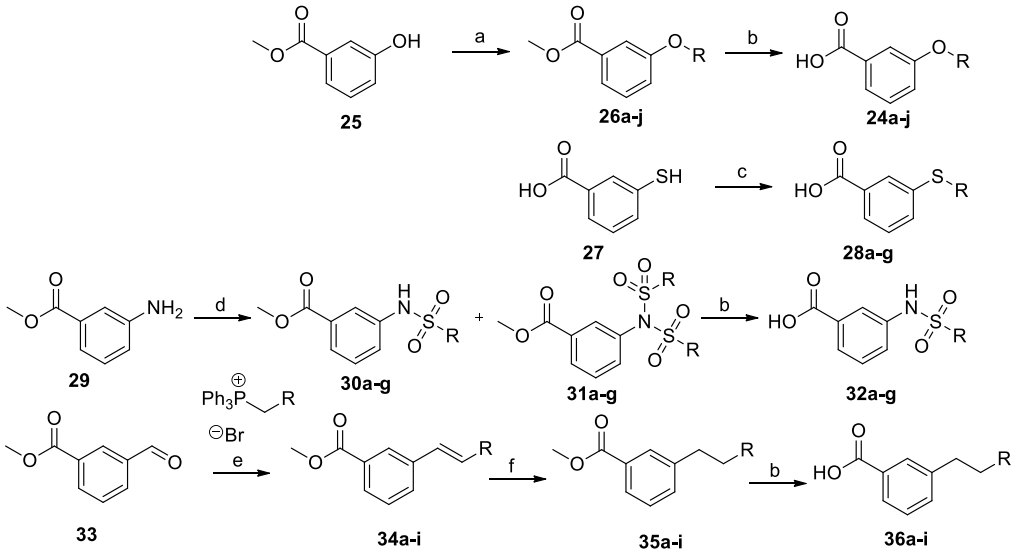
https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

Scheme 2. Preparation of the 7-Substituted Coumarin Cores[a]



[a](a) BnBr, K_2CO_3, KI, acetone, reflux, 5 h; (b) NaH, $EtO_2(CO)$, toluene, 140 °C, 16 h; (c) $NaNO_2$, HNO_3 (w = 65%), AcOH, 60 °C, 15 min; (d) H_2, Pd/C, MeOH, RT, 16 h; (e) Ac_2O, NEt_3, DMAP, EtOAc, 80 °C, 16 h; (f) (i) Boc_2O, $NaHCO_3$, DMAP, H_2O/dioxane, RT, 5 h; (ii) KOH, EtOH, RT, 3 h; (g) (i) $SOCl_2$, DCM, reflux, 5 h; (ii) 2-((*tert*-butoxycarbonyl)amino)-3-ethoxy-3-oxopropanoic acid, NEt_3, $MgCl_2$, THF, 0 °C → RT, 16 h; (iii) $NaOH_{aq}$, MeOH, RT, 3 h; (h) 4M HCl in dioxane, MeOH, 0 °C → RT, 5 h.

Scheme 3. Preparation of the Benzoic Acid Derivatives[a]



[a](a) $(MeO)_2SO_2$ or R−X (X = Cl, Br, I), K_2CO_3, KI, acetone or ACN, 6 h, reflux; (b) KOH, THF/MeOH/H_2O (2:1:1), 6 h, reflux; (c) (a) R−X (X = Cl, Br, I), NaOH, EtOH, 4 h, reflux; (d) R-SO_2Cl, NEt_3, DCM, 3 h, 0 °C; (e) alkyl triphenyl phosphonium bromide, LiHMDS, THF, 0 °C → RT, 3 h; (f) H_2, Pd/C (w = 10%), MeOH, RT, 5 h.

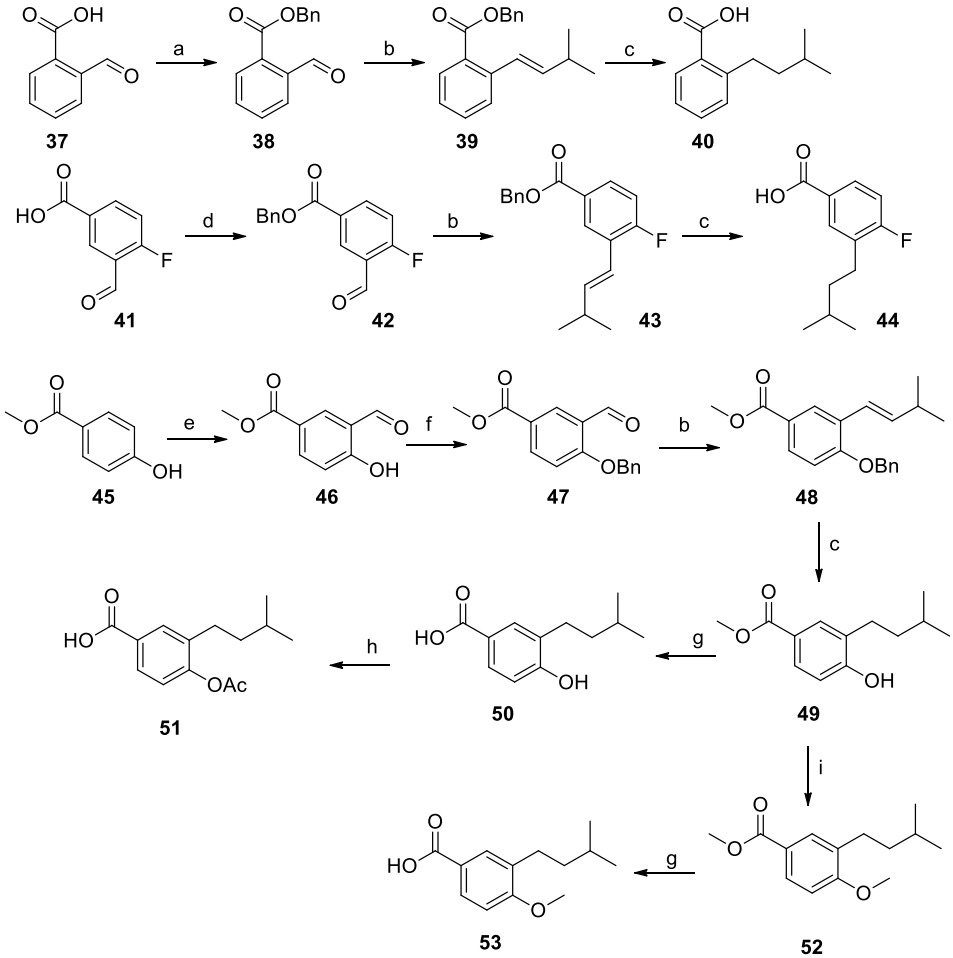
= 2.3/8.7 Hz, 1H), 6.72 (d, J = 2.2 Hz, 1H), 2.59−2.55 (m, 2H), 1.57 (non, J = 6.5 Hz, 1H), 1.48−1.43 (m, 2H), 0.93 (d, J = 6.5 Hz, 6H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6) δ = 166.6, 161.3, 160.9, 159.8, 158.4, 153.4, 130.0, 128.4, 127.4, 125.0, 124.2, 114.3, 113.0, 108.2, 101.9, 100.7, 38.7, 27.5, 27.4, 22.5 (2 × C) ppm; MS (ESI−) m/z: 382.00 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{21}H_{21}NO_6+Na]^+$: 406.12611, found: 406.12603, Δ = 0.2 ppm; HPLC: t_R (Method A) = 15.92 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-isopentylbenzamide (**6a**).* According to general procedure A: 236 mg (1.22 mmol) of **15a**, 235 mg (1.22 mmol) of **36a**, 212 mg (1.34 mmol) of HOBt, 258 mg (1.34 mmol) of EDC × HCl, 15 mL of DMF, and 0.3 mL of triethylamine. The desired product (96 mg, 0.26 mmol, 21%) was obtained as a pale-yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.80 (bs, 1H), 10.55 (s, 1H), 9.37 (s, 1H), 7.86−7.77 (m, 2H), 7.72 (d, J = 8.7 Hz, 1H), 7.43−7.38 (m, 2H), 6.83 (dd, J = 2.2/8.7 Hz, 1H), 6.73 (d, J = 2.2 Hz, 1H), 2.66 (t, J = 7.8 Hz, 2H), 1.64−1.47 (m, 3H), 0.93 (d, J = 6.3 Hz, 6H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 166.7, 161.4, 160.8, 160.2, 153.5, 142.5, 133.9, 131.5, 128.1, 127.8, 125.4, 125.0, 113.0, 108.0, 101.9, 100.2, 40.3, 32.9, 27.1, 22.4 (2 × C) ppm; MS (ESI−) m/z: 366.30 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{21}H_{21}NO_5 + H]^+$: 368.14925, found: 368.14926, Δ = 0.03 ppm; HPLC: t_R (Method A) = 17.78 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-propylbenzamide (**6c**).* According to general procedure A: 209 mg (1.08 mmol) of **15a**, 178 mg (1.08 mmol) of **36c**, 188 mg (1.19 mmol) of HOBt, 229 mg

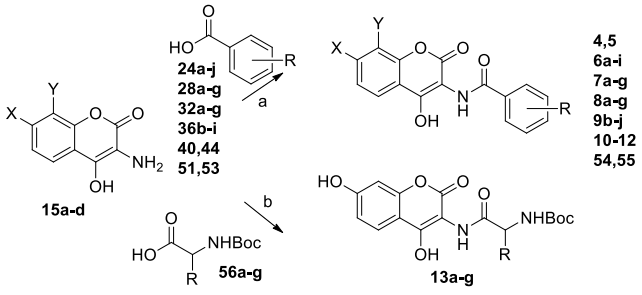
https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

Scheme 4. Synthesis of Benzoic Acids[a]



[a](a) BnBr, K_2CO_3, acetone, reflux, 1 h; (b) isobutyl triphenyl phosphonium bromide, LiHMDS, THF, 0 °C → RT, 3 h; (c) H_2, Pd/C (w = 10%), MeOH, RT, 5 h; (d) (a) BnBr (1.2 equiv), $NaHCO_3$, DMF, RT, 4 h; (e) paraformaldehyde, $MgCl_2$, $(Et)_3N$, DCE, 70°C, 12 h; (f) benzyl bromide, K_2CO_3, DMF, 5.5 h, 85 °C; (g) KOH, THF/MeOH/H_2O (2:1:1), 6 h, reflux; (h) Ac_2O, pyridine, DCM, 0 °C → RT, 12 h; (i) $(MeO)_2SO_2$, K_2CO_3, acetone, 80 °C, 2.5 h.

Scheme 5. Amide Coupling Procedure[a]



[a](a) HOBt, EDC × HCl, NEt_3, DMF, 16 h, RT; (b) HOBt, EDC × HCl, DMF, 16 h, RT.

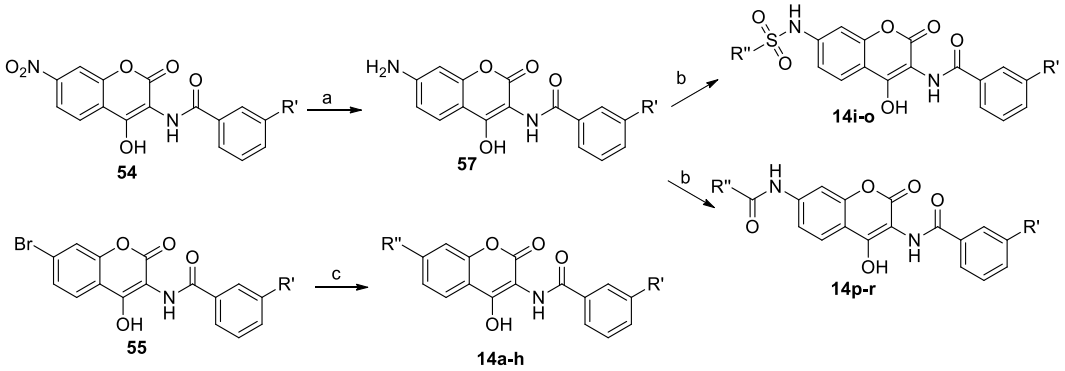
(1.19 mmol) of EDC × HCl, 15 mL of DMF, and 3 mL of triethylamine. The desired product (45 mg, 0.13 mmol, 12%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.80 (1H, bs), 10.54 (s, 1H), 9.37 (s, 1H), 7.86−7.77 (m, 2H), 7.72 (d, J = 8.6 Hz, 1H), 7.45−7.38 (m, 2H), 6.83 (dd, J = 2.3/8.6 Hz, 1H), 6.73 (d, J = 2.3 Hz, 1H), 2.64 (t, J = 7.5 Hz, 2H), 1.64 (m, 2H), 0.92 (t, J = 7.26, 3H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 166.7, 161.4, 160.8, 160.3, 153.5, 142.1, 133.9, 131.6, 128.0, 127.9, 125.5, 125.0, 112.9, 108.0, 101.9, 100.2, 37.1, 24.0, 13.6 ppm; MS (ESI−) m/z: 338.25 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{19}H_{17}NO_5 + H]^+$: 340.11795, found: 340.11811, Δ = 0.5 ppm; HPLC: t_R (Method A) = 16.33 min.

*3-Butyl-N-(4,7-dihydroxy-2-oxo-2H-chromen-3-yl)benzamide (**6d**).* According to general procedure A: 365 mg (1.89 mmol) of **15a**, 337 mg (1.89 mmol) of **36d**, 328 mg (2.08 mmol) of HOBt, 399 mg (2.08 mmol) of EDC × HCl, 16 mL DMF, and 3 mL of triethylamine. The desired product (75 mg, 0.21 mmol, 11%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.82 (bs, 1H), 10.55 (s, 1H), 9.37 (s, 1H), 7.85−7.79 (m, 2H), 7.72 (d, J = 8.7 Hz, 1H), 7.42−7.39 (m, 2H), 6.83 (dd, J = 2.2/8.7 Hz, 1H), 6.73 (d, J = 2.2 Hz, 1H), 2.66 (t, J = 7.6 Hz, 2H), 1.66−1.56 (m, 2H), 1.41−1.27 (m, 2H), 0.92 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 166.7, 161.4, 160.8, 160.2, 153.5, 142.3, 133.9, 131.5, 128.1, 127.9, 125.4, 125.1, 113.0, 108.0, 101.9, 100.2, 34.7, 33.1, 21.7, 13.7 ppm; MS (ESI−) m/z: 352.25 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{20}H_{19}NO_5 + H]^+$: 354.13360, found: 354.13379, Δ = 0.5 ppm; HPLC: t_R (Method A) = 17.19 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-pentylbenzamide (**6e**).* According to general procedure A: 297 mg (1.54 mmol) of **15a**, 296 mg (1.54 mmol) of **36e**, 267 mg (1.69 mmol) of HOBt, 325 mg (1.69 mmol) of EDC × HCl, 15 mL of DMF and 3 mL of triethylamine. The desired product (85 mg, 0.23 mmol, 15%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.79 (bs, 1H), 10.55 (s, 1H, *OH*), 9.37 (s, 1H), 7.85−7.78 (m, 2H), 7.72 (d, J = 8.7 Hz, 1H), 7.44−7.38 (m, 2H), 6.83 (dd, J = 2.3/8.7

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

Scheme 6. Introduction of Substituents in the 7-Position of the Coumarin Core[a]



[a](a) H_2, Pd/C, EtOH, RT, 12 h; (b) R″-SO_2Cl or R″-C(═O)OC(═O)-R″, pyridine, DCM, 0 °C → RT, 16 h; (c) R″-$B(OR)_2$, $NaHCO_3$, $Pd(PPh_3)_4$, 80 °C, 24 h.

Hz, 1H), 6.73 (d, *J* = 2.3 Hz, 1H), 2.65 (t, *J* = 7.6 Hz, 2H), 1.66−1.58 (m, 2H), 1.37−1.24 (m, 4H), 0.88 (t, *J* = 7.0 Hz, 3H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 166.7, 161.4, 160.8, 160.2, 153.5, 142.4, 133.9, 131.5, 128.1, 127.9, 125.4, 125.0, 113.0, 108.0, 101.9, 100.2, 35.0, 30.9, 30.6, 21.9, 13.9 ppm; MS (ESI−) *m*/*z*: 366.30 [M − H]$^-$; HRMS (MALDI+) *m*/*z*: calcd for $[C_{21}H_{21}NO_5 + H]^+$: 368.14925, found: 368.14942, Δ = 0.5 ppm; HPLC: t_R (Method A) = 17.98 min.

*3-(2-Cyclopropylethyl)-N-(4,7-dihydroxy-2-oxo-2H-chromen-3-yl)benzamide (**6f**).* According to general procedure A: 349 mg (1.81 mmol) of **15a**, 378 mg (1.99 mmol) of **36f**, 314 mg (1.99 mmol) of HOBt, 381 mg (1.99 mmol) of EDC × HCl, 15 mL of DMF, and 3 mL of triethylamine. The desired product (130 mg, 0.36 mmol, 20%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.80 (bs, 1H), 10.55 (s, 1H), 9.36 (s, 1H), 7.86−7.79 (m, 2H), 7.72 (d, *J* = 8.7 Hz, 1H), 7.44−7.38 (m, 2H), 6.83 (dd, *J* = 2.3/8.7 Hz, 1H), 6.73 (d, *J* = 2.3 Hz, 1H), 2.78−2.72 (m, 2H), 1.57−1.49 (m, 2H), 0.74−0.65 (m, 1H), 0.44−0.38 (m, 2H), 0.09−0.06 (m, 2H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 166.7, 161.4, 160.8, 160.2, 153.5, 142.2, 133.9, 131.6, 128.0, 127.9, 125.4, 125.0, 113.0, 108.0, 101.9, 100.2, 39.5, 36.1, 35.3, 10.6, 4.4 (2 × C) ppm; MS (ESI−) *m*/*z*: 364.25 [M − H]$^-$; HRMS (MALDI+) *m*/*z*: calcd for $[C_{21}H_{19}NO_5 + H]^+$: 366.13360, found: 366.13370, Δ = 0.3 ppm; HPLC: t_R (Method A) = 17.01 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-isobutylbenzamide (**6g**).* According to general procedure A: 107 mg (0.56 mmol) of **15a**, 109 mg (0.61 mmol) of **36g**, 97 mg (0.61 mmol) of HOBt, 117 mg (0.61 mmol) of EDC × HCl, 8 mL of DMF, and 3 mL of triethylamine. The desired product (46 mg, 0.13 mmol, 23%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.82 (bs, 1H), 10.54 (s, 1H), 9.37 (s, 1H), 7.85−7.79 (m, 2H), 7.72 (d, *J* = 8.7 Hz, 1H), 7.44−7.35 (m, 2H), 6.83 (dd, *J* = 2.3/8.7 Hz, 1H), 6.73 (d, *J* = 2.3 Hz, 1H), 2.53 (d, *J* = 7.1 Hz, 2H), 1.91 (sept., *J* = 6.7 Hz, 1H), 0.89 (d, *J* = 6.7 Hz, 6H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 166.7, 161.4, 160.8, 160.2, 153.5, 141.2, 133.8, 132.2, 128.5, 127.9, 125.5, 125.0, 113.0, 108.0, 101.9, 100.2, 44.5, 29.6, 22.1 (2 × C) ppm; MS (ESI−) *m*/*z*: 352.30 [M − H]$^-$; HRMS (MALDI +) *m*/*z*: calcd for $[C_{20}H_{19}NO_5 + H]^+$: 354.13360, found: 354.13358, Δ = 0.1 ppm; HPLC: t_R (Method A) = 16.98 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-phenethylbenzamide (**6i**).* According to general procedure A: 138 mg (0.72 mmol) of **15a**, 162 mg (0.72 mmol) of **36i**, 124 mg (0.79 mmol) of HOBt, 151 mg (0.79 mmol) of EDC × HCl, 10 mL of DMF, and 3 mL of triethylamine. The desired product (40 mg, 0.10 mmol, 14%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.82 (bs, 1H), 10.55 (s, 1H), 9.38 (s,1H), 7.93 (s, 1H), 7.84−7.81 (m, 1H), 7.72 (d, *J* = 8.7 Hz, 1H), 7.42−7.40 (m, 2H), 7.29−7.25 (m, 4H), 7.21−7.18 (m, 1H), 6.83 (dd, *J* = 2.3/8.7 Hz, 1H), 6.73 (d, *J* = 2.3 Hz, 1H), 2.99−2.90 (m, 4H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 166.6, 161.4, 160.8, 153.5, 141.5, 133.9, 131.6, 128.4 (2 × C), 128.3 (2 × C), 128.1, 128.0, 125.9, 125.6, 125.0, 112.9, 108.0, 101.9, 100.1, 37.0, 37.0 ppm; MS (ESI−) *m*/*z*: 399.90 [M − H]$^-$; HRMS (MALDI+) *m*/*z*: calcd for $[C_{24}H_{19}NO_5 + H]^+$: 402.13360, found: 402.13363, Δ = 0.01 ppm; HPLC: t_R (Method A) = 16.94 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-(isobutylthio)-benzamide (**7a**).* According to general procedure A: 384 mg (1.99 mmol) of **15a**, 376 mg (1.79 mmol) of **28a**, 343 mg (1.79 mmol) of HOBt, 274 mg (1.79 mmol) of EDC × HCl, and 10 mL of DMF. The desired product (112 mg, 0.29 mmol, 15%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.81 (bs, 1H), 10.55 (s, 1H), 9.45 (s, 1H), 7.93 (s, 1H), 7.76 (d, *J* = 7.8 Hz, 1H), 7.72 (d, *J* = 8.7, 1H), 7.52 (d, *J* = 8.1 Hz, 1H), 7.44 (t, *J* = 7.8 Hz, 1H), 6.83 (dd, *J* = 2.2/8.7 Hz, 1H), 6.73 (d, *J* = 2.2 Hz, 1H), 2.94 (d, *J* = 6.6 Hz, 2H), 1.83 (sept., *J* = 6.6 Hz, 1H), 1.01 (d, *J* = 6.6 Hz, 6H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 166.0, 161.4, 160.8, 160.6, 153.5, 137.1, 134.7, 130.7, 128.8, 127.3, 125.1, 125.0, 112.9, 108.0, 101.9, 99.8, 40.8, 27.7, 21.7 ppm; MS (ESI−) *m*/*z*: 383.85 [M − H]$^-$; HRMS (MALDI+) *m*/*z*: calcd for $[C_{20}H_{19}NO_5S + H]^+$: 386.10567, found:386.10580, Δ = 0.3 ppm; HPLC t_R (Method B) = 10.97 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-(ethylthio)-benzamide (**7c**).* According to general procedure A: 148 mg (0.77 mmol) of **15a**, 126 mg (0.69 mmol) of **28c**, 106 mg (0.69 mmol) of HOBt, 133 mg (0.69 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (82 mg, 0.23 mmol, 30%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.80 (bs, 1H), 10.55 (s, 1H), 9.46 (s, 1H), 7.93 (s, 1H), 7.77 (d, *J* = 7.7 Hz, 1H), 7.72 (d, *J* = 8.7 Hz, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.44 (t, *J* = 7.7 Hz, 1H), 6.83 (dd, *J* = 2.2/8.7 Hz, 1H), 6.73 (d, *J* = 2.2 Hz, 1H), 3.06 (q, *J* = 7.3 Hz, 2H), 1.27 (t, *J* = 7.3 Hz, 3H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 166.0, 161.5, 160.8, 160.5, 153.5, 136.5, 134.7, 130.7, 128.9, 127.3, 125.2, 125.0, 113.0, 108.0, 101.9, 99.9, 26.1, 14.2 ppm; MS (ESI−) *m*/*z*: 355.80 [M − H]$^-$; HRMS (MALDI+) *m*/*z*: calcd for $[C_{18}H_{15}NO_5S + H]^+$: 358.07366, found: 358.07437, Δ = 2.0 ppm; HPLC t_R (Method B) = 8.57 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-(propylthio)-benzamide (**7d**).* According to general procedure A: 179 mg (0.93 mmol) of **15a**, 164 mg (0.84 mmol) of **28d**, 128 mg (0.84 mmol) of HOBt, 160 mg (0.84 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (87 mg, 0.23 mmol, 25%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.81 (bs, 1H), 10.55 (s, 1H), 9.45 (s, 1H), 7.93 (s, 1H), 7.76 (d, *J* = 7.7 Hz, 1H), 7.72 (d, *J* = 8.7 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.45 (t, *J* = 7.7 Hz, 1H), 6.82 (d, *J* = 2.2/8.7 Hz, 1H), 6.73 (d, *J* = 2.2 Hz, 1H), 3.03 (t, *J* = 7.2 Hz, 2H), 1.62 (sext, *J* = 7.2 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 166.0, 161.4, 160.8, 160.6, 153.5, 136.7, 134.7, 130.8, 128.8, 127.3, 125.2, 125.1, 112.9, 108.0, 101.9, 99.8, 33.9, 21.9, 13.2 ppm; MS (ESI−) *m*/*z*: 369.85 [M − H]$^-$; HRMS (MALDI+) *m*/*z*: calcd for $[C_{19}H_{17}NO_5S + H]^+$: 372.09002, found: 372.09042, Δ = 1.1 ppm; HPLC t_R (Method B) = 9.87 min.

