

Article

# Design, Synthesis and Antibacterial Activity of Coumarin-1,2,3-triazole Hybrids Obtained from Natural Furocoumarin Peucedanin

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**Abstract:** Synthesis of 1,2,3-triazole-substituted coumarins and also 1,2,3-triazolyl or 1,2,3-triazolylalk-1-ynyl-linked coumarin-2,3-furocoumarin hybrids was performed by employing the cross-coupling and copper catalyzed azide-alkyne cycloaddition reaction approaches. The synthesized compounds were evaluated for their in vitro antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Actinomyces viscosus* and *Escherichia coli* bacterial strains. Coumarin-benzoic acid hybrids **4c**, **42c** and 3-((4-acetylamino-3-(methoxycarbonyl)phenyl)ethynyl)coumarin (**29**) showed promising activity against *S. aureus* strains, and the 1,2,3-triazolyl-oct-1-ynyl linked coumarin-2,3-furocoumarin hybrid **37c** was endowed with high selectivity against *B. subtilis* and *E. coli* species. The in vitro antibacterial activity of **4c**, **29**, **37c** and **42c** can potentially be compared with that of a number of modern antibiotic drugs used in the clinic, suggesting promising prospects for further research. A detailed study of the molecular interactions with the targeted protein MurB was performed using docking simulations and the obtained results are quite promising.

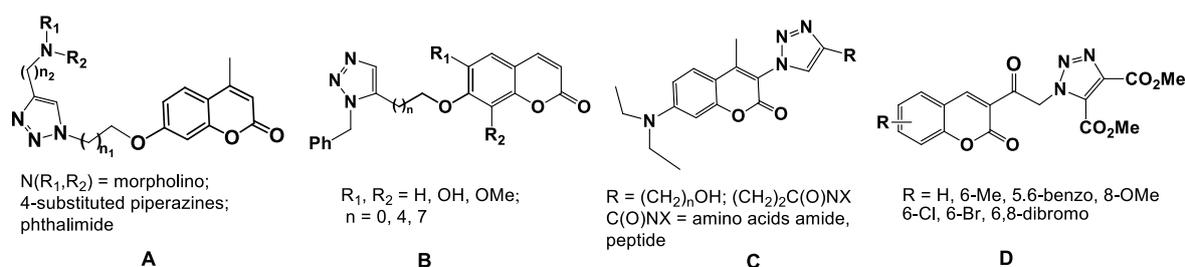
**Keywords:** coumarin; furocoumarin; Sonogashira coupling; CuAAC reaction; antibacterial activity

## 1. Introduction

As stated in a WHO report, in the last few decades, the incidence of microbial infections has increased dramatically together with emergence of antimicrobial-resistant strains [1]. Increasing instances of antimicrobial drug resistance requires the design and synthesis of new small molecules with higher affinity and specificity for their potential targets to serve as antibiotics. Coumarins, naturally plant-derived compounds with a benzopyrone moiety, possess a wide variety of biological activities. Series of coumarin derivatives are being extensively studied due to their broad array of biological activities, low toxicity, and lower drug resistance properties [2].

Various naturally-isolated coumarins, as well as their chemically modified analogs, are active against numerous bacterial strains, including those which have developed multidrug resistance [2]. Among these compounds of interest are the coumarin-1,2,3-triazole hybrids (Figure 1) [3,4].

1,2,3-Triazoles have been nuclei of choice in recent years because of their excellent favorable safety profile, latent ability to form hydrogen bonds, moderate dipole character, rigidity and stability under in vivo conditions and capability of interacting with biomolecular targets [5,6]. A set of coumarin hybrids **A** (Figure 1) having a 1,2,3-triazole moiety in the C-7 position on the coumarin core was assessed for their in vitro antimicrobial activities against Gram-positive and Gram-negative pathogens [7]. The obtained results showed that all hybrids of type **A** displayed considerable activity against the tested strains, and SAR studies revealed that the substituent and the length of alkyl spacers in the 1 and 4 position of the triazole ring have profound effects on the antimicrobial potency. Morpholinylmethyl- and piperazinylmethyl (N-R<sub>1</sub>,R<sub>2</sub>)-containing hybrids exhibited noticeable activity against various bacterial pathogens and were more potent than the hybrids incorporating a phthalimidomethyl moiety in the 4 position of the triazole ring. The 6,8-disubstituted coumarin-1,2,3-triazole conjugates **B** (Figure 1) exhibited significant in vitro antibacterial activity [8,9]. Investigation of the antimicrobial activity of this type of hybrid compounds led to the identification of several different structural frameworks. The obtained results suggested that both the length of the spacer (n) and also the nature of the substituent (H, OH, OMe) in the 6 and 8 positions modified the lipophilicity of the hybrids, and this in turn affected the antibacterial activity. In these molecules, the triazole rings are linked with the coumarins by a methylene oxygen. The antibacterial activity was correlated with the length of the spacer (n), and the contribution order of substituents was the following: H > OMe > OH.



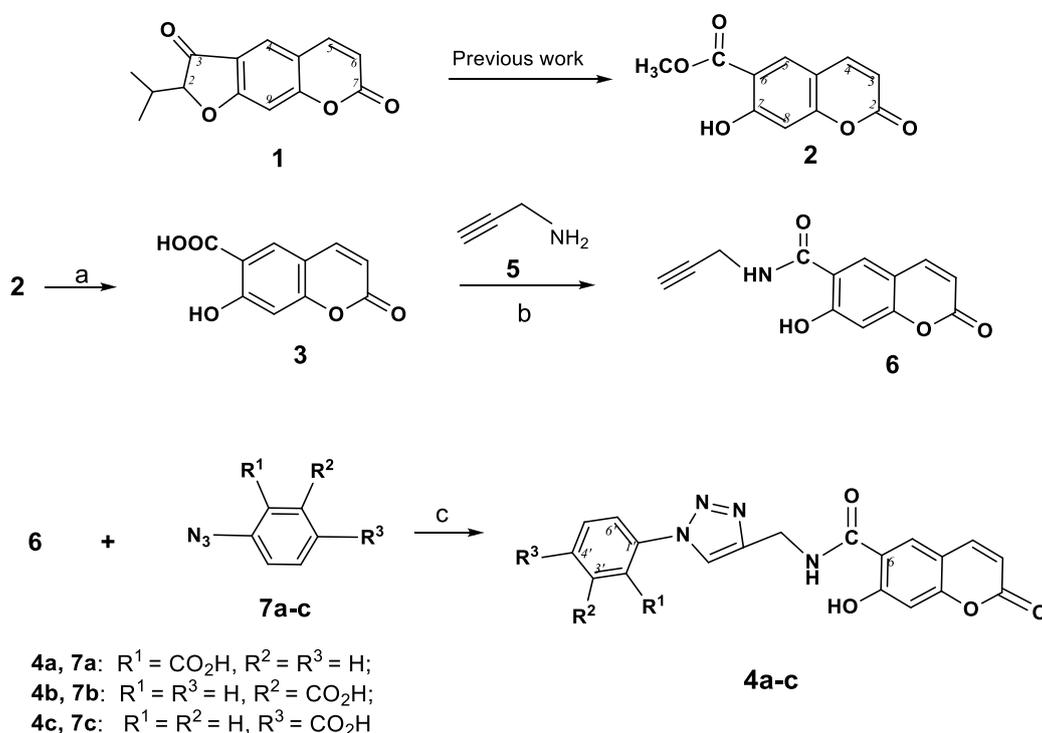
**Figure 1.** Chemical structures of coumarin-1,2,3-triazole hybrids of types A–D.

The substituted coumarins with 1,2,3-triazole substituent in the 3 position (compounds **C**) exhibited activity which was dependent on the nature of the substituent on the triazole ring (alkylamide, alkylpeptide) [10].

All coumarin-1,2,3-triazole hybrids of the type **D** (Figure 1) displayed promising antibacterial and antifungal properties, which were comparable with the reference drug griseofulvin [11]. The SAR revealed that the substituent at C-5, C-6, and/or C-8 position of coumarin moiety have a great influence on the antimicrobial activity, and 6-Br was favorable for activity against the bacterial strain *E. coli* and the fungus *A. niger*, while a 6-Me benefitted the activity against *B. subtilis*; monohalo hybrids were more potent than their bis-halo counterparts (in the 6 and 8 positions) against all strains.

Thus, the design and synthesis of novel coumarinotriazole derivatives offers a prospective route for accessing new molecules with improved antibacterial activity profiles. Our research interests include the design of convenient ways to access polysubstituted natural furocoumarins [12–16] and coumarins [17,18] and the assessment of their potential as bioactive agents. Herein we report the synthesis of a range of coumarins containing a triazole substituent in the 3 or 6 position of the coumarin core. Our attention was concentrated on the synthesis of compounds bearing both a previously identified pharmacophoric moiety and a substituted triazole ring. Thus, we describe the synthesis of a range of mixed compounds having coumarin-2,3-dihydrofurocoumarin hybrid structures linked through an 1*H*-1,2,3-triazole ring or alkyne-methylene-triazolyl bridge at the C2-C3' atoms. As starting compounds we used the natural linear furocoumarin peucedanin (1) and the coumarin peuruthenicin (officinalin, 7-hydroxy-6-(methoxycarbonyl)coumarin, 2, Scheme 1). The Pd-catalyzed coupling methods and Cu-catalyzed azide-alkyne cycloaddition (CuAAC reaction) were the main routes of

synthesis. The antimicrobial activity of the triazolyl-substituted coumarins against a panel of clinically relevant bacterial strains were also investigated and discussed.