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

*3-(Butylthio)-N-(4,7-dihydroxy-2-oxo-2H-chromen-3-yl)-benzamide (**7e**).* According to general procedure A: 169 mg (0.88 mmol) of **15a**, 166 mg (0.79 mmol) of **28e**, 121 mg (0.79 mmol) of HOBt, 151 mg (0.79 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (94 mg, 0.24 mmol, 28%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.81 (bs, 1H), 10.55 (s, 1H), 9.45 (s, 1H), 7.93 (s, 1H), 7.76 (d, J = 7.7 Hz, 1H), 7.72 (d, J = 8.7 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.45 (t, J = 7.7 Hz, 1H), 6.83 (dd, J = 2.2/8.7 Hz, 1H), 6.73 (d, J = 2.2 Hz, 1H), 3.05 (t, J = 7.2 Hz, 1H), 1.59 (quint., J = 7.1 Hz, 2H), 1.42 (sext, J = 7.4 Hz, 2H), 0.90 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): 166.0, 161.4, 160.8, 160.6, 153.5, 136.8, 134.7, 130.7, 128.8, 127.2, 125.1, 125.0, 112.9, 108.0, 101.9, 99.9, 31.6, 30.6, 21.3, 13.5 ppm; MS (ESI−) m/z: 383.85 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{20}H_{19}NO_5S + H]^+$: 386.10567, found: 386.10614, Δ = 1.2 ppm; HPLC t_R (Method B) = 11.08 min.

*3-((Cyclopropylmethyl)thio)-N-(4,7-dihydroxy-2-oxo-2H-chromen-3-yl)benzamide (**7f**).* According to general procedure A: 102 mg (0.53 mmol) of **15a**, 99 mg (0.48 mmol) of **28f**, 73 mg (0.48 mmol) of HOBt, 91 mg (0.48 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (28 mg, 0.07 mmol, 14%) was obtained as a colorless solid. ^{1}H NMR (250 MHz, DMSO-d_6) δ = 11.88 (bs, 1H), 10.52 (s, 1H), 9.41 (s, 1H), 7.94 (s, 1H), 7.76 (d, J = 7.7 Hz, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.52 (d, J = 8.1 Hz, 1H), 7.43 (t, J = 7.7 Hz, 1H), 6.81 (dd, J = 2.2/8.7, 1H), 6.71 (d, J = 2.2 Hz, 1H), 3.00 (d, J = 7.1 Hz, 2H), 1.09−0.99 (m, 1H), 0.57−0.51 (m, 2H), 0.30−0.25 (m, 2H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 166.0, 161.3, 161.0, 160.9, 153.6, 137.1, 134.8, 130.8, 128.8, 127.3, 125.2, 125.1, 112.8, 108.4, 101.9, 99.7, 37.7, 10.4, 5.5 (2 × C) ppm; MS (ESI−) m/z: 381.85 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{20}H_{17}NO_5S + H]^+$: 384.09002, found: 384.08981, Δ = 0.5 ppm; HPLC t_R (Method B) = 9.64 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-(isopropylthio)-benzamide (**7g**).* According to general procedure A: 171 mg (0.88 mmol) of **15a**, 156 mg (0.80 mmol) of **28g**, 122 mg (0.80 mmol) of HOBt, 152 mg (0.80 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (73 mg, 0.20 mmol, 23%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.82 (bs, 1H), 10.55 (s, 1H), 9.47 (s, 1H), 8.00 (s, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.72 (d, J = 8.7 Hz, 1H), 7.57 (d, J = 7.9 Hz, 1H), 7.47 (t, J = 7.7 Hz, 1H), 6.83 (dd, J = 2.2/8.7 Hz, 1H), 6.73 (d, J = 2.2 Hz, 1H), 3.60 (sept, J = 6.6 Hz, 1H), 1.27 (d, J = 6.6 Hz, 6H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 166.0, 161.4, 160.8, 160.6, 153.5, 135.6, 134.8, 133.3, 129.9, 128.9, 126.1, 125.0, 112.9, 108.0, 101.9, 99.8, 37.1, 22.8 ppm; MS (ESI−) m/z: 369.85 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{19}H_{17}NO_5S + H]^+$: 372.09002, found: 372.09008, Δ = 0.2 ppm; HPLC t_R (Method B) = 9.60 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-(2-methylpropylsulfonamido)benzamide (**8a**).* According to general procedure A: 280 mg (1.45 mmol) of **15a**, 410 mg (1.59 mmol) of **32a**, 252 mg (1.59 mmol) of HOBt, 305 mg (1.59 mmol) of EDC × HCl, 20 mL of DMF, and 3 mL of triethylamine. The desired product (65 mg, 0.15 mmol, 10%) was obtained as a colorless solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.84 (bs, 1H), 10.56 (s, 1H), 9.97 (s, 1H), 9.42 (s, 1H), 7.77−7.74 (m, 2H), 7.72 (d, J = 8.6 Hz, 1H), 7.47 (t, J = 7.9 Hz, 1H), 7.42−7.39 (m, 1H), 6.83 (dd, J = 2.1/8.6 Hz, 1H) 6.73 (d, J = 2.1 Hz, 1H), 3.02 (d, J = 6.6 Hz, 2H), 2.15 (non., J = 6.6 Hz, 1H), 0.99 (d, J = 6.6 Hz, 6H) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ = 166.2, 161.4, 160.7, 160.3, 153.5, 138.5, 135.3, 129.1, 125.0, 123.0, 122.4, 119.2, 112.9, 107.9, 101.9, 100.0, 58.4, 48.6, 24.3, 22.1 ppm; MS (ESI−) m/z: 431.30 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{20}H_{20}N_2O_7S + H]^+$: 433.10640, found: 433.10563, Δ = 1.8 ppm; HPLC t_R (Method A) = 13.79 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-(methylsulfonamido)benzamide (**8b**).* According to general procedure A: 117 mg (0.60 mmol) of **15a**, 143 mg (0.66 mmol) of **32b**, 105 mg (0.66 mmol) of HOBt, 127 mg (0.66 mmol) of EDC × HCl, 12 mL of DMF, and 3 mL of triethylamine. The desired product (25 mg, 0.06 mmol, 11%) was obtained as a colorless solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.86 (bs, 1H), 10.55 (s, 1H), 9.91 (s, 1H), 9.42 (s, 1H), 7.79−7.76 (m, 2H), 7.72 (d, J = 8.7 Hz, 1H), 7.48 (t, J = 7.8 Hz, 1H), 7.41 (dd, J = 1.1/8.2 Hz, 1H), 6.83 (dd, J = 2.2/8.7 Hz, 1H), 6.73 (d, J = 2.2 Hz, 1H), 3.04 (s, 3H) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ = 166.2, 161.4, 160.7, 160.4, 153.4, 138.4, 135.3, 129.0, 125.0, 123.2, 122.9, 119.6, 112.9, 108.0, 101.9, 100.0, 39.1 ppm; MS (ESI−) m/z: 389.15 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{17}H_{14}N_2O_7S + H]^+$: 391.05945, found: 391.05921, Δ = 0.6 ppm; HPLC t_R (Method A) = 12.03 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-(ethylsulfonamido)benzamide (**8c**).* According to general procedure A: 174 mg (0.90 mmol) of **15a**, 227 mg (0.99 mmol) of **32c**, 156 mg (0.99 mmol) of HOBt, 190 mg (0.99 mmol) of EDC × HCl, 15 mL of DMF, and 3 mL of triethylamine. The desired product (65 mg, 0.16 mmol, 18%) was obtained as a colorless solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.84 (bs, 1H), 10.56 (s, 1H), 9.97 (s, 1H), 9.42 (s, 1H), 7.79−7.77 (m, 1H), 7.75 (d, J = 7.9 Hz, 1H), 7.72 (d, J = 8.7 Hz, 1H), 7.47 (t, J = 7.9 Hz, 1H), 7.42 (dd, J = 1.3/8.1 Hz, 1H), 6.83 (dd, J = 2.2/8.7 Hz, 1H), 6.73 (d, J = 2.2 Hz, 1H), 3.14 (q, J = 7.3 Hz, 2H), 1.21 (t, J = 7.3 Hz, 3H) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ = 166.2, 161.4, 160.7, 160.3, 153.5, 138.5, 135.3, 129.1, 125.0, 123.0, 122.5, 119.2, 112.9, 107.9, 101.9, 100.0, 45.3, 8.0 ppm; MS (ESI−) m/z: 403.20 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{18}H_{16}N_2O_7S + H]^+$: 405.07454, found: 405.07510, Δ = 1.4 ppm; HPLC t_R (Method A) = 12.47 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-(propylsulfonamido)benzamide (**8d**).* According to general procedure A: 92 mg (0.48 mmol) of **15a**, 127 mg (0.52 mmol) of **32d**, 82 mg (0.52 mmol) of HOBt, 100 mg (0.52 mmol) of EDC × HCl, 10 mL of DMF, and 3 mL of triethylamine. The desired product (20 mg, 0.05 mmol, 10%) was obtained as a colorless solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.84 (bs, 1H), 10.55 (s, 1H), 9.96 (s, 1H), 9.41 (s, 1H), 7.79−7.77 (m, 1H), 7.75 (d, J = 7.9 Hz, 1H), 7.72 (d, J = 8.7 Hz, 1H), 7.46 (t, J = 7.9 Hz, 1H), 7.41 (dd, J = 1.2/8.1, 1H), 6.84 (dd, J = 2.1/8.7 Hz, 1H), 6.73 (d, J = 2.1 Hz, 1H), 3.11 (t, J = 7.7 Hz, 2H), 1.70 (sext, J = 7.6 Hz, 2H), 0.94 (t, J = 7.6 Hz, 3H) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ = 166.2, 161.4, 160.7, 160.3, 153.5, 138.5, 135.3, 129.0, 125.0, 123.0, 122.4, 119.2, 112.9, 107.9, 101.9, 100.0, 52.5, 16.8, 12.5 ppm; MS (ESI−) m/z: 416.90 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{19}H_{18}N2O_7S + H]^+$: 419.09075, found: 419.09005, Δ = 1.7 ppm; HPLC t_R (Method A) = 13.18 min.

*3-(Butylsulfonamido)-N-(4,7-dihydroxy-2-oxo-2H-chromen-3-yl)benzamide (**8e**).* According to general procedure A: 246 mg (1.28 mmol) of **15a**, 361 mg (1.40 mmol) of **32e**, 221 mg (1.40 mmol) of HOBt, 269 mg (1.40 mmol) of EDC × HCl, 20 mL of DMF, and 3 mL of triethylamine. The desired product (60 mg, 0.14 mmol, 11%) was obtained as a colorless solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.84 (bs, 1H), 10.55 (s, 1H), 9.96 (s, 1H), 9.41 (s, 1H), 7.79−7.76 (m, 1H), 7.75 (d, J = 7.6 Hz, 1H), 7.72 (d, J = 8.8 Hz, 1H), 7.47 (t, J = 8.0 Hz, 1H), 7.41 (dd, J = 1.2/8.0 Hz, 1H), 6.83 (dd, J = 2.2/8.8 Hz, 1H), 6.73 (d, J = 2.2 Hz, 1H), 3.14−3.11 (m, 2H), 1.69−1.63 (m, 2H), 1.39−1.33 (m, 2H), 0.89 (t, J = 7.3 Hz, 3H) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ = 166.2, 161.4, 160.7, 160.3, 153.5, 138.5, 135.3, 129.0, 125.0, 122.4, 119.2, 112.9, 107.9, 101.9, 100.0, 50.5, 25.1, 20.6, 13.4 ppm; MS (ESI−) m/z: 431.30 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{20}H_{20}N_2O_7S + H]^+$: 433.10640, found: 433.10559, Δ = 1.9 ppm; HPLC t_R (Method A) = 13.79 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-(1-methylethylsulfonamido)benzamide (**8g**).* According to general procedure A: 130 mg (0.67 mmol) of **15a**, 180 mg (0.74 mmol) of **32g**, 117 mg (0.74 mmol) of HOBt, 142 mg (0.74 mmol) of EDC × HCl, 10 mL of DMF, and 3 mL of triethylamine. The desired product (72 mg, 0.17 mmol, 26%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.92 (bs, 1H), 10.55 (s, 1H), 9.93 (s, 1H), 9.40 (s, 1H), 7.84−7.71 (m, 3H), 7.48−7.42 (m, 2H), 6.83 (dd, J = 2.2/8.8 Hz, 1H), 6.73 (d, J = 2.2 Hz, 1H), 1.26 (d, J = 6.9 Hz, 6H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 166.2, 161.5, 160.8, 160.4, 153.5, 138.8, 135.3, 129.1, 125.0, 122.9, 122.3, 119.1, 113.0, 107.9, 101.9, 100.0, 51.5, 16.1 ppm; MS (ESI−) m/z: 417.25 $[M - H]^-$;

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

HRMS (MALDI+) m/z: calcd for $[C_{19}H_{18}N_2O_7S + H]^+$: 419.09074, found: 419.09041, Δ = 0.8 ppm; HPLC t_R (Method A) = 12.91 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-methoxybenzamide (**9b**).* According to general procedure A: 193 mg (1.0 mmol) of **15a**, 152 mg (1.0 mmol) of **24b**, 168 mg (1.1 mmol) of HOBt, 211 mg (1.1 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (96 mg, 0.29 mmol, 29%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.81 (bs, 1H), 10.54 (s, 1H), 9.39 (s, 1H), 7.72 (d, J = 8.7 Hz, 1H), 7.59−7.56 (m, 2H), 7.42 (t, J = 8.1 Hz, 1H), 7.14 (dd, J = 2.0/8.1 Hz, 1H), 6.83 (dd, J = 2.2/8.7 Hz, 1H), 6.73 (d, J = 2.2 Hz, 1H), 3.83 (s, 3H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 166.3, 161.4, 160.7, 160.4, 159.0, 153.5, 135.4, 129.3, 125.0, 120.3, 117.4, 113.1, 112.9, 108.0, 101.9, 100.1, 55.3 ppm; MS (ESI+) m/z: 327.80 $[M + H]^+$; HRMS (MALDI+) m/z: calcd for $[C_{17}H_{13}NO_6 + H]^+$: 328.08156, found: 328.08159, Δ = 0.1 ppm; HPLC t_R (Method A) = 13.93 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-ethoxybenzamide (**9c**).* According to general procedure A: 193 mg (1.0 mmol) of **15a**, 166 mg (1.0 mmol) of **24c**, 168 mg (1.1 mmol) of HOBt, 211 mg (1.1 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (50 mg, 0.15 mmol, 15%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.87 (bs, 1H), 10.51 (s, 1H), 9.34 (s, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.57−7.54 (m, 2H), 7.40 (t, J = 8.1 Hz, 1H), 7.11 (dd, J = 2.0/8.1 Hz, 1H), 6.81 (dd, J = 2.2/8.7 Hz, 1H), 6.71 (d, J = 2.2 Hz, 1H), 4.10 (q, J = 7.0 Hz, 2H), 1.36 (t, J = 7.0 Hz, 3H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 166.2, 161.3, 160.8, 158.3, 153.5, 135.4, 129.3, 125.0, 120.1, 117.9, 113.6, 112.8, 101.9, 99.9, 63.3, 14.6 ppm; MS (ESI+) m/z: 341.85 $[M + H]^+$; HRMS (MALDI+) m/z: calcd for $[C_{18}H_{15}NO_6 + H]^+$: 342.09721, found: 342.09715, Δ = 0.2 ppm; HPLC t_R (Method A) = 14.91 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-propoxybenzamide (**9d**).* According to general procedure A: 193 mg (1.0 mmol) of **15a**, 180 mg (1.0 mmol) of **24d**, 168 mg (1.1 mmol) of HOBt, 211 mg (1.1 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (90 mg, 0.25 mmol, 25%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.80 (bs, 1H), 10.54 (s, 1H), 9.38 (s, 1H), 7.72 (d, J = 8.7 Hz, 1H), 7.58−7.54 (m, 2H), 7.41 (t, J = 8.2 Hz, 1H), 7.13 (dd, J = 1.8/8.1 Hz, 1H), 6.83 (dd, J = 2.2/8.7 Hz, 1H), 6.73 (d, J = 2.2 Hz, 1H), 4.01 (t, J = 6.5 Hz, 2H), 1.76 (sext, J = 7.0 Hz, 2H), 1.00 (t, J = 7.5 Hz, 3H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 166.3, 161.4, 160.7, 160.3, 158.5, 153.5, 135.3, 129.3, 125.0, 120.2, 117.9, 113.7, 112.9, 108.0, 101.9, 100.1, 69.2, 22.0, 10.4 ppm; MS (ESI+) m/z: 355.85 $[M + H]^+$; HRMS (MALDI+) m/z: calcd for $[C_{19}H_{17}NO_6 + H]^+$: 356.11286, found: 356.11262, Δ = 0.7 ppm; HPLC t_R (Method A) = 15.91 min.

*3-Butoxy-N-(4,7-dihydroxy-2-oxo-2H-chromen-3-yl)benzamide (**9e**).* According to general procedure A: 193 mg (1.0 mmol) of **15a**, 194 mg (1.0 mmol) of **24e**, 168 mg (1.1 mmol) of HOBt, 211 mg (1.1 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (82 mg, 0.22 mmol, 22%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.82 (bs, 1H), 10.54 (s, 1H), 9.37 (s, 1H), 7.72 (d, J = 8.7 Hz, 1H), 7.57−7.54 (m, 2H), 7.40 (t, J = 8.1 Hz, 1H), 7.13 (dd, J = 1.6/8.1 Hz, 1H), 6.83 (dd, J = 2.2/8.7 Hz, 1H), 6.73 (d, J = 2.2 Hz, 1H), 4.04 (t, J = 6.5 Hz, 2H), 1.77−169 (m, 2H), 1.51−1.42 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 166.3, 161.4, 160.8, 160.4, 158.5, 153.5, 135.3, 129.3, 125.0, 120.7, 120.2, 118.1, 117.9, 113.7, 112.9, 108.0, 101.9, 100.0, 67.4, 30.7, 18.7, 13.7 ppm; MS (ESI+) m/z: 369.90 $[M + H]^+$; HRMS (MALDI+) m/z: calcd for $[C_{20}H_{19}NO_6 + H]^+$: 370.12851, found: 370.12870, Δ = 0.5 ppm; HPLC t_R (Method A) = 16.71 min.

*3-(Cyclopropylmethoxy)-N-(4,7-dihydroxy-2-oxo-2H-chromen-3-yl)benzamide (**9f**).* According to general procedure A: 193 mg (1.0 mmol) of **15a**, 192 mg (1.0 mmol) of **24f**, 168 mg (1.1 mmol) of HOBt, 211 mg (1.1 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (94 mg, 0.26 mmol, 26%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.86 (bs, 1H), 10.53 (s, 1H), 9.35 (s, 1H), 7.71 (d, J = 8.7 Hz, 1H), 7.57−7.53 (m, 2H), 7.39 (t, J = 8.1 Hz, 1H), 7.12 (dd, J = 1.9/8.1 Hz, 1H), 6.82 (dd, J = 2.2/8.7 Hz, 1H), 6.72 (d, J = 2.2 Hz, 1H), 3.89 (d, J = 7.0 Hz, 2H), 1.31−1.21 (m, 1H), 0.61−0.55 (m, 2H), 0.36−0.32 (m, 2H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 166.3, 161.4, 160.8, 160.5, 158.4, 153.5, 135.3, 129.2, 125.0, 120.1, 118.0, 113.6, 112.9, 108.1, 101.9, 100.0, 72.2, 10.1, 3.1 (2 × C) ppm; MS (ESI+) m/z: 367.80 $[M + H]^+$; HRMS (MALDI+) m/z: calcd for $[C_{20}H_{17}NO_6 + H]^+$ 368.11286, found: 368.11301, Δ = 0.4 ppm; HPLC t_R (Method A) = 15.54 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-isopropoxybenzamide (**9g**).* According to general procedure A: 193 mg (1.0 mmol) of **15a**, 180 mg (1.0 mmol) of **24g**, 168 mg (1.1 mmol) of HOBt, 211 mg (1.1 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (67 mg, 0.19 mmol, 19%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.84 (bs, 1H), 10.52 (s, 1H), 9.34 (s, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.54−7.52 (m, 2H), 7.39 (t, J = 8.1 Hz, 1H), 7.10 (dd, J = 1.8/8.1 Hz, 1H), 6.81 (dd, J = 2.2/8.7 Hz, 1H), 6.72 (d, J = 2.2 Hz, 1H), 4.69 (sept, J = 6.1 Hz, 1H), 1.30 (d, J = 6.1 Hz, 6H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 166.3, 161.3, 160.8, 160.7, 157.2, 153.5, 135.4, 129.3, 125.0, 120.1, 119.0, 115.0, 112.8, 108.2, 101.9, 99.9, 69.5, 21.8 (2 × C) ppm; MS (ESI+) m/z: 355.85 $[M + H]^+$; HRMS (MALDI+) m/z: calcd for $[C_{19}H_{17}NO_6 + H]^+$: 356.11286, found: 356.11282, Δ = 0.1 ppm; HPLC t_R (Method A) = 15.56 min.

*3-(Allyloxy)-N-(4,7-dihydroxy-2-oxo-2H-chromen-3-yl)-benzamide (**9h**).* According to general procedure A: 193 mg (1.0 mmol) of **15a**, 178 mg (1.0 mmol) of **24h**, 168 mg (1.1 mmol) of HOBt, 211 mg (1.1 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (49 mg, 0.14 mmol, 14%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.90 (bs, 1H), 10.51 (s, 1H), 9.34 (s, 1H), 7.71 (d, J = 8.7 Hz, 1H), 7.59−7.55 (m, 2H), 7.41 (t, J = 8.1 Hz, 1H), 7.19−7.14 (m, 1H), 6.81 (dd, J = 2.2/8.7 Hz, 1H), 6.71 (d, J = 2.2 Hz, 1H), 6.13−6.03 (m, 1H), 5.42 (dd, J = 1.5/17.3 Hz, 1H), 5.28 (dd, J = 1.5/10.6 Hz, 1H), 4.64 (d, J = 5.2 Hz, 2H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 166.2, 161.3, 160.8, 158.0, 153.5, 135.4, 133.6, 129.3, 125.1, 120.4, 118.0, 117.5, 114.0, 112.8, 101.9, 68.3 ppm; MS (ESI+) m/z: 353.85 $[M + H]^+$; HRMS (MALDI+) m/z: calcd for $[C_{19}H_{15}NO_6 + H]^+$: 354.09721, found: 354.09699, Δ = 0.6 ppm; HPLC t_R (Method A) = 15.18 min.