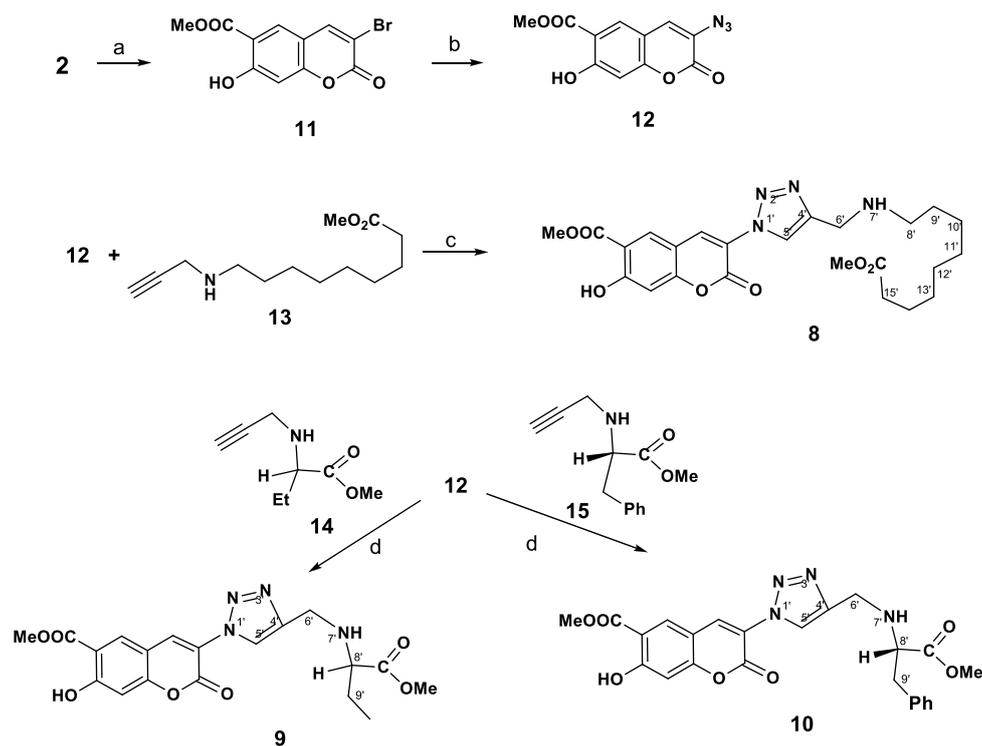
**Scheme 1.** Synthesis 6-(1-(carboxyphenyl)-1H-1,2,3-triazol-4-ylcarbamoyl)umbelliferones **4a–c**.  
*Reagents and Conditions:* (a) 20% eq. NaOH, dioxane, reflux, 20 min; (b) DCC, HOBT, Et<sub>3</sub>N, DMF, rt, 48 h; (c) CuSO<sub>4</sub>·5H<sub>2</sub>O (5 mol%), sodium ascorbate (15 mol%), H<sub>2</sub>O-CH<sub>2</sub>Cl<sub>2</sub>, 20→40 °C.

## 2. Results and Discussion

### 2.1. Chemical Synthesis

The umbelliferon carboxylic acid **3**, the starting compound for the synthesis of triazolyl substituted coumarins **4a–c** (Scheme 1), was conveniently obtained by treatment of peuruthenicin (**2**) with NaOH in dioxane [19]. The peptide coupling reaction of **3** with propargyl amine **5** using DCC as a coupling reagent gave the corresponding 6-(prop-2-ynylcarbamoyl)umbelliferone (**6**) in 67% yield. We have chosen the most simple and well-characterized variant, namely, carrying out the reaction in a CH<sub>2</sub>Cl<sub>2</sub>-water mixture, with catalysis of the Cu(I) ions generated in situ from CuSO<sub>4</sub> and sodium ascorbate [12]. CuAAC-reaction of ethynyl coumarin **6** with three positional azidobenzoic acid isomers **7a–c** led to the 6-[1-(carboxyphenyl)-1,2,3-triazol-4-yl]carbamoyl-umbelliferones **4a–c** in 50–62% yields.