*3-(Benzyloxy)-N-(4,7-dihydroxy-2-oxo-2H-chromen-3-yl)-benzamide (**9i**).* According to general procedure A: 193 mg (1.0 mmol) of **15a**, 228 mg (1.0 mmol) of **24i**, 168 mg (1.1 mmol) of HOBt, 211 mg (1.1 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (87 mg, 0.22 mmol, 22%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.87 (bs, 1H), 10.53 (s, 1H), 9.37 (s, 1H), 7.72 (d, J = 8.7 Hz, 1H), 7.66 (s, 1H), 7.59 (d, J = 7.7 Hz, 1H), 7.49−7.47 (m, 2H), 7.43−7.38 (m, 3H), 7.36−7.32 (m, 1H), 7.21 (dd, J = 2.1/8.1 Hz, 1H), 6.82 (dd, J = 2.2 Hz, 8.7 Hz, 1H), 6.72 (d, J = 2.2 Hz, 1H), 5.19 (s, 2H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 166.2, 161.3, 160.8, 160.7, 158.1, 153.5, 136.9, 135.4, 129.3, 128.5, 127.9, 127.6, 125.0, 120.5, 118.1, 114.2, 112.8, 108.2, 101.9, 99.9, 69.4 ppm; MS (ESI+) m/z: 403.90 $[M + H]^+$; HRMS (MALDI+) m/z: calcd for $[C_{23}H_{17}NO_6 + H]^+$: 404.11286, found: 404.11198, Δ = 2.2 ppm; HPLC t_R (Method A) = 16.11 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-phenoxybenzamide (**9j**).* According to general procedure A: 232 mg (1.2 mmol) of **15a**, 257 mg (1.2 mmol) of 3-phenoxybenzoic acid (**24j**), 235 mg (1.3 mmol) of HOBt, 253 mg (1.3 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (300 mg, 0.77 mmol, 64%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.82 (bs, 1H), 10.53 (s, 1H), 9.42 (s, 1H), 7.78 (d, J = 7.7 Hz, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.62 (s, 1H), 7.53 (t, J = 7.9 Hz, 1H), 7.43 (t, J = 7.8 Hz, 2H), 7.24 (dd, J = 2.0/8.1 Hz, 1H), 7.18 (t, J = 7.4 Hz, 1H), 7.05 (d, J = 8.1 Hz, 2H), 6.83 (dd, J = 1.9/8.7 Hz, 1H), 6.71 (d, J = 1.9 Hz, 1H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6) δ = 166.1, 161.6, 161.0, 160.7, 156.7, 156.6, 153.7, 136.1, 130.4, 130.2, 125.2, 124.0, 123.2, 119.0, 118.2, 113.2, 108.0, 102.1, 100.0 ppm; MS (ESI+) m/z: 390.11 $[M + H]^+$; HRMS (MALDI+) m/z: calcd for $[C_{22}H_{15}NO_6 + H]^+$: 390.09721, found: 390.09650, Δ = 1.8 ppm; HPLC t_R (Method A) = 9.24 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-4-fluoro-3-isopentylbenzamide (**10**).* According to general procedure A: 386 mg (2.0 mmol) of **15a**, 463 mg (2.2 mmol) of **44**, 422 mg (2.2 mmol) of

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

HOBt, 347 mg (2.2 mmol) of EDC × HCl, 10 mL of DMF, and 0.6 mL of triethylamine. The desired product (146 mg, 0.38 mmol, 19%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.79 (bs, 1H), 10.57 (s, 1H), 9.39 (s, 1H), 7.95 (dd, J = 2.1/7.4 Hz, 1H), 7.89−7.85 (m, 1H), 7.72 (d, J = 8.7 Hz, 1H), 7.30−7.25 (m, 1H), 6.83 (dd, J = 2.3/8.7 Hz, 1H), 6.73 (d, J = 2.3 Hz), 2.69−2.65 (m, 2H), 1.62 (m, 3H), 0.94 (d, J = 6.4 Hz, 6H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 165.7, 161.5, 160.8, 160.4, 153.5, 130.9, 130.3, 129.0, 128.0, 125.1, 115.1, 114.9, 113.0, 107.9, 101.9, 100.0, 40.2, 27.3, 26.2, 22.3 (2 × C) ppm; MS (ESI−) m/z: 384.16 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{21}H_{20}FNO_5 + H]^+$: 386.13983, found: 386.13985, Δ = 0.1 ppm; HPLC: t_R (Method A) = 18.08 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-3-isopentyl-4-methoxybenzamide (**11**).* According to general procedure A: 155 mg (0.80 mmol) of **15a**, 213 mg (0.96 mmol) of **53**, 152 mg (0.96 mmol) of HOBt, 184 mg (0.96 mmol) of EDC × HCl, 5 mL of DMF, and 0.3 mL of triethylamine. The desired product (25 mg, 0.06 mmol, 8%) was obtained as a colorless solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 11.85 (bs, 1H), 10.55 (s, 1H), 9.27 (s, 1H), 7.86 (dd, J = 2.2/8.6 Hz, 1H), 7.81 (d, J = 2.2 Hz, 1H), 7.71 (d, J = 8.7 Hz, 1H), 7.05 (d, J = 8.6 Hz, 1H), 6.83 (dd, J = 2.3/8.7 Hz, 1H), 6.73 (d, J = 2.3 Hz, 1H), 3.86 (s, 3H), 2.62−2.58 (m, 2H), 1.57 (non, J = 6.7 Hz, 1H), 1.47−1.42 (m, 2H), 0.93 (d, J = 6.7 Hz, 6H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6) δ = 166.4, 161.4, 160.9, 160.0, 159.8, 153.4, 130.0, 129.4, 127.7, 125.6, 125.0, 113.0, 110.0, 108.1, 101.9, 100.5, 55.7, 38.7, 27.5, 27.4, 22.5 (2 × C) ppm; MS (ESI−) m/z: 395.95 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{22}H_{23}NO_6 + H]^+$: 398.15981, found: 398.15979, Δ = 0.05 ppm; HPLC: t_R (Method A) = 18.40 min.

*N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)-2-isopentylbenzamide (**12**).* According to general procedure A: 115 mg (0.59 mmol) of **15a**, 137 mg (0.71 mmol) of **40**, 113 mg (0.71 mmol) of HOBt, 137 mg (0.71 mmol) of EDC × HCl, 8 mL of DMF, and 3 mL of triethylamine. The desired product (33 mg, 0.09 mmol, 15%) was obtained as a colorless solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 11.86 (bs, 1H), 10.56 (s, 1H), 9.28 (s, 1H), 7.75 (d, J = 8.7 Hz, 1H), 7.73−7.71 (m, 1H), 7.40−7.37 (m, 1H), 7.29−7.26 (m, 2H), 6.83 (dd, J = 2.3/8.7 Hz, 1H), 6.73 (d, J = 2.3 Hz, 1H), 2.81−2.77 (m, 2H), 1.56−1.51 (m, 1H), 1.50−1.44 (m, 2H), 0.88 (d, J = 6.5 Hz, 6H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6) δ = 170.2, 161.9, 161.0, 160.3, 153.9, 141.9, 136.2, 130.2, 130.0, 128.8, 125.6, 125.5, 113.4, 108.5, 102.4, 100.9, 41.0, 31.1, 28.2, 22.9 (2 × C) ppm; MS (ESI−) m/z: 366.00 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{21}H_{21}NO_5 + H]^+$: 368.14925, found: 368.14951, Δ = 0.7 ppm; HPLC: t_R (Method A) = 17.19 min.

*tert-Butyl (2-((4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)amino)-2-oxoethyl)carbamate (**13a**).* According to general procedure A: 193 mg (1.0 mmol) of **15a**, 161 mg (0.9 mmol) of Boc-Gly (**56a**), 138 mg (0.9 mmol) of HOBt, 173 mg (0.9 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (118 mg, 0.34 mmol, 37%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.02 (bs, 1H), 10.56 (s, 1H), 9.17 (s, 1H), 7.69 (d, J = 8.7 Hz, 1H), 7.02 (t, J = 5.8 Hz, 1H), 6.82 (dd, J = 2.2/8.7 Hz, 1H). 6.70 (d, J = 2.2 Hz. 1H), 3.84 (d, J = 5.8 Hz, 2H), 1.39 (s, 9H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 170.6, 161.4, 160.5, 158.3, 155.8, 153.1, 125.1, 113.1, 108.0, 101.9, 100.1, 78.2, 43.2, 28.2 (3 × C) ppm; MS (ESI−) m/z: 349,20 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{16}H_{18}N_2O_7+Na]^+$: 373.10062, found: 373.10061, Δ = 0.03 ppm; HPLC: t_R (Method A) = 12.67 min.

*(S)-tert-Butyl (1-((4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)amino)-1-oxopropan-2-yl)carbamate (**13b**).* According to general procedure A: 193 mg (1.0 mmol) of **15a**, 170 mg (0.9 mmol) of Boc-L-Ala (**56b**), 138 mg (0.9 mmol) of HOBt, 173 mg (0.9 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (115 mg, 0.32 mmol, 35%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.14 (bs, 1H), 10.57 (s, 1H), 9.31 (s, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.14 (d, J = 7.1 Hz, 1H), 6.82 (dd, J = 2.2/8.7 Hz, 1H), 6.72 (d, J = 2.2 Hz, 1H), 4.30 (quint, J = 7.1 Hz, 1H), 1.40 (s, 9H), 1.28 (d, J = 7.1 Hz, 3H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 174.1, 161.4, 160.2, 157.3, 155.2, 152.9, 125.1, 113.2, 108.0, 101.9, 100.7, 78.5, 49.7, 28.2 (3 × C), 18.1 ppm; MS (ESI−) m/z: 363.25 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{17}H_{20}N_2O_7+Na]^+$: 387.11627, found: 387.11592, Δ = 0.9 ppm; HPLC: t_R (Method A) = 13.38 min.

*(S)-tert-Butyl (1-((4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)amino)-1-oxopropan-2-yl)carbamate (**13c**).* According to general procedure A: 193 mg (1.0 mmol) of **15a**, 196 mg (0.9 mmol) of Boc-L-Val (**56c**), 138 mg (0.9 mmol) of HOBt, 173 mg (0.9 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (128 mg, 0.33 mmol, 36%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.03 (bs, 1H), 10.57 (s, 1H), 9.35 (s, 1H), 7.70 (d, J = 8.7 Hz, 1H), 6.87−6.80 (m, 2H), 6.71 (d, J = 2.2 Hz, 1H), 4.20−4.15 (m, 1H), 2.18−2.03 (m, 1H), 1.40 (s, 9H), 0.92 (d, J = 6.7 Hz, 3H), 0.88 (d, J = 6.7 Hz, 3H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 172.8, 161.4, 160.2, 157.9, 155.7, 153.1, 125.1, 113.2, 108.0, 101.9, 100.6, 78.4, 59.3, 30.6, 28.2 (3 × C), 19.2, 17.8 ppm; MS (ESI−) m/z: 391.95 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{19}H_{24}N_2O_7+Na]^+$: 415.14757, found: 415.14737, Δ = 0.5 ppm; HPLC: t_R (Method A) = 14.66 min.

*(R)-tert-Butyl (1-((4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)amino)-4-methyl-1-oxopentan-2-yl)-carbamate (**13d**).* According to general procedure A: 193 mg (1.0 mmol) of **15a**, 208 mg (0.9 mmol) of Boc-D-Leu (**56d**), 138 mg (0.9 mmol) of HOBt, 173 mg (0.9 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (87 mg, 0.21 mmol, 24%) was obtained as a colorless solid. ^{1}H NMR (250 MHz, DMSO-d_6) δ = 12.13 (bs, 1H), 10.57 (s, 1H), 9.35 (s, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.13 (d, J = 7.6 Hz, 1H), 6.83 (dd, J = 2.2/8.7 Hz, 1H), 6.72 (d, J = 2.2 Hz, 1H), 4.29 (q, J = 7.6 Hz, 1H), 1.68 (sept, J = 6.4 Hz, 1H), 1.54 (t, J = 7.0 Hz), 1.40 (s, 9H), 0.90 (dd, J = 1.3/6.4 Hz, 6H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ = 174.1, 161.4, 160.2, 157.1, 155.6, 152.9, 125.0, 113.2, 108.0, 101.9, 100.9, 78.5, 52.7, 40.6, 28.2 (3 × C), 24.3, 23.2, 21.4 ppm; MS (ESI−) m/z: 405.30 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{20}H_{26}N_2O_7+Na]^+$: 429.16322, found: 429.16269, Δ = 1.2 ppm; HPLC: t_R (Method A) = 15.49 min.

*(S)-tert-Butyl (1-((4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)amino)-4-methyl-1-oxopentan-2-yl)-carbamate (**13e**).* According to general procedure A: 193 mg (1.0 mmol) of **15a**, 224 mg (0.9 mmol) of Boc-L-Leu × H_2O (**56e**), 138 mg (0.9 mmol) of HOBt, 173 mg (0.9 mmol) of EDC × HCl, of 5 mL of DMF. The desired product (97 mg, 0.24 mmol, 27%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.13 (bs, 1H), 10.57 (s, 1H), 9.35 (s, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.12 (d, J = 7.4 Hz, 1H), 6.82 (dd, J = 2.2/8.7 Hz, 1H), 6.72 (d, J = 2.2 Hz, 1H), 4.29 (q, J = 7.4 Hz, 1H)), 1.74−1.51 (m, 3H), 1.40 (s, 9H), 0.91−0.89 (m, 6H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 174.1, 161.4, 160.2, 157.1, 155.6, 152.9, 125.0, 113.2, 108.0, 101.9, 100.8, 78.5, 52.7, 40.6, 28.2 (3 × C), 24.3, 23.2, 21.4 ppm; MS (ESI−) m/z: 405.30 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{20}H_{26}N_2O_7+Na]^+$: 429.16322, found: 429.16256, Δ = 1.5 ppm; HPLC: t_R (Method A) = 15.49 min.

*tert-Butyl ((2S,3S)-1-((4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)amino)-3-methyl-1-oxopentan-2-yl)-carbamate (**13f**).* According to general procedure A: 193 mg (1.0 mmol) of **15a**, 212 mg (0.9 mmol) of Boc-L-Ile × 1/2H_2O (**56f**), 138 mg (0.9 mmol) of HOBt, 173 mg (0.9 mmol) of EDC × HCl, and 5 mL of DMF. The desired product (62 mg, 0.15 mmol, 17%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.03 (bs, 1H), 10.57 (s, 1H), 9.41 (s, 1H), 7.71 (d, J = 8.7 Hz, 1H), 6.94 (d, J = 7.6 Hz, 1H), 6.82 (dd, J = 2.2/8.7 Hz, 1H), 6.71 (d, J = 2.2 Hz, 1H), 4.19 (t, J = 7.6 Hz, 1H), 1.89−1.74 (m, 1H), 1.54−1.33 (m, 10H), 1.25−1.09 (m, 1H), 0.90 (d, J = 6.8 Hz, 3H), 0.84 (t, J = 7.3 Hz, 3H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 173.0, 161.4, 160.2, 157.6, 155.6, 153.0, 125.0, 113.2, 108.0, 101.9, 100.8, 78.4, 58.6, 36.7, 28.2 (3 × C), 24.3, 15.3, 11.1 ppm; MS (ESI−) m/z: 405.35 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{20}H_{26}N_2O_7+Na]^+$: 429.16322, found: 429.16255, Δ = 1.6 ppm; HPLC: t_R (Method A) = 15.35 min.

*(S)-tert-Butyl (1-((4,7-Dihydroxy-2-oxo-2H-chromen-3-yl)amino)-1-oxohexan-2-yl)carbamate (**13g**).* According to general procedure A: 193 mg (1.0 mmol) of **15a**, 208 mg (0.9 mmol) of Boc-L-norleucine (**56g**), 138 mg (0.9 mmol) of HOBt, 173 mg (0.9

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

mmol) of EDC × HCl, and 5 mL of DMF. The desired product (116 mg, 0.29 mmol, 32%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.12 (bs, 1H), 10.57 (s, 1H), 9.36 (s, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.07 (d, J = 7.6 Hz, 1H), 6.82 (dd, J = 2.2/8.7 Hz, 1H), 6.71 (d, J = 2.2 Hz, 1H), 4.29−4.19 (m, 1H), 1.78−1.51 (m, 2H), 1.46−1.23 (m, 13H), 0.87 (t, J = 6.5 Hz, 3H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 173.8, 161.4, 160.2, 157.2, 155.5, 152.9, 125.0, 113.2, 108.0, 101.9, 100.8, 78.4, 54.2, 31.6, 28.2 (3 × C), 27.5, 21.9, 13.9 ppm; MS (ESI−) m/z: 405.35 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{20}H_{26}N_2O_7+Na]^+$: 429.16322, found: 429.16268, Δ = 1.3 ppm; HPLC: t_R (Method A) = 15.54 min.

*N-(4-Hydroxy-7-nitro-2-oxo-2H-chromen-3-yl)-3-isopentylbenzamide (**54**).* According to general procedure A: 1.20 g (4.65 mmol) of **15c** × HCl, 0.98 g (5.12 mmol) of **36a**, 0.98 (5.12 mmol) of EDC × HCl, 0.78 g (5.12 mmol) of HOBt × H_2O, 2.0 mL of triethylamine, and 15 mL of DMF. The desired product (1.60 g, 4.04 mmol, 87%) was obtained as an orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 14.11 (s, 1H), 8.90 (s, 1H), 8.22 (s, 3H), 7.78−7.74 (m, 2H), 7.50−7.45 (m, 2H), 2.72 (t, J = 8.1 Hz, 2H), 1.67−1.53 (m, 3H), 0.97 (d, J = 6.5 Hz, 6H) ppm; MS (ESI−) m/z: 395.25 $[M - H]^-$.

*N-(7-Bromo-4-hydroxy-2-oxo-2H-chromen-3-yl)-3-isopentylbenzamide (**55**).* According to general procedure A: 2.08 g (8.14 mmol) of **15d** × HCl, 1.30 g (6.78 mmol) of **36a**, 1.56 g (8.14 mmol) of EDC × HCl, 1.29 g (8.14 mmol) of HOBt, 2.9 mL of triethylamine, and 20 mL of DMF. The desired product (1.81 g, 4.20 mmol, 62%) was obtained as a light brown solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 14.00 (s, 1H), 8.84 (s, 1H), 7.89 (d, J = 8.6 Hz, 1H), 7.76−7.73 (m, 2H), 7.53 (d, J = 1.6 Hz, 1H), 7.49 (dd, J = 1.8/8.5 Hz, 1H), 7.46−7.41 (m, 2H), 2.74−2.68 (m, 2H), 1.69−1.51 (m, 3H), 0.96 (d, J = 6.3 Hz, 6H) ppm; MS (ESI−) m/z: 428.24 $[M - H]^-$.

General Procedure for Suzuki Coupling (Procedure B). **55** (1.0 equiv), the corresponding boronic acid or boronic ester (1.2 equiv), and $Pd(PPh_3)_4$ (0.1 equiv) were combined under argon atmosphere. Then DMF and a 2 M aqueous Na_2CO_3 solution (6.0 equiv) were added. The reaction mixture was stirred for 12h at 80 °C. The reaction was quenched by the addition of 2 M aqueous HCl, and the aqueous phase was extracted three times with EtOAc. The combined organic phases were washed with brine, dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by preparative HPLC.

*N-(4-Hydroxy-7-(naphthalen-1-yl)-2-oxo-2H-chromen-3-yl)-3-isopentylbenzamide (**14a**).* According to general procedure B: 198 mg (0.46 mmol) of **55**, 95 mg (0.55 mmol) of naphthalen-1-ylboronic acid, 293 mg (2.76 mmol) of $NaHCO_3$, 54 mg (0.05 mmol) of $Pd(PPh_3)_4$, 5 mL of DMF, and 1.4 mL of H_2O. The desired product (167 mg, 0.35 mmol, 76%) was obtained as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 13.90 (s, 1H), 8.84 (s, 1H), 8.05 (d, J = 8.1 Hz, 1H), 7.83 (t, J = 8.2 Hz, 2H), 7.79 (d, J = 8.6 Hz, 1H), 7.70−7.67 (m, 2H), 7.48−7.34 (m, 8H), 2.64−2.61 (m, 2H), 1.54 (non, J = 6.6 Hz, 1H), 1.49−1.44 (m, 2H), 0.87 (d, J = 6.5 Hz, 6H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 167.9, 161.5, 153.0, 150.6, 145.0, 144.6, 138.3, 133.9, 133.6, 131.6, 131.2, 129.2, 128.8, 128.6, 127.7, 127.3, 127.0, 126.7, 126.3, 125.5, 124.9, 124.5, 117.7, 116.2, 104.9, 40.8, 33.8, 27.9, 22.6 (2 × C) ppm; MS (ESI−) m/z: 476.05 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{31}H_{27}NO_4+Na]^+$: 500.18323, found: 500.18327, Δ = 0.08 ppm; HPLC: t_R (Method C) = 17.19 min.

*N-(7-([1,1′-Biphenyl]-2-yl)-4-hydroxy-2-oxo-2H-chromen-3-yl)-3-isopentylbenzamide (**14b**).* According to general procedure B: 198 mg (0.46 mmol) of **55**, 109 mg (0.55 mmol) of [1,1′-biphenyl]-2-ylboronic acid, 293 mg (2.76 mmol) of $NaHCO_3$, 54 mg (0.05 mmol) of $Pd(PPh_3)_4$, 5 mL of DMF, and 1.4 mL of H_2O. The desired product (175 mg, 0.35 mmol, 76%) was obtained as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 13.85 (s, 1H), 8.87 (s, 1H), 7.85 (d, J = 8.2 Hz, 1H), 7.76−7.73 (m, 2H), 7.48−7.41 (m, 6H), 7.24−7.21 (m, 3H), 7.17−7.11 (m, 4H), 2.72−2.69 (m, 2H), 1.66−1.52 (m, 3H), 0.96 (d, J = 6.5 Hz, 6H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 167.8, 161.5, 153.0, 150.3, 145.9, 144.6, 140.9, 140.9, 138.8, 133.5, 131.6, 131.0, 130.6, 129.9 (2 × C), 129.1, 128.6, 128.3 (2 × C), 127.9, 127.7, 127.1, 126.8, 124.9, 124.0, 117.5, 115.5, 104.8, 40.8, 33.8, 27.9, 22.6 (2 × C) ppm; MS (ESI−) m/z: 502.05 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{33}H_{29}NO_4+Na]^+$: 526.19888, found: 526.19886, Δ = 0.04 ppm; HPLC: t_R (Method C) = 16.60 min.

*4-(4-Hydroxy-3-(3-isopentylbenzamido)-2-oxo-2H-chromen-7-yl)benzoic Acid (**14c**).* According to general procedure B: 198 mg (0.46 mmol) of **55**, 95 mg (0.55 mmol) of 4-boronobenzoic acid, 293 mg (2.76 mmol) of $NaHCO_3$, 54 mg (0.05 mmol) of $Pd(PPh_3)_4$, 5 mL of DMF, and 1.4 mL of H_2O. The desired product (100 mg, 0.21 mmol, 46%) was obtained as a colorless solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 13.10 (bs, 1H), 12.20 (bs, 1H), 9.55 (s, 1H), 8.07−8.06 (m, 2H), 8.01 (d, J = 8.2 Hz, 1H), 7.96 (d, J = 8.5 Hz, 2H), 7.87 (s, 1H), 7.85−7.79 (m, 3H), 7.43 (d, J = 4.7 Hz, 2H), 2.69−2.65 (m, 2H), 1.60−1.49 (m, 3H), 0.94 (d, J = 6.4 Hz, 6H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6) δ = 167.0, 166.3, 160.4, 159.2, 152.1, 142.7, 142.6, 142.4, 133.8, 131.7, 130.6, 130.1 (2 × C), 128.2, 127.9, 127.3 (2 × C), 125.5, 124.4, 123.0, 115.9, 114.4, 103.3, 40.6, 33.0, 27.1, 22.4 (2 × C) ppm; MS (ESI−) m/z: 470.05 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{28}H_{25}NO_6 + H]^+$: 494.15741, found: 494.15890, Δ = 3.0 ppm; HPLC: t_R (Method C) = 12.29 min.

*3-(4-Hydroxy-3-(3-isopentylbenzamido)-2-oxo-2H-chromen-7-yl)benzoic Acid (**14d**).* According to general procedure B: 198 mg (0.46 mmol) of **55**, 92 mg (0.55 mmol) of 3-boronobenzoic acid, 293 mg (2.76 mmol) of $NaHCO_3$, 54 mg (0.05 mmol) of $Pd(PPh_3)_4$, 5 mL of DMF, and 1.4 mL of H_2O. The desired product (105 mg, 0.22 mmol, 48%) was obtained as a colorless solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 13.19 (bs, 1H), 12.19 (bs, 1H), 9.55 (s, 1H), 8.30 (s, 1H), 8.08 (d, J = 8.3 Hz, 1H), 8.02−8.00 (m, 2H), 7.87 (s, 1H), 7.84−7.81 (m, 2H), 7.78 (dd, J = 1.6/8.3 Hz, 1H), 7.67 (t, J = 7.8 Hz, 1H), 7.64−7.60 (m, 1H), 7.50−7.46 (m, 1H), 7.42 (d, J = 4.9 Hz, 2H), 2.68−2.65 (m, 2H), 1.62−1.50 (m, 3H), 0.94 (d, J = 6.3 Hz, 6H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6) δ = 167.1, 166.6, 160.5, 159.3, 152.1, 143.0, 142.6, 138.7, 134.7, 134.6, 133.8, 131.7, 131.6, 130.7, 129.6, 129.3, 128.2, 127.9, 127.7, 125.5, 124.5, 122.9, 115.6, 114.2, 103.2, 40.4, 33.0, 27.1, 22.4 (2 × C) ppm; MS (ESI−) m/z: 470.00 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{28}H_{25}NO_6 + H]^+$: 494.15741, found: 494.15812, Δ = 1.4 ppm; HPLC: t_R (Method C) = 12.11 and 12.23 min.

*N-(7-(Benzofuran-3-yl)-4-hydroxy-2-oxo-2H-chromen-3-yl)-3-isopentylbenzamide (**14e**).* According to general procedure B: 198 mg (0.46 mmol) of **55**, 89 mg (0.55 mmol) of benzofuran-3-ylboronic acid, 293 mg (2.76 mmol) of $NaHCO_3$, 54 mg (0.05 mmol) of $Pd(PPh_3)_4$, 5 mL of DMF, and 1.4 mL of H_2O. The desired product (140 mg, 0.30 mmol, 65%) was obtained as a pale-yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 13.98 (s, 1H), 8.89 (s, 1H), 8.10 (d, J = 8.6 Hz, 1H), 7.92 (s, 1H), 7.89−7.86 (m, 1H), 7.77−7.74 (m, 2H), 7.65−7.63 (m, 2H), 7.58−7.56 (m, 1H), 7.46−7.36 (m, 4H), 2.73−2.70 (m, 2H), 1.63 (non, J = 6.6 Hz, 1H), 1.58−1.53 (m, 2H), 0.97 (d, J = 6.5 Hz, 6H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 167.8, 161.3, 156.1, 152.8, 151.1, 144.6, 142.6, 136.2, 133.6, 131.5, 129.2, 127.7, 125.7, 125.2, 125.2, 124.9, 123.9, 123.7, 121.0, 120.3, 116.2, 114.5, 112.2, 104.8, 40.8, 33.8, 27.9, 22.6 (2 × C) ppm; MS (ESI−) m/z: 466.00 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{29}H_{25}NO_5+Na]^+$: 490.16249, found: 490.16242, Δ = 0.1 ppm; HPLC: t_R (Method C) = 15.40 min.

*N-(7-(1-Benzyl-1H-pyrazol-5-yl)-4-hydroxy-2-oxo-2H-chromen-3-yl)-3-isopentylbenzamide (**14f**).* According to general procedure B: 198 mg (0.46 mmol) of **55**, 157 mg (0.55 mmol) of 1-benzyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole, 293 mg (2.76 mmol) of $NaHCO_3$, 54 mg (0.05 mmol) of $Pd(PPh_3)_4$, 5 mL of DMF, and 1.4 mL of H_2O. The desired product (130 mg, 0.26 mmol, 57%) was obtained as a pale-yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 13.90 (s, 1H), 8.81 (s, 1H), 7.96 (d, J = 8.8 Hz, 1H), 7.70−7.67 (m, 2H), 7.59 (d, J = 2.0 Hz, 1H), 7.40−7.36 (m, 2H), 7.27−7.18 (m, 5H), 6.98 (d, J = 6.9 Hz, 2H), 6.39 (d, J = 1.9 Hz, 1H), 5.35 (s, 2H), 2.66−2.62 (m, 2H), 1.55 (non, J = 6.6 Hz, 1H), 1.50−1.45 (m, 2H), 0.89 (d, J = 6.4 Hz, 6H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 168.0, 161.1, 152.4, 150.4, 144.6, 142.4, 139.6, 137.1, 134.1, 133.7, 131.4, 129.2, 129.0 (2 × C), 127.9, 127.7, 126.8 (2 × C), 125.3, 125.1, 124.9, 117.2, 116.3, 107.5, 105.3, 53.7, 40.8,

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

33.8, 27.9, 22.6 (2 × C) ppm; MS (ESI−) m/z: 506.20 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{31}H_{29}N_3O_4 + H]^+$: 508.22308, found: 508.22289, Δ = 0.4 ppm; HPLC: t_R (Method C) = 12.77 min.

*N-(7-(1-Ethyl-1H-pyrazol-5-yl)-4-hydroxy-2-oxo-2H-chromen-3-yl)-3-isopentylbenzamide (**14g**).* According to general procedure B: 198 mg (0.46 mmol) of **55**, 123 mg (0.55 mmol) of 1-ethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole, 293 mg (2.76 mmol) of $NaHCO_3$, 54 mg (0.05 mmol) of $Pd(PPh_3)_4$, 5 mL of DMF, and 1.4 mL of H_2O. The desired product (63 mg, 0.14 mmol, 30%) was obtained as a pale-yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 13.91 (s, 1H), 8.80 (s, 1H), 8.01 (d, *J* = 8.1 Hz, 1H), 7.68−7.65 (m, 2H), 7.49 (d, *J* = 1.9 Hz, 1H), 7.38−7.33 (m, 2H), 7.31 (dd, *J* = 1.6/8.1 Hz, 1H), 7.29 (d, *J* = 1.6 Hz, 1H), 6.28 (d, *J* = 1.9 Hz, 1H), 4.14 (q, *J* = 7.3 Hz, 2H), 2.63−2.59 (m, 2H), 1.52 (non, *J* = 6.6 Hz, 1H), 1.47−1.42 (m, 2H), 1.37 (t, *J* = 7.3 Hz, 3H), 0.86 (d, *J* = 6.5 Hz, 6H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 168.0, 161.2, 152.5, 150.5, 144.6, 141.4, 139.1, 134.4, 133.7, 129.2, 127.7, 125.3, 125.1, 124.9, 117.1, 116.2, 107.0, 105.3, 45.0, 40.8, 33.8, 27.9, 22.6 (2 × C), 15.9 ppm; MS (ESI−) m/z: 444.00 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{26}H_{27}N_3O_4 + H]^+$: 446.20743, found: 446.20745, Δ = 0.04 ppm; HPLC: t_R (Method C) = 12.31 min.

*N-(4-Hydroxy-7-(1-methyl-1H-pyrazol-5-yl)-2-oxo-2H-chromen-3-yl)-3-isopentylbenzamide (**14h**).* According to general procedure B: 198 mg (0.46 mmol) of **55**, 157 mg (0.55 mmol) of 1-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazol, 293 mg (2.76 mmol) of $NaHCO_3$, 54 mg (0.05 mmol) of $Pd(PPh_3)_4$, 5 mL of DMF, and 1.4 mL of H_2O. The desired product (110 mg, 0.25 mmol, 54%) was obtained as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 13.91 (s, 1H), 8.80 (s, 1H), 8.01 (d, *J* = 8.3 Hz, 1H), 7.68−7.65 (m, 2H), 7.46 (d, *J* = 1.9 Hz, 1H), 7.37−7.33 (m, 3H), 7.32 (d, *J* = 1.3 Hz, 1H), 6.33 (d, *J* = 1.9 Hz, 1H), 3.88 (s, 3H), 2.63−2.60 (m, 2H), 1.52 (non, *J* = 6.7 Hz, 1H), 1.47−1.43 (m, 2H), 0.86 (d, *J* = 6.5 Hz, 6H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 168.0, 161.1, 152.4, 150.5, 144.7, 142.0, 138.9, 134.0, 133.7, 131.4, 129.2, 127.7, 125.1, 124.9, 117.1, 116.1, 107.1, 105.3, 40.8, 38.0, 33.8, 27.9, 22.6 (2 × C) ppm; MS (ESI−) m/z: 430.00 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{25}H_{25}N_3O_4 + H]^+$: 432.19178, found: 432.19154, Δ = 0.6 ppm; HPLC: t_R (Method C) = 11.60 min.

General Procedure for 7-*N*-Sulfonation and Acylation (Procedure C). **57** (1.0 equiv) and pyridine (1.2−2.0 equiv) were dissolved in dry DCM and cooled to 0 °C. Then the corresponding alkylsulfonyl chloride or acid anhydride (1.2 equiv) was added, and the reaction mixture was stirred for 12 h, while it was allowed to warm up to room temperature. The reaction was quenched by addition of 2 M aqueous HCl, and the aqueous phase was extracted three times with EtOAc. The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by preparative HPLC.

*N-(4-Hydroxy-2-oxo-7-(2-phenylethylsulfonamido)-2H-chromen-3-yl)-3-isopentylbenzamide (**14i**).* According to general procedure C: 150 mg (0.41 mmol) of **57**, 100 mg (0.49 mmol) of 2-phenylethanesulfonyl chloride, and 66 μL (0.81 mmol) of pyridine in 5 mL of dry DCM. The desired product (74 mg, 0.14 mmol, 34%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.01 (bs, 1H), 10.53 (s, 1H), 9.45 (s, 1H), 7.85−7.79 (m, 3H), 7.42−7.40 (m, 2H), 7.29−7.16 (m, 7H), 3.59−3.53 (m, 2H), 3.05−2.99 (m, 2H), 2.72−2.62 (m, 2H), 1.64−1.47 (m, 3H), 0.93 (d, *J* = 6.2 Hz, 6H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 166.6, 160.4, 159.6, 152.5, 142.6, 142.1, 137.8, 133.8, 131.6, 128.5 (2 × C), 128.4 (2 × C), 128.1, 127.8, 126.5, 125.4, 125.0, 114.6, 111.4, 104.8, 101.7, 52.1, 40.4, 32.9, 29.1, 27.1, 22.4 (2 × C) ppm; MS (ESI−) m/z: 533.00 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{29}H_{30}N_2O_6S + H]^+$: 535.18973, found: 535.18792, Δ = 3.4 ppm; HPLC: t_R (Method A) = 19.98 min.

*N-(4-Hydroxy-2-oxo-7-(phenylmethylsulfonamido)-2H-chromen-3-yl)-3-isopentylbenzamide (**14j**).* According to general procedure C: 150 mg (0.41 mmol) of **57**, 95 mg (0.49 mmol) of phenylmethanesulfonyl chloride, and 66 μL (0.81 mmol) of pyridine in 5 mL of dry DCM. The desired product (92 mg, 0.18 mmol, 43%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.00 (bs, 1H), 10.45 (s, 1H), 9.45 (s, 1H), 7.86−7.80 (m, 3H), 7.43−7.40 (m, 2H), 7.37−7.32 (m, 3H), 7.30−7.26 (m, 2H), 7.17−7.13 (m, 2H), 2.70−2.64 (m, 2H), 1.62−1.47 (m, 3H), 0.93 (d, *J* = 6.2 Hz, 6H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 166.7, 160.5, 159.7, 152.5, 142.6, 142.4, 133.8, 131.6, 131.0 (2 × C), 129.2, 128.4 (2 × C), 128.4, 128.1, 127.8, 125.4, 124.9, 114.2, 111.0, 104.2, 101.5, 52.3, 40.4, 32.9, 27.1, 22.4 (2 × C) ppm; MS (ESI−) m/z: 519.00 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{28}H_{28}N_2O_6S + H]^+$: 521.17408, found: 521.17214, Δ = 3.7 ppm; HPLC: t_R (Method A) = 19.32 min.

*N-(4-Hydroxy-7-(2-methylpropylsulfonamido)-2-oxo-2H-chromen-3-yl)-3-isopentylbenzamide (**14k**).* According to general procedure C: 150 mg (0.41 mmol) of **57**, 66 μL (0.49 mmol) of 2-methylpropane-1-sulfonyl chloride, and 66 μL (0.81 mmol) of pyridine in 5 mL of dry DCM. The desired product (56 mg, 0.12 mmol, 28%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.06 (bs, 1H), 10.45 (s, 1H), 9.44 (s, 1H), 7.86−7.80 (m, 3H), 7.46−7.38 (m, 2H), 7.21−7.17 (m, 2H), 3.15 (d, *J* = 6.3 Hz, 2H), 2.69−2.63 (m, 2H), 2.15 (non, *J* = 6.8 Hz, 1H), 1.64−1.47 (m, 3H), 1.02 (d, *J* = 6.8 Hz, 6H), 0.94 (d, *J* = 6.3 Hz, 6H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 166.6, 160.5, 159.7, 152.6, 142.6, 142.3, 133.8, 131.6, 128.1, 127.8, 125.4, 125.0, 114.4, 111.2, 104.3, 101.6, 58.6, 40.4, 32.9, 27.1, 24.4, 22.4 (2 × C), 22.0 (2 × C) ppm; MS (ESI−) m/z: 485.05 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{25}H_{30}N_2O_6S + H]^+$: 487.18973, found: 487.18796, Δ = 3.6 ppm; HPLC: t_R (Method A) = 19.66 min.

*N-(4-Hydroxy-2-oxo-7-(phenylsulfonamido)-2H-chromen-3-yl)-3-isopentylbenzamide (**14l**).* According to general procedure C: 300 mg (0.82 mmol) of **57**, 126 μL (0,98 mmol) of benzolsulfonyl chloride, and 132 μL (1,64 mmol) of pyridine in 10 mL of dry DCM. The desired product (293 mg, 0.58 mmol, 71%) was obtained as a colorless solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 12.02 (bs, 1H), 10.99 (s, 1H), 9.42 (s, 1H), 7.87−7.85 (m, 2H), 7.83 (s, 1H), 7.80−7.77 (m, 1H), 7.76 (d, *J* = 8.6 Hz, 1H), 7.67−7.63 (m, 1H), 7.61−7.58 (m, 2H), 7.40−7.39 (m, 2H), 7.15 (dd, *J* = 2.0/8.6 Hz, 1H), 7.08 (d, *J* = 2.0 Hz, 1H), 2.65 (t, *J* = 7.8 Hz, 2H) 1.60−1.47 (m, 3H), 0.93 (d, *J* = 6.4 Hz, 6H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6) δ = 166.6, 160.3, 159.5, 152.2, 142.5, 141.5, 139.1, 133.8, 133.4, 131.5, 129.6 (2 × C), 128.1, 127.8, 126.7 (2 × C), 125.4, 125.0, 115.0, 111.7, 105.0, 101.8, 40.3, 32.9, 27.1, 22.4 (2 × C) ppm; MS (ESI−) m/z: 504.95 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{27}H_{26}N_2O_6S + H]^+$: 507.15843, found: 507.15781, Δ = 1.2 ppm; HPLC: t_R (Method C) = 10.35 min.

*N-(4-Hydroxy-7-(4-methylphenylsulfonamido)-2-oxo-2H-chromen-3-yl)-3-isopentylbenzamide (**14m**).* According to general procedure C: 302 mg (0.82 mmol) of **57**, 191 mg (0.98 mmol) of *para*-toluenesulfonyl chloride, and 132 μL (1.64 mmol) of pyridine in 10 mL of dry DCM. The desired product (269 mg, 0.52 mmol, 63%) was obtained as a colorless solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 12.09 (s, 1H), 10.92 (s, 1H), 9.42 (s, 1H), 7.84−7.73 (m, 4H), 7.40−7.37 (m, 3H), 7.13 (dd, *J* = 1.6/8.6 Hz, 1H), 7.08 (d, *J* = 1.6 Hz, 1H), 2.64 (t, *J* = 7.8 Hz, 2H), 2.33 (s, 3H), 1.60−1.47 (m, 3H), 0.92 (d, *J* = 6.4 Hz, 6H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6) δ = 166.6, 160.3, 159.6, 152.3, 143.9, 142.5, 141.7, 136.2, 133.8, 131.5, 130.0 (2 × C), 128.1, 127.8, 126.8 (2 × C), 125.4, 124.9, 114.9, 111.7, 104.9, 101.7, 40.3, 32.9, 27.1, 22.4 (2 × C), 21.0 ppm; MS (ESI−) m/z: 518.95 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{28}H_{28}N_2O_6S + H]^+$: 521.17408, found = 521.17342, Δ = 1.3 ppm; HPLC: t_R (Method C) = 10.84 min.

*N-(4-Hydroxy-7-(2-methylphenylsulfonamido)-2-oxo-2H-chromen-3-yl)-3-isopentylbenzamide (**14n**).* According to general procedure C: 293 mg (0.8 mmol) of **57**, 191 mg (0.96 mmol) of *ortho*-toluenesulfonyl chloride, and 128 μL (1.6 mmol) of pyridine in 10 mL of dry DCM. The desired product (234 mg, 0.45 mmol, 56%) was obtained as a colorless solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 12.00 (s, 1H), 11.26 (s, 1H), 9.40 (s, 1H), 8.00 (d, *J* = 7.6 Hz, 1H), 7.82 (s, 1H), 7.79−7.76 (m, 1H), 7.73 (d, *J* = 8.8 Hz, 1H), 7.54 (td, *J* = 0.9/7.5 Hz, 1H), 7.44−7.37 (m, 4H), 7.10 (dd, *J* = 1.9/8.7 Hz, 1H), 7.00 (d, *J* = 1.9 Hz, 1H), 2.65−2.62 (m, 5H), 1.59−1.47 (m, 3H), 0.92 (d, *J* = 6.4 Hz, 6H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6)

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

δ = 166.6, 160.3, 159.6, 152.3, 142.5, 141.4, 137.0, 136.9, 133.8, 133.5, 132.8, 131.5, 129.5, 128.1, 127.8, 126.6, 125.4, 125.0, 114.0, 111.2, 103.9, 101.6, 40.3, 32.9, 27.1, 22.4 (2 × C), 19.6 ppm; MS (ESI−) m/z: 519.00 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{28}H_{28}N_2O_6S + H]^+$: 521.17408, found: 521.17351, Δ = 1.1 ppm; HPLC: t_R (Method C) = 10.84 min.

*N-(4-Hydroxy-7-(naphthalene-1-sulfonamido)-2-oxo-2H-chromen-3-yl)-3-isopentylbenzamide (**14o**).* According to general procedure C: 300 mg (0.82 mmol) of **57**, 223 mg (0.98 mmol) of naphthalene-1-sulfonyl chloride, and 132 μL (1.64 mmol) of pyridine in 10 mL of dry DCM. The desired product (213 mg, 0.38 mmol, 46%) was obtained as a colorless solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 11.98 (bs, 1H), 11.42 (s, 1H), 9.41 (s, 1H), 8.75 (d, J = 8.6 Hz, 1H), 8.38 (d, J = 7.0 Hz, 1H), 8.24 (d, J = 8.1 Hz, 1H), 8.08 (d, J = 8.1 Hz, 1H), 7.82−7.77 (m, 3H), 7.70−7.66 (m, 3H), 7.39−7.35 (m, 2H), 7.09 (dd, J = 1.9/8.7 Hz, 1H), 7.03 (d, J = 1.9 Hz, 1H), 2.62 (t, J = 7.8 Hz, 2H), 1.55−1.45 (m, 3H), 0.89 (d, J = 6.3 Hz, 6H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6) δ = 166.6, 160.3, 159.4, 152.3, 142.6, 141.4, 135.0, 133.8, 133.8, 133.7, 131.6, 130.4, 129.3, 128.5, 128.1, 127.8, 127.3, 127.2, 125.4, 125.0, 124.6, 124.0, 114.1, 111.3, 104.0, 101.7, 40.3, 32.9, 27.1, 22.4 (2 × C) ppm; MS (ESI−) m/z: 555.00 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{31}H_{28}N_2O_6S + H]^+$: 557.17408, found: 557.17344, Δ = 1.1 ppm; HPLC: t_R (Method C) = 11.24 min.

*4-((4-Hydroxy-3-(3-isopentylbenzamido)-2-oxo-2H-chromen-7-yl)amino)-4-oxobutanoic Acid (**14p**).* According to general procedure C: 149 mg (0.41 mmol) of **57**, 50 mg (0.49 mmol) of succinic anhydride, and 66 μL (0.81 mmol) of pyridine in 5 mL of dry DCM. The desired product (51 mg, 0.11 mmol, 27%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.16 (bs, 1H), 11.97 (bs, 1H), 10.44 (s, 1H), 9.43 (s, 1H), 7.85−7.77 (m, 4H), 7.51 (dd, J = 1.9/8.7 Hz, 1H), 7.42−7.40 (m, 2H), 2.69−2.61 (m, 4H), 2.58−2.54 (m, 2H), 1.64−1.47 (m, 3H), 0.94 (d, J = 6.2 Hz, 6H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 173.7, 171.0, 166.7, 160.6, 159.6, 152.3, 142.8, 142.6, 133.8, 131.6, 128.1, 127.8, 125.4, 124.3, 115.0, 111.0, 105.1, 101.5, 40.4, 33.0, 31.3, 28.6, 27.1, 22.4 ppm; MS (ESI−) m/z: 465.30 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{25}H_{26}N_2O_7 + H]^+$: 467.18128, found: 467.17978, Δ = 3.2 ppm; HPLC: t_R (Method A) = 17.09 min.

*5-((4-Hydroxy-3-(3-isopentylbenzamido)-2-oxo-2H-chromen-7-yl)amino)-5-oxopentanoic Acid (**14q**).* According to general procedure C: 149 mg (0.41 mmol) of **57**, 56 mg (0.49 mmol) of glutaric anhydride, and 66 μL (0.81 mmol) of pyridine in 5 mL of dry DCM. The desired product (97 mg, 0.20 mmol, 50%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.09 (bs, 1H), 11.97 (bs, 1H), 10.36 (s, 1H), 9.43 (s, 1H), 7.85−7.78 (m, 4H), 7.52 (dd, J = 1.9/8.7 Hz, 1H), 7.42−7.40 (m, 2H), 2.66 (t, J = 7.7 Hz, 2H), 2.43 (d, J = 7.3 Hz, 2H), 2.30 (d, J = 7.3 Hz, 2H), 1.83 (quint, J = 7.3 Hz, 2H), 1.64−1.47 (m, 3H), 0.94 (d, J = 6.2 Hz, 6H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 174.1, 171.6, 166.7, 160.6, 159.7, 152.3, 142.8, 142.6, 133.8, 131.6, 128.1, 127.8, 125.4, 124.3, 115.1, 111.0, 105.2, 101.5, 40.3, 35.5, 32.9, 32.9, 27.1, 22.4 (2 × C), 20.2 ppm; MS (ESI−) m/z: 479.15 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{26}H_{28}N_2O_7$+Na$]^+$: 503.17887, found: 503.17773, Δ = 2.3 ppm; HPLC: t_R (Method A) = 17.83 min.

*N-(7-Acetamido-4-hydroxy-2-oxo-2H-chromen-3-yl)-3-isopentylbenzamide (**14r**).* According to general procedure C: 149 mg (0.41 mmol) of **57**, 46 μL (0.49 mmol) of acetic anhydride, and 66 μL (0.81 mmol) of pyridine in 5 mL of dry DCM. The desired product (60 mg, 0.15 mmol, 36%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.94 (bs, 1H), 10.40 (s, 1H), 9.44 (s, 1H), 7.85−7.78 (m, 4H), 7.50 (dd, J = 1.9/8.7 Hz, 1H), 7.43−7.39 (m, 2H), 2.67 (t, J = 7.7 Hz, 2H), 2.11 (s, 3H), 1.62−1.48 (m, 3H), 0.94 (d, J = 6.3 Hz, 6H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 169.1, 166.7, 160.6, 159.6, 152.3, 142.9, 142.6, 133.8, 131.5, 128.1, 127.8, 125.4, 124.3, 115.1, 111.0, 105.1, 101.5, 40.4, 32.9, 27.1, 24.2, 22.4 ppm; MS (ESI−) m/z: 407.10 $[M - H]^-$; HRMS (MALDI+) m/z: calcd for $[C_{23}H_{24}N_2O_5$+Na$]^+$: 431.15774, found: 431.15700, Δ = 1.7 ppm; HPLC: t_R (Method A) = 18.33 min.

General Procedure for Debenzylation and Reduction of the Nitro Group (Procedure D). The corresponding 7-benzyloxy-4-hydroxy-3-nitro-2*H*-chromen-2-one (1.0 equiv) was suspended in MeOH or EtOH, and palladium on charcoal (w = 10%) was added. The reaction mixture was stirred under hydrogen atmosphere at room temperature for 6 h before it was filtered through a Celite pad, which was rinsed with MeOH or EtOH (3 × 10 mL). The filtrate was dried in vacuo to obtain the desired product.

*3-Amino-4,7-dihydroxy-2H-chromen-2-one (**15a**).* According to general procedure D: 3.0 g (9.6 mmol) of **19a** and 0.3 g of palladium on charcoal (w = 10%) in 180 mL of EtOH. The desired product (1.8 g, 9.2 mmol, 96%) was obtained as a brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 10.06 (bs, 1H), 7.64 (d, J = 8.5 Hz, 1H), 6.65 (dd, J = 2.2/8.5 Hz, 1H), 6.53 (d, J = 2.2 Hz, 1H) ppm; MS (ESI+) m/z: 234.85 $[M + H$+ACN$]^+$.

*3-Amino-4,7-dihydroxy-8-methyl-2H-chromen-2-one (**15b**).* According to general procedure D: 1.60 g (4.89 mmol) of **19b**, 0.16 g palladium on charcoal (w = 10%) in 100 mL of EtOH. The desired product (943 mg, 4.55 mmol, 93%) was obtained as a brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.90 (bs, 1H), 7.50 (d, J = 8.4 Hz, 1H), 6.72 (d, J = 8.4 Hz, 1H), 3.65 (bs, 2H), 2.11 (s, 3H) ppm; MS (ESI+) m/z: 208.06 $[M + H]^+$.

*Methyl 4-Hydroxy-3-isopentylbenzoate (**49**).* According to general procedure D: 1.27 g (4.09 mmol) of **48** and 127 mg of palladium on charcoal w = 10% in 35 mL of MeOH. The desired product (894 mg, 4.02 mmol, 98%) was obtained as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.84 (d, J = 2.0 Hz, 1H), 7.78 (dd, J = 2.0/8.3 Hz, 1H), 6.79 (d, J = 8.3 Hz, 1H), 3.89 (s, 3H), 2.66−2.59 (t, J = 8.1 Hz, 2H), 1.69−1.46 (m, 3H), 0.95 (d, J = 6.4 Hz, 6H) ppm; MS (ESI+) m/z: 222.90 $[M + H]^+$.