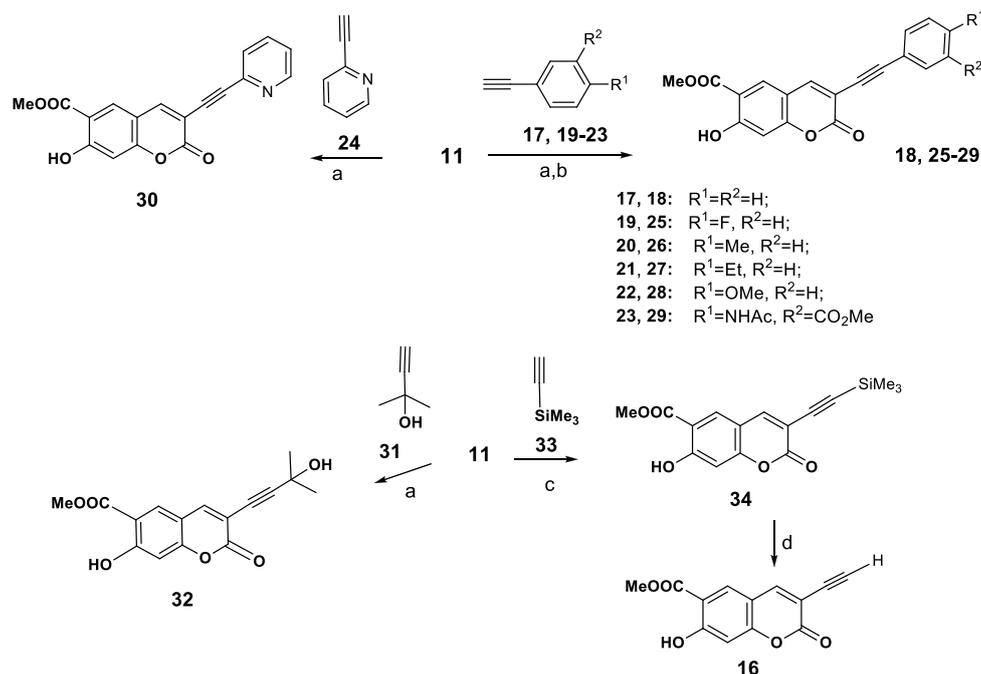
The synthetic route followed for the synthesis of the desired novel coumarins substituted with a triazole ring in the C-3 position **8–10** is outlined in Scheme 2. Bromination of peuruthenicin (**2**) with dioxane dibromide (2.2 eq) in CH<sub>2</sub>Cl<sub>2</sub> proceeds selectively and led to the formation of 3-bromopeuruthenicin (**11**) as the sole product in 88% isolated yield. 3-Azidocoumarin **12** was conveniently obtained in 68% yield, by reacting the corresponding 3-bromopeuruthenicin (**11**) with sodium azide in DMF. The second alkyne building block was obtained from amino acids in two steps. At the first step, 9-aminopelargonic acid, D,L-2-aminobutyric acid or L-phenylalanine hydrochloride were transformed to subsequent methyl ethers of the amino acid hydrochloride in nearly quantitative yield requiring no further purification. Reaction of amino acids methyl esters hydrochloride with propargyl bromide in the presence of K<sub>2</sub>CO<sub>3</sub> in dry DMF gave alkynes **13–15**, which were purified through the column chromatography on silica gel using a CHCl<sub>3</sub>/EtOH mixture as an eluent.



**Scheme 2.** Synthesis of 3-(triazolyl)-substituted coumarins **8–10**. *Reagents and Conditions:* (a): dioxane dibromide,  $\text{CH}_2\text{Cl}_2$ , rt, 16 h; (b):  $\text{NaN}_3$ , DMF,  $40^\circ\text{C}$ , 14 h; (c)  $\text{CuSO}_4$ , sodium ascorbate, eq.  $\text{CH}_2\text{Cl}_2$  (1:1), rt, 3 h, then  $40^\circ\text{C}$ , 1 h; (d):  $\text{CuI}$ ,  $\text{Et}_3\text{N}$ , MeCN, rt, 10 h.

The interaction of the new azide **12** with methyl 9-(prop-2-ynylamino)nonanoate **13** under above catalytic conditions was carried out at  $20^\circ\text{C}$  for 4 h (monitoring by TLC) and led to the target compound **8** in the isolated yield 67%. The reaction of azide **12** with terminal alkynes **14**, **15**, proceeds with the formation of substituted coumarins **9** or **10** in high yield by performing this reaction in the presence of  $\text{CuI}$  and  $\text{Et}_3\text{N}$  (method *d*). By using of  $\text{CuSO}_4$  and sodium ascorbate as the catalysts in the reaction of **12** with **15**, the isolated yield of coumarin **10** was decreased to 34%.