*N-(7-Amino-4-hydroxy-2-oxo-2H-chromen-3-yl)-3-isopentylbenzamide (**57**).* According to general procedure D: 1.39 g (3.50 mmol) of **54** and 100 mg of palladium on charcoal (w = 10%) in 100 mL of EtOH, H_2 atmosphere. The desired product (1.25 g, 3.41 mmol, 97%) was obtained as a light brown solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 11.48 (bs, 1H), 9.30 (s, 1H), 7.85 (s, 1H), 7.82−7.79 (m, 1H), 7.54 (d, J = 8.6 Hz, 1H), 7.43−7.38 (m, 2H), 6.59 (dd, J = 1.9/8.6 Hz, 1H), 6.43 (d, J = 1.9 Hz, 1H), 6.12 (bs, 2H), 2.67 (t, J = 7.8 Hz, 2H), 1.60−1.49 (m, 3H), 0.94 (d, J = 6.4 Hz, 6H) ppm; MS (ESI−) m/z: 365.30 $[M - H]^-$.

*2-Isopentylbenzoic Acid (**40**).* According to general procedure D: 299 mg (1.0 mmol) of **39** and 45 mg of palladium on charcoal (w = 10%) in 15 mL of MeOH. The desired product (137 mg, 0.7 mmol, 66%) was obtained as a colorless solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.02 (d, J = 8.0 Hz, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.30−7.24 (m, 2H), 3.02 (t, J = 7.8 Hz, 2H), 1.74−1.46 (m, 3H), 0.96 (d, J = 6.2 Hz, 6H) ppm; MS (ESI+) m/z: 215.12 $[M + H]^+$.

*4-Fluoro-3-isopentylbenzoic Acid (**44**).* According to general procedure D: 1.54 g (5.2 mmol) of **43** and 150 mg of palladium on charcoal (w = 10%) in 20 mL of MeOH. The desired product (1.05 g, 5.0 mmol, 96%) was obtained as a colorless solid and directly used for amide coupling.

General Procedure for Boc Cleavage (Procedure E). The corresponding Boc protected 3-aminocoumarin was dissolved in MeOH and cooled to 0 °C. Then 4 M HCl in 1,4-dioxane (10 equiv) was added dropwise, and after addition was completed the reaction mixture was stirred for 5 h at room temperature. The resulting precipitate was filtrated and washed three times with diethyl ether and dried in vacuo.

*3-Amino-4-hydroxy-7-nitro-2H-chromen-2-one (**15c**).* According to general procedure E: 2.35 g (7.28 mmol) of **23a** and 18 mL (72 mmol) of 4 M HCl in 1,4-dioxane in 15 mL of MeOH. The desired product (2.20 g, 6.35 mmol, 99%) was obtained in the form of its hydrochloride 1,4-dioxane adduct as a yellow solid. ^{1}H NMR (250 MHz, DMSO-d_6) δ = 8.51 (bs, 4H), 8.12−8.07 (m, 2H), 8.03−7.99 (m, 1H) ppm; MS (ESI−) m/z: 221.10 $[M - H]^-$.

*3-Amino-7-bromo-4-hydroxy-2H-chromen-2-one (**15d**).* According to general procedure E: 4.70 g (13.2 mmol) of **23b** and 33 mL (132 mmol) of 4 M HCl in 1,4-dioxane in 27 mL of MeOH. The desired product (4.67, 12.3 mmol, 93%) was obtained in the form of

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

its hydrochloride 1,4-dioxane adduct as a yellow solid. ^{1}H NMR (250 MHz, DMSO-d_6) δ = 7.86 (d, J = 8.4 Hz, 1H), 7.60 (d, J = 1.8 Hz, 1H), 7.48 (dd, J = 1.8/8.4 Hz, 1H), 6.90 (bs, 4H) ppm; MS (ESI−) m/z: 255.75 $[M - H]^-$.

General Procedure for Benzylation of 2′,4′-Dihydroxyacetophenones (Procedure F). The respective 2′,4′-dihydroxyacetophenone (1.0 equiv) was dissolved in acetone, and potassium carbonate (2.0 equiv) and potassium iodide (0.1 equiv) were added. The suspension was stirred at 50 °C for 30 min before benzyl bromide (1.1 equiv) was added slowly. After the addition was completed, the reaction mixture was heated to reflux for 5 h. The suspension was cooled to room temperature and filtered, and the solvent was removed in vacuo. The crude product was purified by column chromatography (hexane/EtOAc 4:1).

*1-(4-(Benzyloxy)-2-hydroxyphenyl)ethanone (**17a**).* According to general procedure F: 2.0 g (13.2 mmol) of 2′,4′-dihydroxyacetophenone (**16a**), 1.7 mL (14.5 mmol) of benzyl bromide, 3.6 (26.3 mmol) of K_2CO_3, and 0.2 g (1.3 mmol) of potassium iodide in 66 mL of acetone. The desired product (3.1 g, 12.8 mmol, 97%) was obtained as an orange-colored solid. ^{1}H NMR (250 MHz, DMSO-d_6) δ = 12.61 (s, 1H), 7.84 (d, J = 8.9 Hz, 1H), 7.47−7.34 (m, 5H), 6.60 (dd, J = 2.4/8.9 Hz, 1H), 6.55 (d, J = 2.4 Hz, 1H), 5.19 (s, 2H), 2.56 (s, 3H) ppm; MS (ESI+) m/z: 242.90 $[M + H]^+$.

*1-(4-(Benzyloxy)-2-hydroxy-3-methylphenyl)ethanone (**17b**).* According to general procedure F: 5.0 g (30.0 mmol) of 2′,4′-dihydroxy-3′methylacetophenone (**16b**), 4.0 mL (33.0 mmol) of benzyl bromide, 8.3 g (60.0 mmol) of potassium carbonate, and 0.5 g (3.0 mmol) of potassium iodide in 150 mL of acetone. The desired product (7.3 g, 28.5 mmol, 95%) was obtained as an orange-colored solid. ^{1}H NMR (250 MHz, DMSO-d_6) δ = 12.86 (s, 1H), 7.81 (d, J = 9.0 Hz, 1H), 7.51−7.29 (m, 5H), 6.74 (d, J = 9.0 Hz, 1H), 5.27 (s, 2H), 2.58 (s, 3H), 2.06 (s, 3H) ppm; MS (ESI−) m/z: 255.00 $[M - H]^-$.

General Procedure for Cyclization of Acetophenones (Procedure G). 4-Benzyloxy-2-hydroxyacetophenone (1.0 equiv) was added slowly to a solution of sodium hydride (w = 60% in mineral oil, 4.0 equiv) in dry toluene, and the mixture was heated up to 120 °C. Diethyl carbonate (4.0 equiv) was added dropwise, and after completion the reaction mixture was stirred at 140 °C overnight. Most of the solvent was removed under reduced pressure, and the residue was treated with ice cold water. The two phases were separated, and the organic phase was extracted with water (2 × 100 mL). The toluene phase was discarded, while all aqueous phases were combined. The combined aqueous phases were acidified with 2 M aqueous HCl and extracted with EtOAc (3 × 100 mL). The combined organic phases were dried over $MgSO_4$ and filtered, and the solvent was removed in vacuo. The products were pure enough without any further purification.

*7-(Benzyloxy)-4-hydroxy-2H-chromen-2-one (**18a**).* According to general procedure G: 4.90 g (20.3 mmol) of **17a**, 9.6 mL (78.0 mmol) of diethyl carbonate, 2.00 g (78 mmol) of sodium hydride (w = 60% in mineral oil), and 200 mL of toluene. The desired product (4.90 g, 18.3 mmol, 90%) was obtained as a pale orange-colored solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 12.38 (s, 1H), 7.71 (d, J = 8.7, 1H), 7.50−7.30 (m, 5H), 7.05−6.97 (m, 2H), 5.47 (s, 1H), 5.21 (s, 2H) ppm; MS (ESI−) m/z: 267.09 $[M - H]^-$.

*7-(Benzyloxy)-4-hydroxy-8-methyl-2H-chromen-2-one (**18b**).* According to general procedure G: 4.0 g (15.6 mmol) of **17b**, 7.6 mL (62.4 mmol) of diethyl carbonate, 2.5 g (62,4 mmol) of sodium hydride (w = 60% in mineral oil), and 150 mL of toluene. The desired product (3.95 g, 13.99 mmol, 90%) was obtained as a pale orange-colored solid. ^{1}H NMR (250 MHz, DMSO-d_6) δ = 12.27 (s, 1H), 7.64 (d, J = 8.8, 1H), 7.52−7.28 (m, 5H), 7.12 (d, J = 8.8 Hz, 1H), 5.46 (s, 1H), 5.25 (s, 2H), 2.22 (s, 3H) ppm; MS (ESI−) m/z: 281.30 $[M - H]^-$.

General Procedure for Nitration of 4-Hydroxycoumarins (Procedure H). The corresponding 4-hydroxycoumarin (1.0 equiv) and sodium nitrite (0.05 equiv) were suspended in acetic acid. A solution of nitric acid (w = 65%, 2.0 equiv) and acetic acid was added slowly. After the addition was completed, the reaction mixture was heated up and stirred for 15 min at 60 °C. The suspension was cooled to room temperature, and the precipitate was separated by filtration and washed with ice cold water and dried in vacuo.

*7-(Benzyloxy)-4-hydroxy-3-nitro-2H-chromen-2-one (**19a**).* According to general procedure H: 2.15 g (8.0 mmol) of **18a**, 0.03 g (0.38 mmol) of $NaNO_2$, 1.1 mL of HNO_3 (w = 65%, 16.0 mmol), and 15 mL of AcOH. The desired product (1.72 g, 5.49 mmol, 69%) was obtained as a light brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.80 (d, J = 8.8 Hz, 1H), 7.41 (m, 5H), 6.88 (m, 2H), 5.18 (s, 2H) ppm; MS (ESI−) m/z: 311.80 $[M - H]^-$.

*7-(Benzyloxy)-4-hydroxy-8-methyl-3-nitro-2H-chromen-2-one (**19b**).* According to general procedure H: 2.09 g (7.4 mmol) of **18b**, 0.03 g (0.35 mmol) of $NaNO_2$, 1,0 mL of HNO_3 (w = 65%, 14.8 mmol), and 15 mL of AcOH. The desired product (1.62 g, 4.95 mmol, 67%) was obtained as a light brown solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.73 (d, J = 8.8 Hz, 1H), 7.49−7.33 (m, 5H), 7.02 (d, J = 8.8 Hz, 1H), 5.22 (s, 2H), 2.17 (s, 3H) ppm; MS (ESI−) m/z: 325.80 $[M - H]^-$.

General Procedure for Acetylation of 2-Hydroxy Benzoic Acid Derivatives (Procedure I). The reactions were realized under exclusion of light. The corresponding 2-hydroxybenzoic acid (1.0 equiv) was dissolved in EtOAc. Then triethylamine (2.0 equiv), acetic anhydride (2.0 equiv) and DMAP (0.1 equiv) were added. The reaction mixture was stirred for 12 h at 80 °C. After the reaction was completed, the reaction mixture was filtered over Celite. The filtrate was acidified with 2 N aqueous HCl and extracted with EtOAc (3 × 50 mL). The combined organic phase was washed with brine (50 mL), dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The product could be isolated after purification through column chromatography (DCM/MeOH 95:5 + 1% acetic acid).

*2-Acetoxy-4-nitrobenzoic Acid (**21a**).* According to general procedure I: 3.96 (21 mmol) of 2-hydroxy-4-nitrobenzoic acid (**20a**), 5.9 mL (42 mmol) of triethylamine, 3.97 mL (42 mmol) of Ac_2O, 0.26 g (2.1 mmol) of DMAP, and 21 mL of EtOAc. The desired product (4.10 g, 18.2 mmol, 87%) was obtained as a fawn solid. ^{1}H NMR (250 MHz, DMSO-d_6) δ = 8.19 (d, J = 2.6 Hz, 1H), 8.15 (s, 1H), 8.12 (d, J = 1.7 Hz, 1H), 2.29 (s, 3H) ppm; MS (ESI+) m/z: 225.95 $[M + H]^+$.

*2-Acetoxy-4-bromobenzoic Acid (**21b**).* According to general procedure I: 4.99 g (23.0 mmol) of 4-bromo-2-hydroxybenzoic acid (**20b**), 4.39 mL (46.0 mmol) of Ac_2O, 6.46 mL (46.0 mmol) of triethylamine, 0.28 g (2.30 mmol) of DMAP, and 23 mL of EtOAc. The desired product (5.23 g, 20.2 mmol, 88%) was obtained as a fawn solid. ^{1}H NMR (250 MHz, DMSO-d_6) δ = 12.88 (bs, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.59 (dd, J = 2.0/8.4 Hz, 1H), 7.52 (d, J = 2.0 Hz, 1H), 2.24 (s, 3H) ppm; MS (ESI−) m/z: 216.70 $[M - Ac]^-$.

*2-((tert-Butoxycarbonyl)amino)-3-ethoxy-3-oxopropanoic Acid (**22b**).* $NaHCO_3$ (4.15 g, 49.3 mmol, 1.05 equiv) was added slowly to a suspension of diethyl aminomalonate hydrochloride (**22a**) (10.2 g, 47.0 mmol, 1.0 equiv) in a mixture of water (60 mL) and 1,4-dioxane (88 mL) and stirred at room temperature. After the solution turned clear, a catalyst amount of DMAP (0.06 g, 0.47 mmol, 1.0 equiv) was added. A solution of Boc_2O (10.8 g, 49.3 mmol, 1.05 equiv) in 1,4-dioxane (32 mL) was added dropwise, and the reaction mixture was stirred for an additional 5 h at room temperature. The solvents were evaporated in vacuo, and the residue was dissolved in EtOAc. The organic phase was washed with aqueous solutions of $KHSO_4$ (w = 5%), sat. $NaHCO_3$, and brine. The organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated in vacuo. The desired product (12.3 g, 44.7 mmol, 95%) was obtained as a colorless viscous oil and was pure enough for the next step without further purification needed.

The crude product was dissolved in abs. EtOH (30 mL) ,and a solution of KOH (2.51 g, w = 85%, 38 mmol, 1.0 equiv) in abs. EtOH (50 mL) was added dropwise. The reaction mixture was stirred at room temperature for 3 h. After the reaction was completed, the solvent was removed in vacuo. The residue was taken up in aqueous 1N $NaHCO_3$ solution (80 mL) and washed two times with EtOAc (2 × 25 mL). The aqueous phase was cooled to 0 °C and acidified by solid $KHSO_4$ and extracted three times with EtOAc (3 × 50 mL). The

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

organic phase was washed with brine, dried over anhydrous $MgSO_4$, filtered, and evaporated in vacuo. The desired product (8.2 g, 33.2 mmol, 87%) was obtained as a colorless viscous oil and was pure enough for the next step without further purification needed. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.34 (bs, 1H), 7.47 (d, J = 8.0 Hz, 1H), 4.71 (d, J = 8.0 Hz, 1H), 4.18−4.10 (m, 2H), 1.39 (s, 9H), 1.19 (t, J = 7.1 Hz, 3H) ppm; MS (ESI−) m/z: 245.90 $[M - H]^-$.

General Procedure for Benzoic Acid Chloride Formation (Procedure J). The corresponding 2-acetoxybenzoic acid (1.0 equiv) was dissolved in dry DCM under argon atmosphere. After the dropwise addition of thionyl chloride (20 equiv), the reaction mixture was refluxed for 5 h. Then the solvent and unreacted thionyl chloride were removed under reduced pressure. The resulting residue was dried in vacuo and used in the next synthesis step without further purification.

General Procedure for C−C Coupling (Procedure K). To an ice-cooled solution of **22b** (1.4 equiv) in dry THF and triethylamine (6.3 equiv), anhydrous $MgCl_2$ (3.4 equiv) was added. Then a solution of the corresponding benzoic acid chloride (1.0 equiv) in dry THF was added, and the resulting suspension was stirred for 12 h, while it was allowed to warm up to room temperature. The reaction was quenched by addition of sat. aqueous NH_4Cl solution until the reaction mixture turned clear and then was extracted three times with EtOAc (3 × 50 mL). The combined organic phase was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The resulting oil was used in the next synthesis step without further purification.

General Procedure for Cyclization of Salicyl Derivatives (Procedure L). The corresponding product of the C−C coupling (1.0 equiv) was dissolved in MeOH, and 1.5 M aqueous NaOH solution (6.0 equiv) was added. The reaction mixture was stirred for 3 h at room temperature. Then the solution was adjusted to pH 3 using 1.5 M aqueous HCl. The aqueous phase was extracted three times with EtOAc (3 × 30 mL). The combined organic phase was washed with brine, dried over $MgSO_4$, and filtered, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (DCM/MeOH 9:1).

*tert-Butyl (4-Hydroxy-7-nitro-2-oxo-2H-chromen-3-yl)-carbamate (**23a**).* The acyl chloride intermediate was prepared according to general procedure J and was used without purification: 6.08 g (27.0 mmol) of **21a** and 39 mL (540 mmol) of $SOCl_2$ in 54 mL of DCM. Coupling was performed according to procedure K and used without purification: 9.35 g (37.8 mmol) of **22b**, 8.74 g (91.8 mmol) of $MgCl_2$, and 23.9 mL (170 mmol) of triethylamine in 160 mL of THF. Cyclization was performed according to procedure L: 6.48 g (162 mmol) of NaOH in 100 mL of H_2O and 81 mL of MeOH. 4.70 g (14.6 mmol, 54% over three steps) of product **23a** was obtained. ^{1}H NMR (250.0 MHz, DMSO-d_6) δ = 12.43 (s, 1H), 8.22 (d, J = 1.9 Hz, 1H), 8.19 (d, J = 2.2 Hz, 1H), 8.16 (d, J = 2.2 Hz, 1H), 8.10 (s, 1H), 1.41 (s, 9H) ppm; MS (ESI−) m/z: 321.10 $[M - H]^-$.

*tert-Butyl (7-Bromo-4-hydroxy-2-oxo-2H-chromen-3-yl)-carbamate (**23b**).* The acyl chloride intermediate was prepared according to general procedure J and was used without purification: 5.96 g (23.0 mmol) of **21b** and 33 mL (460 mmol) of $SOCl_2$ in 46 mL of DCM. Coupling was performed according to procedure K and used without purification: 7.96 g (32.2 mmol) of **22b**, 7.45 g (78.2 mmol) of $MgCl_2$, and 20.4 mL (145 mmol) of triethylamine in 130 mL of THF. Cyclization was performed according to procedure L: 5.52 g (138 mmol) of NaOH in 92 mL of H_2O and 70 mL of MeOH. 4.70 g (13.2 mmol, 57% over three steps) of product **23b** was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 12.24 (bs, 1H), 8.01 (bs, 1H), 7.77 (d, J = 8.5 Hz, 1H), 7.72 (d, J = 1.8 Hz, 1H), 7.56 (dd, J = 1.8/8.5 Hz, 1H), 1.42 (s, 9H) ppm; MS (ESI−) m/z: 355.75 $[M - H]^-$.

General Procedure for Alkaline Hydrolysis of Esters (Procedure M). The respective product of alkene reduction (1.0 equiv) was dissolved in a 1:2 mixture of MeOH and THF. An aqueous solution of NaOH or KOH (1M, 5.0−6.0 equiv) was added, and the reaction mixture was heated to reflux for 5 h. After cooling to room temperature, the organic solvents were removed in vacuo and the aqueous residue was acidified with 2 M aqueous HCl. After extraction with DCM (3 × 15 mL), the combined organic phases were dried over $MgSO_4$ and filtered and the solvent was removed in vacuo. The obtained products could be used in following synthesis without further purification.

*3-Methoxybenzoic Acid (**24b**).* According to general procedure M: 0.98 g (5.9 mmol) of **26b**, 1.95 g (29.5 mmol) of KOH in 15 mL of THF, 15 mL of MeOH, and 30 mL of H_2O. The desired product (0.82 g, 5.39 mmol, 91%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 12.97 (bs, 1H), 7.53 (d, J = 7.6 Hz, 1H), 7.44−7.39 (m, 2H), 7.20−7.17 (m, 1H), 3.80 (s, 3H) ppm; MS (ESI−) m/z: 151.90 $[M - H]^-$.

*3-Ethoxybenzoic Acid (**24c**).* According to general procedure M: 1.09 g (6.05 mmol) of **26c**, 2.00 g (30.3 mmol) of KOH in 15 mL of THF, 15 mL of MeOH, and 30 mL of H_2O. The desired product (0.88 g, 5.30 mmol, 88%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 12.95 (bs, 1H), 7.51 (d, J = 7.6 Hz, 1H), 7.42−7.37 (m, 2H), 7.18−7.15 (m, 1H), 4.06 (q, J = 7.0 Hz, 2H), 1.33 (t, J = 7.0 Hz, 3H) ppm; MS (ESI−) m/z: 164.85 $[M - H]^-$.

*3-Propoxybenzoic Acid (**24d**).* According to general procedure M: 0.32 g (1.65 mmol) of **26d**, 0.55 g (8.25 mmol) of KOH in 10 mL of THF, 10 mL of MeOH, and 20 mL of H_2O. The desired product (0.27 g, 1.50 mmol, 91%) was obtained as a colorless solid. ^{1}H NMR (250 MHz, DMSO-d_6) δ = 12.94 (bs, 1H), 7.51 (d, J = 7.6 Hz, 1H), 7.42−7.36 (m, 2H), 7.19−7.15 (m, 1H), 3.97 (t, J = 6.5 Hz, 2H), 1.73 (sext, J = 7.0 Hz, 2H), 0.98 (t, J = 7.4 Hz, 3H) ppm; MS (ESI−) m/z: 178.90 $[M - H]^-$.

*3-Butoxybenzoic Acid (**24e**).* According to general procedure M: 1.29 g (6.20 mmol) of **26e**, 2.05 g (31.0 mmol) of KOH in 20 mL of THF, 20 mL of MeOH, and 40 mL of H_2O. The desired product (1.14 g, 5.87 mmol, 95%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 12.96 (bs, 1H), 7.53 (d, J = 7.7 Hz, 1H), 7.44−7.38 (m, 2H), 7.20−7.16 (m, 1H), 4.01 (t, J = 6.3 Hz, 2H), 1.75−1.68 (m, 2H), 1.50−1.40 (m, 2H), 0.94 (t, J = 7.4 Hz, 3H) ppm; MS (ESI−) m/z: 193.00 $[M - H]^-$.

*3-(Cyclopropylmethoxy)benzoic Acid (**24f**).* According to general procedure M: 1.22 g (5.92 mmol) of **26f**, 1.95 g (29.6 mmol) of KOH in 25 mL of THF, 25 mL of MeOH, and 50 mL of H_2O. The desired product (0.94 g, 4.89 mmol, 83%) was obtained as a pale-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 12.94 (bs, 1H), 7.52−7.49 (m, 1H), 7.41−7.37 (m, 2H), 7.19−7.16 (m, 1H), 3.85 (d, J = 7.0 Hz, 2H), 1.27−1.16 (m, 1H), 0.58−0.55 (m, 2H), 0.36−0.32 (m, 2H) ppm; MS (ESI−) m/z: 190.85 $[M - H]^-$.

*3-Isopropoxybenzoic Acid (**24g**).* According to general procedure M: 0.98 g (5.05 mmol) of **26g**, 1.67 g (25.3 mmol) of KOH in 15 mL of THF, 15 mL of MeOH, and 30 mL of H_2O. The desired product (0.83 g, 4.61 mmol, 91%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 12.95 (bs, 1H), 7.52−7.50 (m, 1H), 7.43−7.37 (m, 2H), 7.18−7.15 (m, 1H), 4.66 (sept, J = 6.0 Hz, 1H), 1.28 (d, J = 6.0 Hz, 6H) ppm; MS (ESI−) m/z: 178.95 $[M - H]^-$.

*3-(Allyloxy)benzoic Acid (**24h**).* According to general procedure M: 1.15 g (5.98 mmol) of **26h**, 1.97 g (29.9 mmol) of KOH in 25 mL of THF, 25 mL of MeOH, and 50 mL of H_2O. The desired product (1.02 g, 5.72 mmol, 96%) was obtained as a pale-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 12.98 (bs, 1H), 7.56−7.51 (m, 1H), 7.47−7.44 (m, 1H), 7.40 (t, J = 7.8 Hz, 1H), 7.21−7.18 (m, 1H), 6.09−5.99 (m, 1H), 5.42−5.36 (m, 1H), 5.28−5.25 (m, 1H) ppm; MS (ESI−) m/z: 176.85 $[M - H]^-$.

*3-(Benzyloxy)benzoic Acid (**24i**).* According to general procedure M: 1.58 g (6.52 mmol) of **26i**, 2.15 g (32.6 mmol) of KOH in 25 mL of THF, 25 mL of MeOH, and 50 mL of H_2O. The desired product (1.41 g, 6.18 mmol, 95%) was obtained as a pale-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 12.97 (bs, 1H), 7.56−7.52 (m, 2H), 7.47−7.38 (m, 5H), 7.35−7.33 (m, 1H), 7.28−7.25 (m, 1H), 5.16 (s, 2H) ppm; MS (ESI−) m/z: 226.95 $[M - H]^-$.