Thus we obtained 3-(1*H*-1,2,3-triazol-1-yl)peuruthenicin derivatives **8–10**. It was also important for us to identify the influence of the position of coumarin as the substituent in the C-4 or C-1 of the triazole ring. To obtain 3-(1*H*-1,2,3-triazol-4-yl)peuruthenicins we were interested in the synthesis of 3-(ethynyl)peuruthenicin (**16**, Scheme 3).

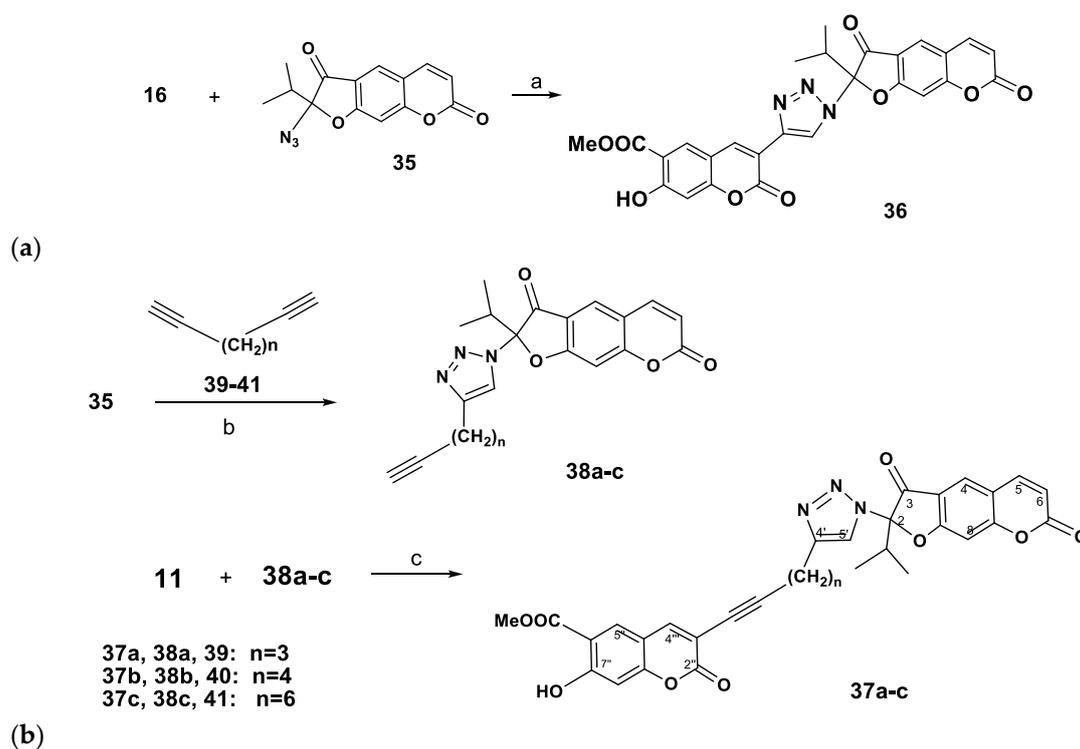


**Scheme 3.** Synthesis 3-arylethynylcoumarins **17**, **24–29**, 3-(alkynyl)coumarins **31**, **33**, and 3-(ethynyl)peuruthenicin (**34**). *Reagents and Conditions:* (a) Pd(PPh<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>, CuI, Et<sub>3</sub>N, benzol, 110 °C, 12 h; (b) Pd(PPh<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>, CuI, Et<sub>3</sub>N, Bu<sub>4</sub>NBr, DMF; (c) Pd(PPh<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>, CuI, Et<sub>3</sub>N, toluene, MAOS, 100 °C, 2 h; (d) CsF, MeOH, TEBA, rt, 10 h.

For this purpose we studied the activity of 3-bromopeuruthenicin (**11**) in the Sonogashira cross-coupling reaction. Besides providing data about the reactivity of 3-bromopeuruthenicin (**11**) in the cross-coupling reaction with various terminal alkynes, this study served to make available a larger panel of peuruthenicin derivatives for testing their antimicrobial activity. Therefore, the reaction between 3-bromopeuruthenicin (**11**) with phenyl acetylene (**17**) was optimized first, attempting to obtain the cross-coupling derivative **18** under different conditions. After considerable experimentation, we found that the cross-coupling proceeds in a benzene solution in the presence of a catalytic amounts of *trans*-dichlorobis(triphenylphosphine)palladium(II), copper(I) iodide, and Et<sub>3</sub>N as a base (conditions a, Scheme 3) and led to the corresponding 3-(phenylethynyl)coumarin (**18**) in 65% yield after column chromatography on silica gel. Using Bu<sub>4</sub>NBr as an additive, DMF as a solvent and Et<sub>3</sub>N as a base did not improve the yield of compound **18** (conditions b). On the other hand, using K<sub>2</sub>CO<sub>3</sub> as a base in benzene or DMF at 80–100 °C also met with failure, and the reaction afforded yields of 45% or 42%, respectively, and a yield of 20% was recorded when the transformation was performed using (*i*Pr)<sub>2</sub>NH as a base. With the set of optimum conditions in hands, we found that the reaction of coumarin **11** with aryl(hetaryl)acetylenes **19–24** proceeds with the formation of compounds **25–30** (52–68% yields). A perspective route for synthesis of terminal alkynes (for example, 3-ethynylpeuruthenicin (**16**)) was the Sonogashira coupling of **11** with accessible 2-methylbut-3-yn-2-ol (**31**) and subsequent elimination of acetone from the 3-alkynylcoumarin **32**. We found that the reaction of **11** with alkyne **31** (conditions a) led to the formation of 3-(3-hydroxy-3-methylbut-1-ynyl)coumarin (**32**, (66% yield). However, the transformation of the alcohol **32** into the terminal alkyne using the known conditions [20] was unsuccessful. The copper and palladium-catalyzed Sonogashira coupling of 3-bromopeuruthenicin **11** with trimethylsilylacetylene (**33**) (using conditions b) required a long reaction time and proceeds with the formation of traces of 3-(trimethylsilyl-ethynyl)peuruthenicin (**34**). Those results forced us improve this reaction. Currently, microwave-assisted organic synthesis (MAOS) is receiving increasing attention as a valuable alternative to the conventional heating to speed up chemical reactions, and microwave irradiation has been applied to Sonogashira coupling reactions [21,22]. It should be noted that MAOS has significant advantages that include simplicity in

operation, increased reaction rates, and improved reaction yields. Performing the microwave-assisted reaction of 3-bromopeuruthenicin (**11**) and trimethylsilylacetylene (**33**) using  $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ ,  $\text{CuI}$  as catalysts and  $\text{Et}_3\text{N}$  as a base in toluene solution under microwave irradiation at  $100^\circ\text{C}$  for 2 h give the cross-coupling product **34** in 64% isolated yield. Thus, the use of microwaves was found to significantly improve the reaction yield and shorten the reaction time. Desilylation of compound **34** using  $\text{CsF}$  in  $\text{MeOH}$  in the presence of benzyltriethylammonium chloride (TEBA) afforded 3-ethynylpeuruthenicin (**16**, 85% yield) which was purified by column chromatography on silica gel using  $\text{CHCl}_3$  as an eluent.

At the next step of our study, the acetylene building block **16** was reacted with 2-azidooreozelone (**35**) [14]. Carrying out the reaction in a  $\text{CH}_2\text{Cl}_2$ -water mixture, with catalysis of the  $\text{Cu}(\text{I})$  ions generated in situ from  $\text{CuSO}_4$  and sodium ascorbate we obtained the coumarin-2,3-dihydrofurocoumarin hybrid linked through an 1*H*-1,2,3-triazole ring **36** isolated in 70% yield (Scheme 4).



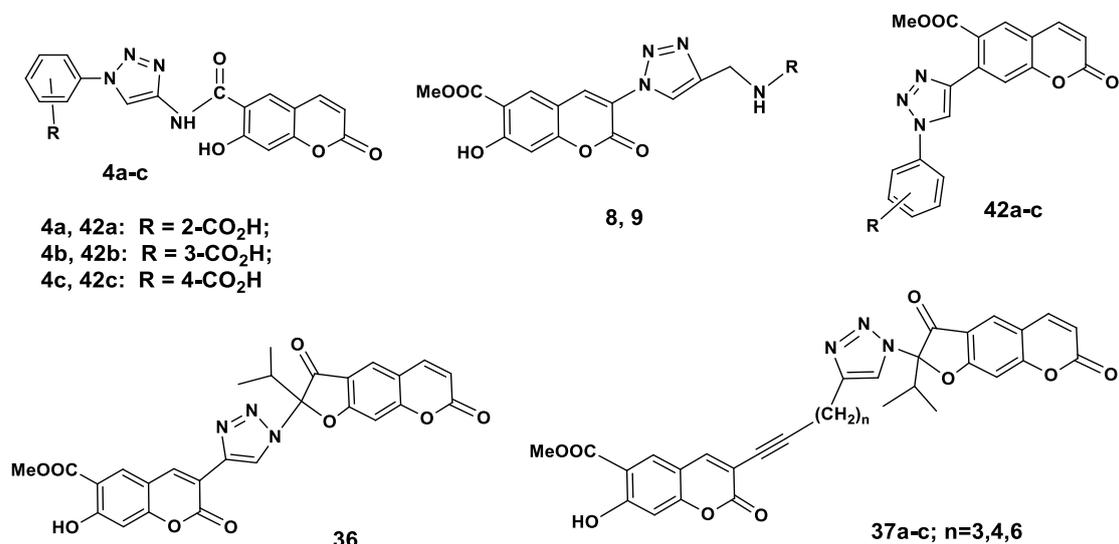
**Scheme 4.** Synthesis of coumarin-2,3-dihydrofurocoumarin hybrids linked with 1*H*-1,2,3-triazole rings. *Reagents and Conditions:* (a):  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (5 mol%), sodium ascorbate (15 mol%),  $\text{H}_2\text{O}-\text{CH}_2\text{Cl}_2$ ,  $20 \rightarrow 40^\circ\text{C}$ ; (b):  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , sodium ascorbate,  $\text{H}_2\text{O}-\text{CH}_2\text{Cl}_2$ ,  $40^\circ\text{C}$ , 5 h; (c)  $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ ,  $\text{CuI}$ ,  $\text{Et}_3\text{N}$ , benzene,  $80^\circ\text{C}$ , 14 h.