*3-Isopentylbenzoic Acid (**36a**).* According to general procedure M: 339 mg (1.64 mmol) of **35a**, 461 mg (8.22 mmol) of NaOH, 20 mL of THF, 10 mL of MeOH, and 10 mL of H_2O. The desired product (235 mg, 1.22 mmol, 75%) was obtained as a colorless solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.95−7.91 (m, 2H), 7.46−7.35 (m,

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

2H), 2.71−2.65 (m, 2H), 1.65−1.49 (m, 3H), 0.95 (d, J = 6.3 Hz, 6H) ppm; MS (ESI+) m/z: 190.95 $[M - H]^-$.

*3-Propylbenzoic Acid (**36c**).* According to general procedure M: 235 mg (1.32 mmol) of **35c**, 435 mg (6.59 mmol) of NaOH, 20 mL of THF, 10 mL of MeOH, and 10 mL of H_2O. The desired product (178 mg, 1.08 mmol, 82%) was obtained as a colorless solid. ^{1}H NMR (250 MHz, $CDCl_3$): 7.96−7.93 (m, 2H), 7.47−7.36 (m, 2H), 2.69−2.63 (m, 2H), 1.68 (sext, J = 7.6 Hz, 2H), 0.96 (t, J = 7.3 Hz, 3H) ppm; MS (ESI+) m/z: 162.85 $[M - H]^-$.

*3-Butylbenzoic Acid (**36d**).* According to general procedure M: 403 mg (2.10 mmol) of **35d**, 692 mg (10.5 mmol) of NaOH, 20 mL of THF, 10 mL of MeOH, and 10 mL of H_2O. The desired product (337 mg, 1.89 mmol, 90%) was obtained as a colorless solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.96−7.92 (m, 2H), 7.46−7.35 (m, 2H), 2.72−2.65 (m, 2H), 1.70−1.58 (m, 2H), 1.48−1.22 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H) ppm; MS (ESI+) m/z: 176.85 $[M - H]^-$.

*3-Pentylbenzoic Acid (**36e**).* According to general procedure M: 379 mg (1.84 mmol) of **35e**, 606 mg (9.19 mmol) of NaOH, 20 mL of THF, 10 mL of MeOH, and 10 mL of H_2O. The desired product (296 mg, 1.54 mmol, 84%) was obtained as a colorless solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.95−7.92 (m, 2H), 7.43−7.38 (m, 2H), 2.71−2.64 (m, 2H), 1.71−1.59 (m, 2H), 1.41−1.25 (m, 4H), 0.91 (t, J = 6.8 Hz, 3H) ppm; MS (ESI+) m/z: 190.85 $[M - H]^-$.

*3-(2-Cyclopropylethyl)benzoic Acid (**36f**).* According to general procedure M: 469 mg (2.30 mmol) of **35f**, 758 mg (11.5 mmol) of NaOH, 20 mL of THF, 10 mL of MeOH, and 10 mL of H_2O. The desired product (378 mg, 1.99 mmol, 86%) was obtained as a colorless solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.96−7.92 (m, 2H), 7.47−7.35 (m, 2H), 2.82−2.65 (m, 2H), 1.68−1.48 (m, 2H), 1.36−1.28 (m, 1H), 0.47−0.40 (m, 2H), 0.08−0.02 (m, 2H) ppm; MS (ESI+) m/z: 188.90 $[M - H]^-$.

*3-Isobutylbenzoic Acid (**36g**).* According to general procedure M: 163 mg (0.85 mmol) of **35g**, 280 mg (4.24 mmol) of NaOH, 20 mL of THF, 10 mL of MeOH, and 10 mL of H_2O. The desired product (109 mg, 0.61 mmol, 72%) was obtained as a colorless solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.97−7.90 (m, 2H), 7.40−7.37 (m, 2H), 2.55 (d, J = 7.2 Hz, 2H), 1.91 (sept., J = 6.8 Hz, 1H), 0.92 (d, J = 6.6 Hz, 6H) ppm; MS (ESI+) m/z: 177.05 $[M - H]^-$.

*3-Phenethylbenzoic Acid (**36i**).* According to general procedure M: 221 mg (0.92 mmol) of **35i**, 304 mg (4.60 mmol) of NaOH, 20 mL of THF, 10 mL of MeOH, and 10 mL of H_2O. The desired product (162 mg, 0.72 mmol, 78%) was obtained as a colorless solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.90−7.86 (m, 2H), 7.33−7.30 (m, 2H), 7.25−7.09 (m, 5H), 2.97−2.84 (m, 4H) ppm; MS (ESI+) m/z: 224.90 $[M - H]^-$.

*3-Isopentyl-4-methoxybenzoic Acid (**53**).* According to general procedure M: 260 mg (1.1 mmol) of **52**, 363 mg (5.5 mmol) of KOH, 12 mL of THF, 6 mL of MeOH, and 6 mL of H_2O. The desired product (232 mg, 1.04 mmol, 95%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.97 (dd, J = 2.2/8.6 Hz, 1H), 7.89 (d, J = 2.2 Hz, 1H), 6.88 (d, J = 8.6 Hz, 1H), 3.90 (s, 3H), 2.66−2.61 (m, 2H), 1.61 (non, J = 6.5 Hz, 1H), 1.52−1.44 (m, 2H), 0.95 (d, J = 6.5 Hz, 6H) ppm; MS (ESI+) m/z: 220.95 $[M - H]^-$.

*4-Hydroxy-3-isopentylbenzoic Acid (**50**).* According to general procedure M: 534 mg (2.4 mmol) of **49**, 793 mg (12.0 mmol) of KOH, 30 mL of THF, 15 mL of MeOH, and 15 mL of H_2O. The desired product (485 mg, 2.33 mmol, 97%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.91 (d, J = 2.1 Hz, 1H), 7.86 (dd, J = 2.1/8.3 Hz, 1H), 6.81 (d, J = 8.3 Hz, 1H), 2.67−2.2.61 (m, 2H), 1.64 (non, J = 6.6 Hz, 1H), 1.56−1.49 (m, 2H), 0.97 (d, J = 6.6 Hz, 6H) ppm; MS (ESI−) m/z: 206.95 $[M - H]^-$.

General Procedure for Alkylation of Methyl 3-Hydroxybenzoate (Procedure N). Methyl 3-hydroxy-benzoate (**25**, 1.0 equiv) was dissolved in acetone, AcN, or DMF, and K_2CO_3 (2.0 equiv) was added. Depending on the alkylating agent, potassium iodide (0.5 equiv) was added. After stirring for 30 min, a solution of the corresponding alkylating agent (1.1 equiv) in 5 mL of solvent was added dropwise and the reaction mixture was refluxed for 6 h. Then the reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated in vacuo, and the residue was purified by column chromatography (hexane/EtOAc 9:1).

*Methyl 3-Methoxybenzoate (**26b**).* According to general procedure N: 1.00 g (6.57 mmol) of methyl 3-hydroxybenzoate (**25**), 0.69 mL (7.23 mmol) of dimethyl sulfate, and 1.82 g (13.1 mmol) of K_2CO_3 in 30 mL of acetone. The desired product (1.0 g, 5.9 mmol, 90%) was obtained as a colorless liquid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.56−7.54 (1H, m), 7.46−7.42 (m, 2H), 7.24−7.21(m, 1H), 3.85 (s, 3H), 3.81 (s,3H) ppm; MS (ESI+) m/z: 167.00 $[M + H]^+$.

*Ethyl 3-Methoxybenzoate (**26c**).* According to general procedure N: 1.00 g (6.57 mmol) of methyl 3-hydroxybenzoate (**25**), 0.54 mL (7.23 mmol) of bromoethane, and 1.82 g (13.1 mmol) of K_2CO_3 in 30 mL of acetone. The desired product (1.09 g, 5.99 mmol, 91%) was obtained as a colorless liquid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.54−7.52 (m, 1H), 7.44−7.40 (m, 2H), 7.22−7.21 (m, 1H), 4.07 (q, J = 7.0 Hz, 2H), 3.84 (s, 3H), 1.34 (t, J = 7.0 Hz, 3H) ppm; MS (ESI+) m/z: 180.90 $[M + H]^+$.

*Methyl 3-Propoxybenzoate (**26d**).* According to general procedure N: 1.00 g (6.57 mmol) of methyl 3-hydroxybenzoate (**25**), 0.64 mL (7.23 mmol) of 1-chloropropane, and 1.82 g (13.1 mmol) of K_2CO_3 in 30 mL of acetone. The desired product (0.32 g, 1.65 mmol, 25%) was obtained as a colorless liquid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.54−7.52 (m, 1H), 7.44−7.40 (m, 2H), 7.22−7.20 (m, 1H), 3.97 (t, J = 6.5 Hz, 2H), 3.84 (s, 3H), 1.74 (sext, J = 7.0 Hz, 2H), 0.98 (t, J = 7.4 Hz, 3H) ppm; MS (ESI+) m/z: 194.40 $[M + H]^+$.

*Methyl 3-Butoxybenzoate (**26e**).* According to general procedure N: 1.00 g (6.57 mmol) of methyl 3-hydroxybenzoate (**25**), 0.78 mL (7.23 mmol) of 1-bromobutane, 1.82 g (13.1 mmol) of K_2CO_3, and 0.55 g (3.29 mmol) of potassium iodide in 30 mL of acetonitrile. The desired product (1.29 g, 6.19 mmol, 94%) was obtained as a colorless liquid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.52 (d, J = 7.6 Hz, 1H), 7.44−7.40 (m, 2H), 7.22−7.19 (m, 1H), 4.02 (t, J = 6.5 Hz, 2H), 3.84 (s, 3H), 1.74−1.66 (m, 2H), 1.49−1.39 (m, 2H), 0.93 (t, J = 7.4 Hz, 3H) ppm; MS (ESI+) m/z: 208.90 $[M + H]^+$.

*Methyl 3-(Cyclopropylmethoxy)benzoate (**26f**).* According to general procedure N: 1.00 g (6.57 mmol) of methyl 3-hydroxybenzoate (**25**), 0.70 mL (7.23 mmol) of (bromomethyl)-cyclopropane, 1.82 g (13.1 mmol) of K_2CO_3, and 0.55 g (3.29 mmol) of potassium iodide in 30 mL of acetonitrile. The desired product (1.22 g, 5.92 mmol, 90%) was obtained as a pale-yellow liquid. H NMR (400 MHz, DMSO-d_6) δ = 7.54−7.51 (m, 1H), 7.44−7.39 (m, 2H), 7.23−7.19 (m, 1H), 3.86 (d, J = 7.2 Hz, 2H), 3.84 (s, 3H), 1.27−1.18 (m, 1H), 0.59−0.55 (m, 2H), 0.36−0.32 (m, 2H) ppm; MS (ESI+) m/z: 206.85 $[M + H]^+$.

*Methyl 3-Isopropoxybenzoate (**26g**).* According to general procedure N: 1.00 g (6.57 mmol) of methyl 3-hydroxybenzoate (**25**), 0.72 mL (7.23 mmol) of 2-iodopropane, and 1.82 g (13.1 mmol) of K_2CO_3 in 30 mL of acetonitrile. The desired product (0.98 g, 5.05 mmol, 77%) was obtained as a colorless liquid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.50 (d, J = 7.7 Hz, 1H), 7.43−7.39 (m, 2H), 7.21−7.18 (m, 1H), 4.66 (sept, J = 6.0 Hz, 1H), 3.84 (s, 3H), 1.27 (d, J = 6.0 Hz, 6H) ppm; MS (ESI+) m/z: 194.95 $[M + H]^+$.

*Methyl 3-(Allyloxy)benzoate (**26h**).* According to general procedure N: 1.00 g (6.57 mmol) of methyl 3-hydroxybenzoate (**25**), 0.62 mL (7.23 mmol) of allyl bromide, 1.82 g (13.1 mmol) of K_2CO_3, 0.55 g (3.29 mmol) of potassium iodide in 30 mL of acetone. The desired product (1.15 g, 5.98 mmol, 91%) was obtained as a colorless liquid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.56−7.53 (m, 1H), 7.46−7.41 (m, 2H), 7.25−7.22 (m, 1H), 6.09−6.00 (m, 1H), 5.43−5.37 (m, 1H), 5.29−5.25 (m, 1H), 4.64−4.62 (m, 2H) ppm; MS (ESI+) m/z: 193.30 $[M + H]^+$.

*Methyl 3-(Benzyloxy)benzoate (**26i**).* According to general procedure N: 1.00 g (6.57 mmol) of methyl 3-hydroxybenzoate (**25**), 0.86 mL (7.23 mmol) of benzyl bromide, 1.82 g (13.1 mmol) of K_2CO_3, and 0.55 g (3.29 mmol) of potassium iodide in 30 mL of acetone. The desired product (1.56 g, 6.44 mmol, 98%) was obtained as a pale-yellow liquid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.57−7.52 (m, 2H), 7.47−7.38 (m, 5H), 7.35−7.29 (m, 2H), 5.17 (s, 2H), 3.84 (s, 3H) ppm; MS (ESI−) m/z: 241.95 $[M - H]^-$.

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

General Procedure for Alkylation of 3-Mercaptobenzoic Acid (Procedure O). 3-Mercaptobenzoic acid (**27**, 1.0 equiv) was dissolved in aqueous ethanol solution (v/v = 30%), and a solution of NaOH (2.0 equiv) in 5 mL of H_2O was added. Then a solution of the corresponding alkyl halide (2.0 equiv) in 5 mL of EtOH was added dropwise, and the reaction mixture was stirred at 60 °C for 12 h. Then the solvent was removed under vacuo, and the aqueous residue was acidified with 2 M aqueous HCl. The resulting precipitate was filtered and dried in vacuo to give the desired product.

*3-(Isobutylthio)benzoic Acid (**28a**).* According to general procedure O: 308 mg (2.0 mmol) of 3-mercaptobenzoic acid (**27**), 431 μL (4.0 mmol) of 1-bromo-2-methylpropane, 160 mg (4.0 mmol) of NaOH in 35 mL of H_2O and 15 mL of EtOH. The desired product (376 mg, 1.79 mmol, 89%) was obtained as a pale-yellow solid. ^{1}H NMR (250 MHz, DMSO-d_6) δ = 13.07 (s, 1H), 7.81 (t, J = 1.6 Hz, 1H), 7.72 (dt, J = 1.3/7.6 Hz, 1H), 7.59−7.52 (m, 1H), 7.43 (t, J = 7.6 Hz, 1H), 2.90 (d, J = 6.7 Hz, 2H), 1.80 (non, J = 6.7 Hz, 1H), 0.99 (d, J = 6.6 Hz, 6H) ppm; MS (ESI−) m/z: 209.05 [M − H]$^-$.

*3-(Ethylthio)benzoic Acid (**28c**).* According to general procedure O: 154 mg (1.0 mmol) of 3-mercaptobenzoic acid (**27**), 150 μL (2.0 mmol) of bromoethane, 80 mg (2.0 mmol) of NaOH in 35 mL of H_2O and 15 mL of EtOH. The desired product (126 mg, 0.69 mmol, 69%) was obtained as a colorless solid. ^{1}H NMR (250 MHz, DMSO-d_6) δ = 13.08 (s, 1H), 7.80 (t, J = 1.6 Hz, 1H), 7.73 (dt, J = 1.3/7.6 Hz, 1H), 7.59−7.52 (m, 1H), 7.44 (t, J = 7.6 Hz, 1H), 3.02 (q, J = 7.3 Hz, 2H), 1.24 (t, J = 7.3 Hz, 3H) ppm; MS (ESI−) m/z: 180.80 [M − H]$^-$.

*3-(Propylthio)benzoic Acid (**28d**).* According to general procedure O: 154 mg (1.0 mmol) of 3-mercaptobenzoic acid (**27**), 176 μL (2.0 mmol) of 1-chloropropane, 80 mg (2.0 mmol) of NaOH in 35 mL of H_2O and 15 mL of EtOH. The desired product (164 mg, 0.84 mmol, 84%) was obtained as a pale-yellow solid. ^{1}H NMR (250 MHz, DMSO-d_6) δ = 13.08 (s, 1H), 7.80 (t, J = 1.6 Hz, 1H), 7.73 (dt, J = 1.3/7.7 Hz, 1H), 7.58−7.54 (m, 1H), 7.44 (t, J = 7.7 Hz, 1H), 2.99 (q, J = 7.3 Hz, 2H), 1.60 (sext, J = 7.3 Hz, 2H) 0.98 (t, J = 7.3 Hz, 3H) ppm; MS (ESI−) m/z: 194.85 [M − H]$^-$.

*3-(Butylthio)benzoic Acid (**28e**).* According to general procedure O: 154 mg (1.0 mmol) of 3-mercaptobenzoic acid (**27**), 216 μL (2.0 mmol) of 1-bromobutane, 80 mg (2.0 mmol) of NaOH in 35 mL of H_2O and 15 mL of EtOH. The desired product (166 mg, 0.79 mmol, 79%) was obtained as a pale-yellow solid. ^{1}H NMR (250 MHz, DMSO-d_6) δ = 13.07 (1H, s), 7.80 (t, J = 1.6 Hz, 1H), 7.73 (dt, J = 1.3/7.5 Hz, 1H), 7.58−7.53 (m, 1H), 7.43 (t, J = 7.5 Hz, 1H), 3.00 (t, J = 7.3 Hz, 2H), 1.62−1.50 (m, 2H), 1.48−1.33 (m, 2H), 0.88 (t, J = 7.3 Hz, 3H) ppm; MS (ESI−) m/z: 208.90 [M − H]$^-$.

*3-((Cyclopropylmethyl)thio)benzoic Acid (**28f**).* According to general procedure O: 154 mg (1.0 mmol) of 3-mercaptobenzoic acid (**27**), 194 μL (2.0 mmol) of (bromomethyl)cyclopropane, 80 mg (2.0 mmol) of NaOH in 35 mL of H_2O and 15 mL of EtOH. The desired product (99 mg, 0.48 mmol, 48%) was obtained as a colorless solid. ^{1}H NMR (250 MHz, DMSO-d_6) δ = 13.07 (s, 1H), 7.83 (t, J = 1.6 Hz, 1H) 7.73 (dt, J = 1.6/7.5 Hz, 1H), 7.60−7.55 (m, 1H), 7.43 (t, J = 7.5 Hz, 1H), 2.97 (d, J = 7.0, 2H), 1.09−0.93 (m, 1H), 0.56−0.18 (m, 4H) ppm; MS (ESI−) m/z: 206.25 [M − H]$^-$.

*3-(Isopropylthio)benzoic Acid (**28g**).* According to general procedure O: 154 mg (1.0 mmol) of 3-mercaptobenzoic acid (**27**), 200 μL (2.0 mmol) of 2-iodopropane, 80 mg (2.0 mmol) of NaOH in 35 mL of H_2O and 15 mL of EtOH. The desired product (156 mg, 0.80 mmol, 80%) was obtained as a pale-yellow solid. ^{1}H NMR (250 MHz, DMSO-d_6) δ = 13.10 (s, 1H), 7.87 (t, J = 1.6 Hz, 1H), 7.79 (dt, J = 1.3/7.7 Hz, 1H), 7.63−7.59 (m, 1H), 7.46 (t, J = 7.7 Hz, 1H), 3.54 (sept, J = 6.6 Hz, 1H), 0.88 (d, J = 6.6 Hz, 6H) ppm; MS (ESI−) m/z: 194.90 [M − H]$^-$.

General Procedure for the Synthesis of 3-Alkylsulfonamidobenzoic Acids (Procedure P). Methyl 3-aminobenzoate (**29**, 1.0 equiv) was dissolved in DCM and cooled to 0 °C. The corresponding alkylsulfonyl chloride (1.2 equiv) and triethylamine (1.2 equiv) were added. The reaction mixture was stirred for 3 h at room temperature. Then the solvent was removed under reduced pressure, and the residue was taken up in EtOAc. The organic phase was washed successive with aqueous solutions of 2 M HCl and sat. $NaHCO_3$, dried over anhydrous $MgSO_4$, and filtered, and the solvent was removed under reduced pressure. The residue was taken up in a mixture of THF and MeOH (4:1), and 1 M aqueous NaOH (3.0 equiv) was added. The reaction mixture was stirred overnight at room temperature before being acidified with 2 M aqueous HCl. The precipitate was dissolved in EtOAc, and the aqueous phase was extracted two times with EtOAc. The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure.

*3-(2-Methylpropylsulfonamido)benzoic Acid (**MH32a**).* According to general procedure P: 284 mg (2.0 mmol) of methyl 3-aminobenzoate (**29**), 323 μL (2.4 mmol) of 2-methylpropane-1-sulfonyl chloride, 337 μL (2.4 mmol) of triethylamine, 240 mg (6.0 mmol) of NaOH, 6 mL of DCM, 4 mL of THF, 1 mL of MeOH, and 6 mL of H_2O. The desired product (410 mg, 1.60 mmol, 80%) was obtained as a pale-yellow solid. ^{1}H NMR (300 MHz, acetone-d_6) δ = 8.80 (bs, 1H), 8.03−8.01 (m, 1H), 7.81−7.78 (m, 1H), 7.59 (ddd, J = 1.1/2.3/8.1 Hz, 1H), 7.49 (t, J = 7.9 Hz, 1H), 3.05 (d, J = 6.5 Hz, 2H), 2.25 (sept, J = 6.6 Hz, 1H), 1.05 (d, J = 6.6 Hz, 6H) ppm; MS (ESI−) m/z: 256.15 [M − H]$^-$.

*3-(Methylsulfonamido)benzoic Acid (**32b**).* According to general procedure P: 284 mg (2.0 mmol) of methyl 3-aminobenzoate (**29**), 186 μL (2.4 mmol) of methanesulfonyl chloride, 337 μL (2.4 mmol) of triethylamine, 240 mg (6.0 mmol) of NaOH, 6 mL of DCM, 4 mL of THF, 1 mL of MeOH, and 6 mL of H_2O. The desired product (143 mg, 0.66 mmol, 33%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, acetone-d_6) δ = 8.76 (bs, 1H), 8.03 (t, J = 1.8 Hz, 1H), 7.81 (dt, J = 1.3/7.6 Hz, 1H), 7.60 (ddd, J = 1.2/2.3/8.1 Hz, 1H), 7.50 (t, J = 7.8 Hz, 1H), 3.04 (s, 3H) ppm; MS (ESI−) m/z: 214.00 [M − H]$^-$.

*3-(Ethylsulfonamido)benzoic Acid (**32c**).* According to general procedure P: 284 mg (2.0 mmol) of methyl 3-aminobenzoate (**29**), 232 μL (2.4 mmol) of ethansulfonyl chloride, 337 μL (2.4 mmol) of triethylamine, 240 mg (6.0 mmol) of NaOH, 6 mL of DCM, 4 mL of THF, 1 mL of MeOH, and 6 mL of H_2O. The desired product (227 mg, 0.99 mmol, 50%) was obtained as a pale-yellow solid. ^{1}H NMR (300 MHz, acetone-d_6) δ = 8.82 (bs, 1H), 8.05−8.03 (m, 1H), 7.81−7.76 (m, 1H), 7.63−7.58 (m, 1H), 7.49 (t, J = 7.9 Hz, 1H), 3.16 (q, J = 7.4 Hz, 2H), 1.30 (t, J = 7.4 Hz, 3H) ppm; MS (ESI−) m/z: 228.05 [M − H]$^-$.

*3-(Propylsulfonamido)benzoic Acid (**32d**).* According to general procedure P: 284 mg (2.0 mmol) of methyl 3-aminobenzoate (**29**), 278 μL (2.4 mmol) of propane-1-sulfonyl chloride, 337 μL (2.4 mmol) of triethylamine, 240 mg (6.0 mmol) of NaOH, 6 mL of DCM, 4 mL of THF, 1 mL of MeOH, and 6 mL of H_2O. The desired product (127 mg, 0.52 mmol, 26%) was obtained as a pale-yellow solid. ^{1}H NMR (300 MHz, acetone-d_6) δ = 8.80 (bs, 1H), 8.04−8.02 (m, 1H), 7.81−7.77 (m, 1H), 7.60 (ddd, J = 1.2/2.3/8.1 Hz, 1H), 7.49 (t, J = 7.8 Hz, 1H), 3.16−3.10 (m, 2H), 1.87−1.74 (m, 2H), 1.00 (t, J = 7.5 Hz, 3H) ppm; MS (ESI−) m/z: 242.15 [M − H]$^-$.

*3-(Butylsulfonamido)benzoic Acid (**32e**).* According to general procedure P: 284 mg (2.0 mmol) of methyl 3-aminobenzoate (**29**), 318 μL (2.4 mmol) of butane-1-sulfonyl chloride, 337 μL (2.4 mmol) of triethylamine, 240 mg (6.0 mmol) of NaOH, 6 mL of DCM, 4 mL of THF, 1 mL of MeOH, and 6 mL of H_2O. The desired product (361 mg, 1.40 mmol, 70%) was obtained as a pale-yellow solid. ^{1}H NMR (300 MHz, acetone-d_6) δ = 8.80 (bs, 1H), 8.04 (t, J = 1.9 Hz, 1H), 7.79 (dt, J = 1.4/7.6 Hz, 1H), 7.60 (ddd, J = 1.1/2.3/8.13 Hz, 1H), 7.49 (t, J = 7.8 Hz, 1H), 3.18−3.13 (m, 2H), 1.81−1.71 (m, 2H), 1.46−1.37 (m, 2H), 0.87 (t, J = 7.3 Hz, 3H) ppm; MS (ESI−) m/z: 256.10 [M − H]$^-$.

*3-(Cyclopropanesulfonamido)benzoic Acid (**32f**).* According to general procedure P: 2.13 g (15.0 mmol) of methyl 3-aminobenzoate (**29**), 1.94 mL (18.0 mmol) of cyclopropanesulfonyl chloride, 2.53 mL (18.0 mmol) of triethylamine, 1.80 g (45.0 mmol) of NaOH, 45 mL of DCM, 30 mL of THF, 7 mL of MeOH, and 45 mL of H_2O. The desired product (1.77 g, 7.34 mmol, 49%) was obtained as a pale-yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = COOH not

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

observed, 9.93 (s, 1H), 7.85−7.83 (m, 1H), 7.69−7.64 (m, 1H), 7.50−7.41 (m, 2H), 2.63 (quint, J = 6.3 Hz, 1H), 0.93 (d, J = 6.3 Hz, 4H) ppm; MS (ESI−) m/z: 240.02 $[M - H]^-$.

*3-(1-Methylethylsulfonamido)benzoic Acid (**32g**).* According to general procedure P: 568 mg (4.0 mmol) of methyl 3-aminobenzoate (**29**), 538 μL (4.8 mmol) of propane-2-sulfonyl chloride, 675 μL (4.8 mmol) of triethylamine, 480 mg (12 mmol) of NaOH, 12 mL of DCM, 8 mL of THF, 2 mL of MeOH, and 12 mL of H_2O. The desired product (180 mg, 0.74 mmol, 19%) was obtained as a pale-yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): 13.04 (s, 1H), 9.98 (s, 1H), 7.84−7.82 (m, 1H), 7.63 (dt, J = 1.7/6.9 Hz, 1H), 7.49−7.40 (m, 2H), 3.23 (sept, J = 6.8 Hz, 1H), 1.24 (d, J = 6.8 Hz, 6H) ppm; MS (ESI−) m/z: 242.04 $[M - H]^-$.

General Procedure for the Wittig Reaction (Procedure Q). Alkyltriphenylphosphonium bromide (1.2 equiv) was suspended in dry THF under argon and cooled with an ice bath. LiHMDS (1 M in THF, 1.2 equiv) was added slowly, and the reaction mixture was stirred for 30 min before a solution of the corresponding methyl 3-formylbenzoate (1.0 equiv) in dry THF was added. The ice bath was removed after the addition was completed, and the reaction mixture was stirred for additional 3 h at room temperature. The reaction was quenched by the addition of a saturated solution of NH_4Cl (20 mL), and the aqueous phase was extracted with EtOAc. The combined organic phases were dried over $MgSO_4$, filtered, and dried in vacuo. The crude product was purified by flash chromatography (hexane/EtOAc 9:1) and was obtained as a colorless liquid, that was not characterized by NMR but rather hydrogenated in the next synthesis step.

*Methyl 3-(3-Methylbut-1-en-1-yl)benzoate (**34a**).* According to general procedure Q: 500 mg (2.98 mmol) of methyl 3-formylbenzoate (**33**), 1.46 g (3.58 mmol) of isobutyltriphenylphosphonium bromide, and 3.6 mL of LiHMDS (1 M in THF, 3.6 mmol) in 15 mL of THF. The desired product (383 mg, 1.87 mmol, 63%) was obtained as a colorless oil. MS (ESI+) m/z: 204.90 $[M + H]^+$.

*Methyl 3-(Prop-1-en-1-yl)benzoate (**34c**).* According to general procedure Q: 500 mg (2.98 mmol) of methyl 3-formylbenzoate (**33**), 1.36 g (3.58 mmol) of ethyltriphenylphosphonium bromide, and 3.6 mL of LiHMDS (1 M in THF, 3.6 mmol) in 15 mL of THF. The desired product (254 mg, 1.44 mmol, 48%) was obtained as a colorless oil. MS (ESI+) m/z: 176.85 $[M + H]^+$.

*Methyl 3-(But-1-en-1-yl)benzoate (**34d**).* According to general procedure Q: 500 mg (2.98 mmol) of methyl 3-formylbenzoate (**33**), 1.39 g (3.58 mmol) of propyltriphenylphosphonium bromide, and 3.6 mL of LiHMDS (1 M in THF, 3.6 mmol) in 15 mL of THF. The desired product (413 mg, 2.17 mmol, 73%) was obtained as a colorless oil. MS (ESI+) m/z: 190.85 $[M + H]^+$.

*Methyl 3-(Pent-1-en-1-yl)benzoate (**34e**).* According to general procedure Q: 500 mg (2.98 mmol) of methyl 3-formylbenzoate (**33**), 1.45 g (3.6 mmol) of butyltriphenylphosphonium bromide, and 3.6 mL of LiHMDS (1 M in THF, 3.6 mmol) in 15 mL of THF. The desired product (363 mg, 1.78 mmol, 60%) was obtained as a colorless oil. MS (ESI+) m/z: 204.80 $[M + H]^+$.

*Methyl 3-(2-Cyclopropylvinyl)benzoate (**34f**).* According to general procedure Q: 500 mg (2.98 mmol) of methyl 3-formylbenzoate (**33**), 1.45 g (3.58 mmol) of (cyclopropylmethyl)-triphenylphosphonium bromide, and 3.6 mL of LiHMDS (1 M in THF, 3.6 mmol) in 15 mL of THF. The desired product (493 mg, 2.44 mmol, 82%) was obtained as a colorless oil. MS (ESI+) m/z: 202.95 $[M + H]^+$.

*Methyl 3-(2-Methylprop-1-en-1-yl)benzoate (**34g**).* According to general procedure Q: 500 mg (2.98 mmol) of methyl 3-formylbenzoate (**33**), 1.58 g (3.58 mmol) of isopropyltriphenylphosphonium bromide, and 3.6 mL of LiHMDS (1 M in THF, 3.6 mmol) in 15 mL of THF. The desired product (183 mg, 0.96 mmol, 32%) was obtained as a colorless oil. MS (ESI+) m/z: 190.90 $[M + H]^+$.

*Methyl 3-Styrylbenzoate (**34i**).* According to general procedure Q: 500 mg (2.98 mmol) of methyl 3-formylbenzoate (**33**), 817 g (3.58 mmol) of dimethyl benzylphosphonate, and 3.6 mL of LiHMDS (1 M in THF, 3.6 mmol) in 15 mL of THF. The desired product (244 mg, 1.02 mmol, 34%) was obtained as a pale-yellow oil. MS (ESI+) m/z: 239.05 $[M + H]^+$.

*Benzyl 2-(3-Methylbut-1-en-1-yl)benzoate (**39**).* According to general procedure Q: 1.0 g (4.2 mmol) of **38**, 2.0 g (5.0 mmol) of isobutyltriphenylphosphonium bromide, and 5.0 mL of LiHMDS (1 M in THF, 5.0 mmol) in 30 mL of THF. The desired product (299 mg, 1.1 mmol, 26%) was obtained as a colorless oil. MS (ESI+) m/z: 303.15 $[M + Na]^+$.

*Benzyl 4-Fluoro-3-(3-methylbut-1-en-1-yl)benzoate (**43**).* According to general procedure Q: 1.50 g (5.7 mmol) of **42**, 2.78 g (6.84 mmol) of isobutyltriphenylphosphonium bromide, and 6.8 mL of LiHMDS (1 M in THF, 6.8 mmol) in 20 mL of THF. The desired product (1.54 g, 5.17 mmol, 43%) was obtained as a pale-yellow oil. MS (ESI+) m/z: 298.95 $[M + H]^+$.

*Methyl 4-(Benzyloxy)-3-(3-methylbut-1-en-1-yl)benzoate (**48**).* According to general procedure Q: 1.40 g (5.18 mmol) of **47**, 2.53 g (6.22 mmol) of isobutyltriphenylphosphonium bromide, and 6.22 mL of LiHMDS (1 M in THF, 6.22 mmol) in 26 mL of THF. The desired product (1.27 g, 4.09 mmol, 79%) was obtained as a pale-yellow oil. MS (ESI+) m/z: 310.95 $[M + H]^+$.

General Procedure for Reduction of Alkenes (Procedure R). The respective product of the Wittig reaction was dissolved in MeOH, and palladium on charcoal (w = 10%) was added. The suspension was stirred for 5 h at room temperature under hydrogen atmosphere. The suspension was poured and filtered through a Celite pad, which was rinsed several times with MeOH (3 × 10 mL). The filtrate was dried in vacuo, and the obtained products were pure enough for further synthesis.

*Methyl 3-Isopentylbenzoate (**35a**).* According to general procedure R: 383 mg (1.87 mmol) of **34a** and 45 mg of palladium on charcoal (w = 10%) in 15 mL of MeOH. The desired product (339 mg, 1.64 mmol, 88%) was obtained as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.87−7.82 (m, 2H), 7.40−7.33 (m, 2H), 3.91 (s, 3H), 2.69−2.62 (m, 2H), 1.68−1.44 (m, 3H), 0.94 (d, J = 6.3 Hz, 6H) ppm; MS (ESI+) m/z: 206.90 $[M + H]^+$.

*Methyl 3-Propylbenzoate (**35c**).* According to general procedure R: 254 mg (1.44 mmol) of **34c** and 45 mg of palladium on charcoal (w = 10%) in 15 mL of MeOH. The desired product (235 mg, 1.32 mmol, 92%) was obtained as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.88−7.83 (m, 2H), 7.39−7.30 (m, 2H), 3.91 (s, 3H), 2.67−2.60 (m, 2H), 1.74−1.59 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H) ppm; MS (ESI+) m/z: 178.90 $[M + H]^+$.

*Methyl 3-Butylbenzoate (**35d**).* According to general procedure R: 413 mg (2.17 mmol) of **34d** and 45 mg of palladium on charcoal (w = 10%) in 15 mL of MeOH. The desired product (403 mg, 2.10 mmol, 97%) was obtained as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.87−7.83 (m, 2H), 7.37−7.33 (m, 2H), 3.91 (s, 3H), 2.66 (t, J = 7.7 Hz, 2H), 1.68−1.56 (m, 2H), 1.44−1.29 (m, 2H), 0.93 (t, J = 7.3 Hz, 3H) ppm; MS (ESI+) m/z: 192.90 $[M + H]^+$.

*Methyl 3-Pentylbenzoate (**35e**).* According to general procedure R: 393 mg (1.92 mmol) of **34e** and 45 mg of palladium on charcoal (w = 10%) in 15 mL of MeOH. The desired product (379 mg, 1.84 mmol, 96%) was obtained as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.87−7.83 (m, 2H), 7.37−7.33 (m, 2H), 3.91 (s, 3H), 2.65 (t, J = 7.7 Hz, 2H), 1.69−1.56 (m, 2H), 1.38−1.28 (m, 4H), 0.89 (t, J = 6.8 Hz, 3H) ppm; MS (ESI+) m/z: 206.85 $[M + H]^+$.

*Methyl 3-(2-Cyclopropylethyl)benzoate (**35f**).* According to general procedure R: 493 mg (2.44 mmol) of **34f** and 45 mg of palladium on charcoal (w = 10%) in 15 mL of MeOH. The desired product (469 mg, 2.30 mmol, 94%) was obtained as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.88−7.83 (m, 2H), 7.40−7.30 (m, 2H), 3.91 (s, 3H), 2.79−2.62 (m, 2H), 1.58−1.48 (m, 2H), 1.38−1.27 (m, 1H), 0.46−0.39 (m, 2H), 0.06−0.01 (m, 2H) ppm; MS (ESI+) m/z: 204.90 $[M + H]^+$.

*Methyl 3-Isobutylbenzoate (**35g**).* According to general procedure R: 183 mg (0.96 mmol) of **34g** and 45 mg of palladium on charcoal (w = 10%) in 15 mL of MeOH. The desired product (163 mg, 0.85 mmol, 89%) was obtained as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.89−7.82 (m, 2H), 7.35−7.32 (m, 2H), 3.91 (s, 3H),

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

2.52 (d, J = 7.2 Hz, 2H), 1.89 (non, J = 6.9 Hz, 1H), 0.90 (d, J = 6.6 Hz, 6H) ppm; MS (ESI+) m/z: 192.90 $[M + H]^+$.

*Methyl 3-Phenethylbenzoate (**35i**).* According to general procedure R: 244 mg (1.02 mmol) of **34i** and 45 mg of palladium on charcoal (w = 10%) in 15 mL of MeOH. The desired product (221 mg, 0.92 mmol, 90%) was obtained as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.91−7.84 (m, 2H), 7.35−7.16 (m, 7H), 3.92 (s, 3H), 3.01−2.91 (m, 4H) ppm; MS (ESI+) m/z: 240.85 $[M + H]^+$.

*Benzyl 2-Formylbenzoate (**38**).* 2-Formylbenzoic acid (**37**, 3.0 g, 20.0 mmol, 1.0 equiv) was dissolved in 50 mL of acetone, and K_2CO_3 (5.5 g, 40.0 mmol, 2.0 equiv) was added. Then benzyl bromide (2.9 mL, 24 mmol, 1.2 equiv) was added, and the reaction mixture was refluxed for 1 h. After cooling to room temperature, the reaction mixture was filtered and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (hexane/EtOAc 9:1) to obtain the desired product (4.50 g, 18.7 mmol, 94%) as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 10.63 (s, 1H), 8.03−7.98 (m, 1H), 7.96−7.92 (m, 1H), 7.69−7.60 (m, 2H), 7.48−7.34 (m, 5H), 5.42 (s, 2H) ppm; MS (ESI+) m/z: 262.80 $[M + Na]^+$.

*Benzyl 4-Fluoro-3-formylbenzoate (**42**).* 4-Fluoro-3-formylbenzoic acid (**41**, 1.0 g, 6.0 mmol, 1.0 equiv) and $NaHCO_3$ (0.6 g, 7.2 mmol, 1.2 equiv) were suspended in dry DMF (15 mL) and stirred for 1 h at room temperature. Then benzyl bromide (871 μL, 7.2 mmol, 1.2 equiv) was added dropwise and stirred for an additional 4 h. The reaction mixture was treated with sat. $NaHCO_3$ solution (10 mL), and the aqueous phase was extracted three times with DCM (3 × 20 mL). The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified through column chromatography (hexane/EtOAc 9:1) to obtain the desired product (1.51 g, 5.85 mmol, 98%) as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 10.36 (s, 1H), 8.58 (dd, J = 2.3/6.7 Hz, 1H), 8.36−8.29 (m, 1H), 7.46−7.35 (m, 5H), 7.26 (t, J = 9.2 Hz, 1H), 5.38 (s, 2H) ppm; MS (ESI+) m/z: 258.90 $[M + H]^+$.

*Methyl 3-Isopentyl-4-methoxybenzoate (**52**).* **49** (262 mg, 1.18 mmol, 1.0 equiv) was dissolved in acetone (10 mL), and K_2CO_3 (326 mg, 2.36 mmol, 2.0 equiv) was added. The suspension was stirred for 30 min at 80 °C before dimethyl sulfate (134 μL, 1.42 mmol, 1.2 equiv) was added, and the reaction mixture was stirred for additional 2 h at 80 °C. The suspension was filtered, and the filtrate was washed with sat. $NaHCO_3$ solution (2 × 30 mL), dried over $MgSO_4$, and filtered. The organic phase was evaporated in vacuo, and the resulting residue was purified by column chromatography (hexane/EtOAc 9:1) to obtain the desired product (255 mg, 1.08 mmol, 91%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.88 (dd, J = 2.2/8.5 Hz, 1H), 7.82 (d, J = 2.2 Hz, 1H), 6.84 (d, J = 8.6 Hz, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 2.65−2.59 (m, 2H), 1.60 (non, J = 6.5 Hz, 1H), 1.50−1.42 (m, 2H), 0.94 (d, J = 6.5 Hz, 6H) ppm; MS (ESI+) m/z: 236.95 $[M + H]^+$.

*Methyl 3-Formyl-4-hydroxybenzoate (**46**).* Methyl *para*-hydroxybenzoate (**45**, 3.00 g, 20.0 mmol, 1.0 equiv) and $MgCl_2$ (9.52 g, 100 mmol, 5.0 equiv) were suspended in 1,2-dichloroethane (80 mL) and triethylamine (17 mL, 120 mmol, 6.0 equiv). The suspension was stirred at 40 °C for 1 h before paraformaldehyde (6.00 g, 200 mmol, 10.0 equiv) was added. The reaction mixture was stirred overnight at 70 °C. The reaction was quenched by the addition of 1 M hydrochloric acid (100 mL). The aqueous phase was extracted with DCM (3 × 100 mL), and the combined organic phases were washed with brine, dried over $MgSO_4$, and filtered, and the solvent was removed in vacuo. The residue was purified by column chromatography (hexane/EtOAc 4:1) to obtain the product (1.09 g, 6.07 mmol, 30%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 11.39 (s, 1H), 9.96 (s, 1H), 8.32 (t, J = 2.0 Hz, 1H), 8.19 (dd, J = 2.0/8.7 Hz, 1H), 7.04 (d, J = 8.7 Hz, 1H), 3.93 (s, 3H) ppm; MS (ESI−) m/z: 178.70 $[M - H]^-$.

*Methyl 4-(Benzyloxy)-3-formylbenzoate (**47**).* **46** (970 mg, 5.38 mmol, 1.0 equiv) was dissolved in dry DFM (10 mL), and K_2CO_3 (1.12 g, 8.07 mmol, 1.5 equiv) was added. The suspension was stirred for 30 min at 85 °C. Thereafter, benzyl bromide (960 μL, 8.07 mmol, 1.1 equiv) was added and the reaction mixture was stirred for an additional 5 h at 85 °C. The reaction was quenched by the addition of water (50 mL), and the aqueous phase was extracted with DCM (3 × 25 mL). The combined organic phases were dried over $MgSO_4$ and filtered, and the solvent was removed in vacuo. Column chromatographic purification (hexane/EtOAc 4:1) gave the product (1.40 g, 5.18 mmol, 96%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 10.53 (s, 1H), 8.53 (d, J = 2.4 Hz, 1H), 8.21 (dd, J = 2.4/8.8 Hz, 1H), 7.46−7.35 (m, 5H), 7.10 (d, J = 8.8 Hz, 1H), 5.27 (s, 2H), 3.91 (s, 3H) ppm; MS (ESI+) m/z: 293.16 $[M + Na]^+$.

*4-Acetoxy-3-isopentylbenzoic Acid (**51**).* **50** (534 mg, 2.56 mmol, 1.0 equiv) and pyridine (414 μL, 5.13 mmol, 2.0 equiv) were dissolved in DCM (10 mL) and cooled to 0 °C with an ice-bath. Acetic anhydride (485 μL, 5.13 mmol, 2.0 equiv) was added, the reaction mixture was stirred overnight, and the solution could warm to room temperature. The solvent was removed in vacuo, and the crude product was purified by column chromatography (hexane/EtOAC 1:1) to give the product (590 mg, 2.36 mmol, 92%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.02 (d, J = 2.0 Hz, 1H), 7.97 (dd, J = 2.0/8.4 Hz, 1H), 7.14 (d, J = 8.4 Hz, 1H), 2.60−2.55 (m, 2H), 2.35 (s, 3H), 1.61 (non, J = 6.6 Hz, 1H), 1.52−1.44 (m, 2H), 0.95 (d, J = 6.6 Hz, 6H) ppm; MS (ESI−) m/z: 248.95 $[M - H]^-$.

Cloning. SNAP is a mutant of the DNA repair protein O^6-alkylguanine-DNA alkyltransferase. It reacts specifically and rapidly with benzylguanine (BG) derivatives. Thereby, the introduced mutations prevent completion of the enzymatic reaction which allows irreversible covalent labeling of fusion proteins via the 20 kDa SNAP-tag (New England Biolabs; NEB).

For the expression of LC3 proteins with N-terminal fusion to SNAP, an expression construct based on the plasmid vector pET29b was prepared. The entire section between the original NdeI site and the fourth base pair following the His-Tag coding sequence of pET29b was replaced, hence essentially leaving only the vector backbone unmodified. First, the section was replaced by a codon optimized sequence encoding a restriction site for NcoI (overlapping with the start codon) and an open reading frame for Met-Gly-[His_{10}-tag]-Asp-Tyr-Asp-Ile-Pro-Thr-Thr followed by a cleavage site for Tobacco Etch Virus protease [TEV site] and restriction sites for *Kpn*I (in frame; first triplet is Gly codon of TEV site) and XhoI, resulting in the pET29b derivative pET29bh4. Next, we amplified by PCR the SNAP open reading frame (residues 2−180) using the plasmid pSNAP-tag(T7)2 (NEB) as the template. Thereby, restriction sites for *Kpn*I (5′ end) and *Bam*HI + XhoI (3′ end) were attached. SNAP was cloned in between *Kpn*I and XhoI restriction sites of the pET29b derivative. The DNA sequences encoding for LC3A (uniprot identifier: Q9H492-1; residues 4−119; preceded by Met) and LC3B (uniprot identifier: Q9GZQ8-1; residues 5−119; preceded by Met-Gly), each immediately followed by the DNA sequence TAAATGATTAGA for stop codons in all reading frames, were then introduced in the frame between the restriction sites for *Bam*HI and XhoI, respectively.

For the HTRF-based LIR binding assay, we generated a fusion protein of LIR peptide with N-terminal fusion to superfolder GFP (sGFP), which served as the FRET acceptor. Cloning followed a comparable strategy starting with pET29bh4. Instead of the SNAP-tag sequence, we introduced the sequence encoding sGFP (without start Met) followed by the *Bam*HI restriction site (in frame), a codon for Gly, and the previously described sequence with stop codons. The resulting plasmid was later also used to generate free sGFP. For sGFP-LIR, we then cloned the LC3-interacting region (LIR) of sequestosome 1 (p62; uniprot identifier Q13501-1; residues 322−347) between the *Bam*HI site and the stop codons. The cysteine within this segment, which corresponds to residue 331 in p62, was mutated to serine. This LIR sequence encompasses all residues that in the crystal structure (PDB entry 2k6q) are in close contact with LC3B.

For all these fusion proteins expression, lysis, initial purification by immobilized metal affinity chromatography (IMAC) followed by cleavage of the His-tag by TEV protease, reverse IMAC, and concentration of the product prior to size exclusion chromatography

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

(SEC) was all performed following the protocol that we published before for expression and purification of free PPARγ LBD.

As a final purification step, volumes of 5 mL of concentrated protein were separated on a HiLoad 16/600 Superdex 75 pg gel filtration column (Ge Healthcare) equilibrated and run in SEC buffer [25 mM HEPES pH 7.5 adjusted with KOH, 150 mM KF, 10% (w/v) glycerol, 5 mM DTT] at 1 mL/min. Fractions from the middle of the peak corresponding to monomeric protein were collected and used for assays.

Screening of Libraries of Compounds That Affect the Interaction between LC3b and LIRtide. The AlphaScreen assay was performed with the GST-detection kit (Streptavidin Donor beads and Anti-GST conjugated AlphaScreen Acceptor beads) essentially as described by the manufacturer (PerkinElmer). The assay (25 μL) contained 50 mM Tris (pH 7.5), 100 mM NaCl, 0.01% Tween 20, 2 mM DTT, 1% DMSO and 2−30 nM GST-LC3B and biotin-LIRtide 2−30 nM. The alphascreen signal was measured in an Envision multiplate reader (PerkinElmer). GST-LC3B was obtained by transient transfection of HEK293 cells and purified in batch on gluthathione beads.[22] LIRtide (SGGDDDWTHLS) and Biotin-LIRtide (biotin-Ahx-GNSSGGDDDWTHLS) were synthesized by Genescript. The concentrations of GST-LC3B and biotin-LIRtide were experimentally chosen from cross-titration assays exploring the different combinations of for each batch of protein used and when a new batch of buffer was prepared. LIRtide inhibited the GST-LC3b interaction with an IC_{50} of 1.6 μM and was used as a control. For the screening, conditions were chosen that could have identified enhancers or inhibitors of the interaction. The signal/background was >35; the estimated Z' was 0.87. We present the data on the screening of the Prestwick Chemical Library (Prestwick Chemical, Illkirch, France) that contains 1280 mostly approved drugs (FDA, EMA and other agencies (http://www.prestwickchemical.com/prestwick-chemical-library.html). The library was screened at 50 μM. Compounds inhibiting <65% of remaining signal or increasing the signal by >120% were identified. Those initial hits from the screening were compared with hits on previous screening campaigns, and hits that appeared previously were eliminated. Eighteen compounds (nine potential inhibitors and nine potential enhancers of the interaction) were selected and reordered. IC_{50}'s were obtained on reordered compounds, and the ability to affect the signal from the LC3b-LIRtide assay was compared with the ability to affect the signal obtained with biotinylated-GST (PerkinElmer). "Hit" compounds that affected the control biotnylated-GST were presumed to affect the alphascreen assay and were discarded. Novobiocin was the only confirmed hit identified from the screening of the Prestwick library. The hit was validated by obtaining the crystal structure of LC3A in complex with Novobiocin.