We also performed the synthesis of coumarin-2,3-dihydrofurocoumarin hybrids **37a–c**, containing a triazolylmethylene-1-ynyl linker group (Scheme 4). The synthetic route involved the Sonogashira's reaction of new 2-ethynylalkyltriazolyl substituted furocoumarins **38a–c** with 3-bromopeuruthenicin (**11**, Scheme 4). Compounds **38a–c** (yield 62–74%) were prepared by the CuAAC reaction of 2-azidooreozelone (**35**), with 1,6-heptadiyne (**39**), 1,7-octadiyne (**40**) or 1,9-decadiyne (**41**). Reaction of 3-bromopeuruthenicin (**16**) with alkynes **38a–c** in the Sonogashira reaction conditions afforded the coumarin-2,3-dihydrofurocoumarin hybrids linked through 1*H*-1,2,3-triazolylalk-1-ynyl groups **37a–c** (52–57% yields).

## 2.2. Biological Screening

The new coumarins **4a–c**, **11**, **29**, **30**, coumarin-dihydrofurocoumarin hybrids **36**, **37a–c**, and the 7-triazolylsubstituted coumarins **42a–c** (Figure 2), the synthesis of which was described in our previous paper [23] were screened for their in vitro antibacterial activity against Gram positive bacterial strain

*Staphylococcus aureus* 209 ATCC 6538P. The antibacterial activity was studied by serial dilution in a liquid nutrient medium [24]. The minimum inhibition concentrations (MIC) of the test cultures were determined. The most promising substances were those with indicators of MIC = 100 µg/mL and less. Table 1 reveals that coumarino-1,2,3-triazole derivatives **4c** and **42c** and 3-arylethynylpeuruthenicin **29** showed excellent antibacterial activity, with MIC values ranging from 0.16–0.41 µg/mL against the tested microorganism and they prove to be better than that of the reference drugs ceftriaxone (0.97 µg/mL) and streptomycin (1.89 µg/mL).



**Figure 2.** Chemical structures of antibacterial coumarin-benzoic acid hybrids and coumarin-furocoumarin hybrids with a 1,2,3-triazolyl linker.

**Table 1.** The in vitro antibacterial activity of compounds **4a–c**, **11**, **26**, **29**, **30**, **37a–c**, **42a–c** and starting compounds **1–3** against bacterial strain *S. aureus* 209p ATCC6538P.

Compound	<i>S. aureus</i> 209p MIC, µg/mL	Compound	<i>S. aureus</i> 209p MIC, µg/mL
1	800 ± 28.87	30	162.5 ± 23.94
2	>1000	37a	250 ± 20.41
3	933.3 ± 66.67	37b	425 ± 47.87
4a	>1000	37c	51.25 ± 3.15
4b	416.7 ± 60.09	42a	>1000
4c	0.16 ± 0.08	42b	>1000
11	92.5 ± 4.79	42c	0.31 ± 0.23
26	412.5 ± 42.7	Ceftriaxonum	0.97 ± 0.02
29	0.41 ± 0.13	Streptomycin	1.89 ± 0.08