Labeling of SNAP-LC3s with Terbium Cryptate. For covalent coupling of terbium cryptate, we utilized SNAP-Lumi4-Tb (Cisbio Bioassays, Codolet, France). A volume of 20 μL with 12 μM protein (0,24 pmol) in SEC buffer was diluted with additional 360 μL of SEC buffer and supplemented with 20 μL of a 10 μM dilution of SNAP-Lumi4-Tb in DMSO. This resulted in a 20% molar excess of SNAP-tag relative to the substrate and prevented remnants of free label. The reaction was allowed to complete overnight at 4 °C. The product had a protein concentration of 600 nM with approximately 80% being labeled. Aliquots were stored at −80 °C.

Displacement Assay. Displacement of LIR-LC3 interaction was studied by homogeneous time-resolved fluorescence resonance energy transfer (HT-FRET or HTRF). The SNAP fused LC3 proteins were incubated with SNAP-Lumi4-Tb labeling reagent (Cisbio assays, France) overnight in a fridge.

Solutions containing 1 mM recombinant LIR-sequence fused to N-terminal sGFP or sGFP alone as FRET acceptor and 10 nM FRET donor complex with either LC3A or LC3B with N-terminal SNAP-tag covalently coupled to terbium cryptate as well as 1% DMSO with test compound at varying concentrations or DMSO alone were prepared in HTRF buffer [25 mM HEPES pH 7.5 adjusted with KOH, 150 mM KF, 10% (w/v) glycerol, 0.1% (w/v) CHAPS, 5 mM DTT]. After 2 h incubation at RT, the fluorescence intensities (FI) at 520 nm (acceptor) and 620 nm (donor reference) after excitation at 340 nm were recorded on a Tecan Infinite F200 instrument (Tecan Group Ltd., Männedorf, Switzerland). FI520 nm was divided by FI620 nm and multiplied by 10 000 to give a dimensionless HTRF signal. Displacement of the LIR-LC3 interaction by test compound separates FRET donor and acceptor resulting in a loss in FRET signal.

Expression and Purification. The expression and purification of LC3A and LC3B used in the TSA, ITC, and crystallization experiments were performed in a similar manner for both proteins.

In brief, TB medium (1.2% (w/v) tryptone, 2.4% (w/v) yeast extract, 0.5% glycerol, and 89 mM phosphate buffer) with kanamycin (100 μg/mL) was inoculated with 1% (v/v) of a preculture of *E. coli* BL21-(DE3) transformed with either the LC3A or LC3B plasmid. Cultures were incubated by shaking at 37 °C and 180 rpm until an OD_{600} of 1.5 was reached, before the incubation temperature and shaking speed were reduced to 20 °C as well as 170 rpm, respectively. Cultures were induced by the addition of 500 μM IPTG at an OD_{600} of 2.5. Cells were harvested by centrifugation (10 866*g*, 20 min) after approximately 16 h. Cell pellets were either stored at −20 °C or directly used in the following protein purification.

For the purification of LC3A and LC3B, cell pellets containing the expressed protein were suspended in buffer A (50 mM Tris pH 8, 500 mM NaCl, 20 mM imidazole, 5 mM β-mercaphtoethanol, and 5% (v/v) glycerol) which was supplemented with one tablet of Complete EDTA free protease inhibitor mix (Roche, Basel, Switzerland) and a trace amount of DNase I (Applichem, Darmstadt, Germany). The resuspended cells were lysed by using a liquid homogenizer (Zelldisruptor, Constant Systems Ltd.), and cell debris were removed by centrifugation (51 632*g*, 60 min, 4 °C). Nickel affinity chromatography (GE Healthcare, Solingen, Germany) was used to further purify the supernatant. Chromatography was performed as a step gradient protocol using buffer A as the running buffer and buffer B (identical to buffer A with an imidazole concentration of 400 mM) as the elution buffer. The fractions containing the target protein were pooled, before 1 mg of TEV protease per 10 mg of LC3 was added and samples were dialyzed overnight against a 100-fold excess of buffer A at 4 °C using a 3500 kDa membrane. Contaminations, uncleaved protein, tags, as well as the TEV protease were removed by reverse nickel affinity chromatography using buffers A and B. The flow through was concentrated by ultrafiltration (3000 Da cutoff membrane) and loaded onto a Superdex 75 HiLoad 16/600 column (GE Healthcare, Germany) equilibrated with either HTRF buffer (150 mM KF, 25 mM HEPES pH 7.5, 10% (w/v) glycerol, 5 mM DTT) or crystallization buffer (20 mM Tris pH 8, 100 mM NaCl) and run at 1 mL/min. The concentrations of the pooled protein stocks were determined by using a Nanodrop spectrophotometer (Implen, Muenchen, Germany), before flash freezing aliquots in liquid nitrogen. Aliquots were stored at −80 °C.

For ITC and NMR experiments, all six human LC3 and GABARAP proteins were cloned and expressed under a modified Ub tag[23] and purified as described previously.[24] Prior to experiments, all proteins were equilibrated against a buffer containing 50 mM Na_2HPO_4, 100 mM NaCl, and 5% DMSO, pH 7.0. Solutions of **1** were prepared in the same buffer.

Differential Scanning Fluorimetry (DSF). DSF experiments of LC3A and LC3B were performed in transparent 96-well PCR plates (MicroAmp, Applied Biosystems). Protein/SYPRO orange solution in HTRF buffer was mixed with and inhibitor solved in DMSO (or pure DMSO in case of the control) in a final assay volume of 40 μL containing 30 μM protein, 0,1% (w/v) CHAPS, 2.5× SYPRO orange, 300 μM inhibitor, as well as 1% (v/v) DMSO. Background fluorescence was measured without enzyme to exclude inhibitor/SYPRO orange interactions. A temperature-dependent increase of fluorescence was measured from 25.0 to 79.8 °C in steps of 1 °C/1 min, using an Icycler IQ single color real time PCR system (BioRad). The fluorescence was measured at an excitation wavelength of 490 nm as well as an emission wavelength of 570 nm. The measurements were performed as triplicate in three independent experiments. The first derivatives of the measured melting curves were automatically calculated with the MyIQ 1.0 software of the Icyler. Data was further

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746

analyzed in Microsoft Excel, were the respective maxima were determined, which were considered as the melting point. For DSF experiments with LC3C, GABARAP, GABARAPL1, and GABARAPL2, recombinant proteins at 2 μM in 10 mM HEPES, pH 7.5 and 100 mM NaCl were mixed with 20 μM inhibitors. The reaction was incubated at room temperature for 10 min. SYPRO orange was added at 1:1000 dilution, and the fluorescence signals corresponding to temperature-dependent protein unfolding were measured using a real-time PCR Mx3005p machine (Stratagene) at a heating rate of 1 °C/min. Melting temperature shifts and the corresponding ΔT_m values were calculated using the previously described method.[25]

ITC Measurements. The ITC experiments were performed at 25 °C using a MicroCal VP-ITC microcalorimeter (Malvern Instruments Ltd., UK). The **1** at concentrations of 0.4 mM were titrated into 0.020 mM LC3 and GABARAP proteins in 21 steps (10 μL per injection). The proteins and **1** concentrations were calculated from the UV absorption at 280 nm with a Nanodrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The ITC data was analyzed with the MicroCal ITC software implemented in Origin 7.0 and fitted based on a "one-side" binding reaction.

ITC experiments were performed on a low volume Affinity ITC instrument (TA Instruments) with the protein solution in the sample cell and the inhibitor solution in the syringe. All measurements were performed at 25 °C and a stirring speed of 75 rpm. The protein and inhibitor were diluted in HTRF buffer from the same batch used in the purification. Samples were supplemented with CHAPS as well as DMSO at final concentrations of 0.1% and 1% respectively. The protein concentration used was 80 μM while the inhibitor concentration was either 500 μM or 1 mM (inhibitor stocks were dissolved in pure DMSO). The number of injections, the delay between the injections, as well as the injection volume were optimized for each compound individually. The linearity of the dilution heat was tested in separate buffer in protein as well as inhibitor in buffer titrations. ITC data was analyzed using NanoAnalyze Data Analysis software (version 3.5.0) software. Baseline subtraction was performed based on observed change in enthalpy in the last data points in each measurement in case of linear reference blank experiments.

NMR Experiments. NMR experiments were performed on a Bruker Avance spectrometers operating at proton frequencies of 600 and 700 MHz at 298 K. Titrations were performed with 150 μM ^{15}N LC3B samples to which **1** was added stepwise with increasing molar ratios to 8 times molar excess according to the recent guidelines.[26] [^{1}H−^{15}N] heteronuclear single quantum coherence (HSQC) spectra were recorded at each step. Spectra were analyzed using the Sparky 3.114 software (University of California, San Francisco, CA, USA).

Crystallization. LC3A in crystallization buffer was concentrated to a final concentration of 20 mg/mL, before spinning the protein sample for 15 min at 4 °C and max speed in an Eppendorf bench top centrifuge. The supernatant was mixed with **2** to a final concentration of 5 mM and 5% DMSO before incubation on ice for 1 h. The solution was centrifuged again for 15 min at 4 °C and max speed in an Eppendorf bench top centrifuge, before the supernatant was used to set up crystallization experiments. Crystallization experiments were performed in 96-well sitting drop plates with a drop volume of 150 nL in the ratios of 2:1, 1:1 and 1:2 with a JCSG7 instrument (MD1-Custom M96-HT96 BN004). Crystallization plates were incubated at 20 °C, and crystals grew over the course of 3 weeks in the well with 0.2 M ammonium nitrate and 20% w/v PEG 3350. Crystals were soaked with a mixture of 25% ethylene glycol and 75% precipitation solution for cryoprotection, before fishing and flash freezing the crystals in liquid nitrogen.

X-ray diffraction data was collected form a single crystal at the SLS beamline. Processing of the obtained data was performed using the XDS software package. The phaser program as part of the PHENIX software package was used for the initial structure determination. As starting model for the molecular replacement in internal LC3A, the structure where coordinates for heteroatoms (water and ligands) were excluded was used. The initial model was further optimized by several iterative rounds of model building with Coot and the model refinement using the PHENIX software package. The final round of refinement showed that Rwork and Rfree factors of 0.1860 and 0.2122, respectively, were reached. Statistics of data collection and structural refinement are summarized in Table S1. Binding of the compound was validated using a polder omit map with a solvent exclusion radius of 4 Å and a resolution factor of 0.25. The graphical representations were made using MOE. The coordinates and structure-factor amplitudes of the structure have been deposited in the Protein Data Bank as entry 6TBE.

Lower abundance secondary conformations of amino acids, unclear electron densities mainly at the surface of the protein, as well as one density close to the inhibitor have not been modeled. RSZR for some AS is higher with the most prominent outlines for the first and the last resolved amino acid due to the low amount of visible density.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01564.

Full [^{1}H−^{15}N]-HSQC spectra of LC3B upon titration with **1** and chemical shift perturbations of LC3B upon interaction with **1**; data collection and refinement statistics; thermodynamic parameters of **1** binding to LC3A and LC3B obtained by ITC (PDF)

Molecular formula strings (CSV)

HPLC-based purity of **6a**, **14o**, and **2** (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Ewgenij Proschak − *Institute of Pharmaceutical Chemistry, Goethe-University Frankfurt, 60438 Frankfurt, Germany; Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, 60596 Frankfurt, Germany; German translational cancer network (DKTK), 60438 Frankfurt, Germany;* orcid.org/0000-0003-1961-1859; Email: proschak@pharmchem.uni-frankfurt.de

Authors

Markus Hartmann − *Institute of Pharmaceutical Chemistry, Goethe-University Frankfurt, 60438 Frankfurt, Germany*

Jessica Huber − *Institute of Biophysical Chemistry and Center for Biomolecular Magnetic Resonance, Goethe-University Frankfurt, 60438 Frankfurt, Germany*

Jan S. Kramer − *Institute of Pharmaceutical Chemistry, Goethe-University Frankfurt, 60438 Frankfurt, Germany*

Jan Heering − *Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, 60596 Frankfurt, Germany;* orcid.org/0000-0002-4922-1993

Larissa Pietsch − *Department of Internal Medicine I, Goethe University Hospital Frankfurt, 60596 Frankfurt, Germany*

Holger Stark − *German translational cancer network (DKTK), 60438 Frankfurt, Germany; Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, 40225 Duesseldorf, Germany;* orcid.org/0000-0003-3336-1710

Dalibor Odadzic − *German translational cancer network (DKTK), 60438 Frankfurt, Germany*

Iris Bischoff − *Institute of Pharmaceutical Biology, Goethe-University Frankfurt, 60438 Frankfurt, Germany*

Robert Fürst − *Institute of Pharmaceutical Biology, Goethe-University Frankfurt, 60438 Frankfurt, Germany*

Martin Schröder − *Institute of Pharmaceutical Chemistry, Goethe-University Frankfurt, 60438 Frankfurt, Germany*

Masato Akutsu − *Buchmann Institute for Molecular Life Sciences, Goethe-University Frankfurt, 60438 Frankfurt, Germany*

Apirat Chaikuad − *Institute of Pharmaceutical Chemistry, Goethe-University Frankfurt, 60438 Frankfurt, Germany;* orcid.org/0000-0003-1120-2209

Volker Dötsch − *Institute of Biophysical Chemistry and Center for Biomolecular Magnetic Resonance, Goethe-University Frankfurt, 60438 Frankfurt, Germany*

Stefan Knapp − *Institute of Pharmaceutical Chemistry, Goethe-University Frankfurt, 60438 Frankfurt, Germany; German translational cancer network (DKTK), 60438 Frankfurt, Germany;* orcid.org/0000-0001-5995-6494

Ricardo M. Biondi − *Department of Internal Medicine I, Goethe University Hospital Frankfurt, 60596 Frankfurt, Germany*

Vladimir V. Rogov − *Institute of Pharmaceutical Chemistry, Goethe-University Frankfurt, 60438 Frankfurt, Germany; Institute of Biophysical Chemistry and Center for Biomolecular Magnetic Resonance, Goethe-University Frankfurt, 60438 Frankfurt, Germany*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jmedchem.0c01564

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Evelyn Süss for technical assistance. E.P. was supported by the Deutsche Forschungsgemeinschaft (DFG, Heisenberg-Professur PR1405/7-1). A.C., S.K., and V.V.R. are grateful for support by the Structural Genomic Consortium (SGC). The SGC is a registered charity (No.: 1097737) that receives funds from AbbVie, Bayer AG, Boehringer Ingelheim, Canada Foundation for Innovation, Eshelman Institute for Innovation, Genentech, Genome Canada through Ontario Genomics Institute [OGI-196], EU/EFPIA/OICR/McGill/KTH/Diamond, Innovative Medicines Initiative 2 Joint Undertaking [EUbOPEN Grant 875510], Janssen, Merck KGaA (aka EMD in Canada and USA), Merck & Co (aka MSD outside Canada and USA), Pfizer, São Paulo Research Foundation-FAPESP, Takeda and Wellcome [106169/ZZ14/Z]. This research was supported by the DFG funded Collaborative Sonderforschungsbereich 1177 Autophagy (SFB1177) at Frankfurt University as well as the German Cancer Consortium (DKTK) and the Frankfurt Cancer Centre (FCI). We acknowledge the Paul Scherrer Institut, Villigen, Switzerland for provision of synchrotron radiation beamtime at beamline Beamline X06SA of the SLS.

■ ABBREVIATIONS

AIM, Atg8-interacting motif; Atg, autophagy related proteins; AUTAC, autophagy-mediated degrader; CSP, chemical shift perturbations; DMAP, 4-dimethylaminopyridine; DSF, differential scanning fluorimetry; HP, hydrophobic pocket; HTRF, homogeneous time-resolved FRET; ITC, isothermal titration calorimetry; LC3, microtubule-associated proteins 1A/1B light chain 3; LDS, LIR docking site; LiHMDS, lithium bis(trimethylsilyl)amide; LIR, LC3-interacting region; PE, phosphatidyl-ethanolamine; UBL, ubiquitin-like proteins

■ REFERENCES

(1) He, C.; Klionsky, D. J. Regulation Mechanisms and Signaling Pathways of Autophagy. *Annu. Rev. Genet.* **2009**, *43*, 67−93.

(2) Mizushima, N.; Yoshimori, T.; Ohsumi, Y. The Role of Atg Proteins in Autophagosome Formation. *Annu. Rev. Cell Dev. Biol.* **2011**, *27*, 107−132.

(3) Dikic, I. Proteasomal and Autophagic Degradation Systems. *Annu. Rev. Biochem.* **2017**, *86*, 193−224.

(4) Kirkin, V.; Rogov, V. V. A Diversity of Selective Autophagy Receptors Determines the Specificity of the Autophagy Pathway. *Mol. Cell* **2019**, *76*, 268.

(5) Dikic, I.; Elazar, Z. Mechanism and Medical Implications of Mammalian Autophagy. *Nat. Rev. Mol. Cell Biol.* **2018**, *19* (6), 349−364.

(6) Ichimura, Y.; Kirisako, T.; Takao, T.; Satomi, Y.; Shimonishi, Y.; Ishihara, N.; Mizushima, N.; Tanida, I.; Kominami, E.; Ohsumi, M.; et al. A Ubiquitin-like System Mediates Protein Lipidation. *Nature* **2000**, *408* (6811), 488−492.

(7) Nakatogawa, H.; Ichimura, Y.; Ohsumi, Y. Atg8, a Ubiquitin-like Protein Required for Autophagosome Formation, Mediates Membrane Tethering and Hemifusion. *Cell* **2007**, *130* (1), 165−178.

(8) Alemu, E. A.; Lamark, T.; Torgersen, K. M.; Birgisdottir, A. B.; Larsen, K. B.; Jain, A.; Olsvik, H.; Øvervatn, A.; Kirkin, V.; Johansen, T. ATG8 Family Proteins Act as Scaffolds for Assembly of the ULK Complex Sequence Requirements for LC3-Interacting Region (LIR) Motifs. *J. Biol. Chem.* **2012**, *287* (47), 39275−39290.

(9) Colecchia, D.; Strambi, A.; Sanzone, S.; Iavarone, C.; Rossi, M.; Dall'Armi, C.; Piccioni, F.; Verrotti di Pianella, A.; Chiariello, M. MAPK15/ERK8 Stimulates Autophagy by Interacting with LC3 and GABARAP Proteins. *Autophagy* **2012**, *8* (12), 1724−1740.

(10) Popovic, D.; Akutsu, M.; Novak, I.; Harper, J. W.; Behrends, C.; Dikic, I. Rab GTPase-Activating Proteins in Autophagy: Regulation of Endocytic and Autophagy Pathways by Direct Binding to Human ATG8Modifiers. *Mol. Cell. Biol.* **2012**, *32* (9), 1733−1744.

(11) Johansen, T.; Lamark, T. Selective Autophagy Mediated by Autophagic Adapter Proteins. *Autophagy* **2011**, *7* (3), 279−296.

(12) Kirkin, V.; McEwan, D. G.; Novak, I.; Dikic, I. A Role for Ubiquitin in Selective Autophagy. *Mol. Cell* **2009**, *34* (3), 259−269.

(13) Noda, N. N.; Ohsumi, Y.; Inagaki, F. Atg8-Family Interacting Motif Crucial for Selective Autophagy. *FEBS Lett.* **2010**, *584* (7), 1379−1385.

(14) Rogov, V. V.; Stolz, A.; Ravichandran, A. C.; Rios-Szwed, D. O.; Suzuki, H.; Kniss, A.; Löhr, F.; Wakatsuki, S.; Dötsch, V.; Dikic, I.; et al. Structural and Functional Analysis of the GABARAP Interaction Motif (GIM). *EMBO Rep.* **2017**, *18* (8), 1382−1396.

(15) Pankiv, S.; Clausen, T. H.; Lamark, T.; Brech, A.; Bruun, J.-A.; Outzen, H.; Øvervatn, A.; Bjørkøy, G.; Johansen, T. P62/SQSTM1 Binds Directly to Atg8/LC3 to Facilitate Degradation of Ubiquitinated Protein Aggregates by Autophagy. *J. Biol. Chem.* **2007**, *282* (33), 24131−24145.

(16) Takahashi, D.; Moriyama, J.; Nakamura, T.; Miki, E.; Takahashi, E.; Sato, A.; Akaike, T.; Itto-Nakama, K.; Arimoto, H. AUTACs: Cargo-Specific Degraders Using Selective Autophagy. *Mol. Cell* **2019**, *76* (5), 797−810.e10.

(17) Meanwell, N. A. Synopsis of Some Recent Tactical Application of Bioisosteres in Drug Design. *J. Med. Chem.* **2011**, *54* (8), 2529−2591.

(18) Gammon, D. W.; Hunter, R.; Wilson, S. A. An Efficient Synthesis of 7-Hydroxy-2,6-Dimethylchromeno[3,4-d]Oxazol-4-One—a Protected Fragment of Novenamine. *Tetrahedron* **2005**, *61* (45), 10683−10688.

(19) Manjappa, K. B.; Peng, Y.-T.; Liou, T.-J.; Yang, D.-Y. Pseudo Three-Component Approach to Coumarin-Annulated Azepines: Synthesis of Coumarin[3,4-b]Azepines. *RSC Adv.* **2017**, *7* (72), 45269−45273.

(20) Stecher, H.; Tengg, M.; Ueberbacher, B. J.; Remler, P.; Schwab, H.; Griengl, H.; Gruber-Khadjawi, M. Biocatalytic Friedel−Crafts Alkylation Using Non-Natural Cofactors. *Angew. Chem., Int. Ed.* **2009**, *48* (50), 9546−9548.
(21) Migliore, M.; Pontis, S.; Fuentes de Arriba, A. L.; Realini, N.; Torrente, E.; Armirotti, A.; Romeo, E.; Di Martino, S.; Russo, D.; Pizzirani, D.; Summa, M.; Lanfranco, M.; Ottonello, G.; Busquet, P.; Jung, K.-M.; Garcia-Guzman, M.; Heim, R.; Scarpelli, R.; Piomelli, D. Second-Generation Non-Covalent NAAA Inhibitors Are Protective in a Model of Multiple Sclerosis. *Angew. Chem., Int. Ed.* **2016**, *55* (37), 11193−11197.
(22) Engel, M.; Hindie, V.; Lopez-Garcia, L. A.; Stroba, A.; Schaeffer, F.; Adrian, I.; Imig, J.; Idrissova, L.; Nastainczyk, W.; Zeuzem, S.; Alzari, P. M.; Hartmann, R. W.; Piiper, A.; Biondi, R. M. Allosteric Activation of the Protein Kinase PDK1 with Low Molecular Weight Compounds. *EMBO J.* **2006**, *25* (23), 5469−5480.
(23) Rogov, V. V.; Rozenknop, A.; Rogova, N. Y.; Löhr, F.; Tikole, S.; Jaravine, V.; Güntert, P.; Dikic, I.; Dötsch, V. A Universal Expression Tag for Structural and Functional Studies of Proteins. *ChemBioChem* **2012**, *13* (7), 959−963.
(24) Huber, J.; Obata, M.; Gruber, J.; Akutsu, M.; Löhr, F.; Rogova, N.; Güntert, P.; Dikic, I.; Kirkin, V.; Komatsu, M.; Dötsch, V.; Rogov, V. V. An Atypical LIR Motif within UBA5 (Ubiquitin like Modifier Activating Enzyme 5) Interacts with GABARAP Proteins and Mediates Membrane Localization of UBA5. *Autophagy* **2020**, *16* (2), 256−270.
(25) Fedorov, O.; Niesen, F. H.; Knapp, S. Kinase Inhibitor Selectivity Profiling Using Differential Scanning Fluorimetry. *Methods Mol. Biol.* **2012**, *795*, 109−118.
(26) Williamson, M. P. Using Chemical Shift Perturbation to Characterise Ligand Binding. *Prog. Nucl. Magn. Reson. Spectrosc.* **2013**, *73*, 1−16.

https://doi.org/10.1021/acs.jmedchem.0c01564
J. Med. Chem. 2021, 64, 3720−3746