In the series of coumarin-benzoic acid hybrids **4a–c**, **42a–c** in view of the position of the carboxylic group in the benzoic acid moiety, we considered that compounds containing this substituent in the 4 position **4c**, **42c** were more active than compounds **4a,b** and **42a,b**, respectively, so the carboxylic acid group is an excellent microbial ligand which can bind more effectively at the active site of receptor. The difference in antibacterial activity profile of 3-bromocoumarin **11** and 3-(arylethynyl) coumarins **26**, **29**, **30** indicates the importance of the nature of the functional substituent on the triple bond in the C-3 position of coumarin. Thus, compound **29** containing an anthranilic acid methyl ester fragment on the 3-ethynylcoumarin nucleus showed greater activity than compounds with 4-tolyl- or pyridine substituents at the triple bond in 3-ethynylcoumarin. It should be noted that the biological effect of anthranilic acid derivatives is based on their ability to act as modulators of the nuclear peroxisome

proliferator activated receptors (PRAR) and the farnesoid X (FXR) receptors, which fulfill crucial roles in metabolic balance [25,26]. In this direction the antibacterial activity of compounds **37a–c**, having a methylene-triazolyl-furocoumarin substituent on the triple bond of 3-ethynylcoumarin was dependent on the length of the C-methylene linker. The activity of the most active hybrid compound **37c** is due the presence of a hexamethylene-1-ynyl linker group. Coumarin-2,3-dihydrofurocoumarin hybrid **37c** was found to exhibit good potency at 51.25 mg/mL of MIC, while the parent compounds **1** and **2** exhibited a lack of activity (Table 1).

The results of study of promising substances **4c**, **29**, **37c** and **42c** on other strains of *Staphylococcus aureus* and *Actinomyces viscosus* U-18 are presented in Table 2. Further study on the *S. aureus* strain confirmed the high activity of compounds **4c**, **29** and **42c**. Compound **4c** (carboxamidotriazolylbenzoic acid substitution at the C-6 position of the coumarin core) showed good activity against *A. viscosus* compared with compound **42c** which showed moderate activity against *A. viscosus*. Characteristically, that compound **42c** with a triazolylbenzoic acid substituent in the C-7 position possessed the highest activity against *S. aureus* “Viotko” bacterial strains. Of interest was also the high antibacterial activity of 3-ethynylcoumarin with methylanthranilate substituent **29** on the all tested *S. aureus* strains. This compound will be further used as the scaffold for structural optimization to develop more potent and selective antibacterial agents.

**Table 2.** The in vitro antibacterial activity results of compounds **4c**, **29**, **37c** and **42c** against *S. aureus* and *A. viscosus* U-18 strains.

Compound	<i>S. aureus</i> C-18 MIC, µg/mL	<i>S. aureus</i> “Viotko” MIC, µg/mL	<i>A. viscosus</i> U-18 MIC, µg/mL
<b>4c</b>	0.65 ± 0.22	3.75 ± 1.73	15.1 ± 1.56
<b>29</b>	1.78 ± 1.10	2.0 ± 1.01	75.2 ± 14.43
<b>37c</b>	68.75 ± 11.97	68.8 ± 18.75	Not Tested
<b>42c</b>	6.28 ± 2.37	0.21 ± 0.08	166.7 ± 44.1
Ceftriaxone	6.50 ± 1.29	1.03 ± 0.32	933.3 ± 33.33

Next set of experiments was dedicated to the analysis of the antibacterial potential of coumarin-2,3-dihydrofurocoumarin hybrids **36**, **37a–c** (Table 3). The in vitro antibacterial activity of those compounds was additionally tested against *Bacillus subtilis* and *Escherichia coli* (JM 109) bacterial strains. The obtained results were compared with the known tumorigenic compound 4-nitroquinolin-1-oxide (NQO) and presented as an average concentration of inhibitory 50% bacterial proliferation (incubation time 20 h). It can be observed that from the series of coumarin-2,3-dihydrofurocoumarin hybrids only dimeric compound **37c** with the 1,2,3-triazolyloct-1-ynyl linker group displayed promising activity against *B. subtilis* and also *E. coli* (JM 109) bacterial strains. On the contrary to this, variously 1,2,3-triazolyl or 1,2,3-triazolylhex-1-ynyl or 1,2,3-triazolylpent-1-ynyl linked hybrids **36**, **37a,b** were deprived of anti-bacterial activities (MICs were >1000 mg/mL).

**Table 3.** Antimicrobial activities of coumarin-furocoumarin hybrids **36**, **37a–c** and parent compounds **1**, **2** against *B. subtilis* and *E. coli* strains (\*  $P < 0.05$  relative to the control).

Compound	MIC $\pm$ SEM, $\mu\text{g/mL}$	
	<i>Bacillus subtilis</i>	<i>Escherichia coli</i> (JM 109)
<b>1</b>	>1000	>1000
<b>2</b>	>1000	>1000
<b>36</b>	>1000	>1000
<b>37a</b>	>1000	>1000
<b>37b</b>	>1000	>1000
<b>37c</b>	0.02 $\pm$ 0.003 *	0.15 $\pm$ 0.04 *
NQO	10.8 $\pm$ 2.50 *	12.79 $\pm$ 1.88 *
Streptomycin	2.0 $\pm$ 0.68	1.0 $\pm$ 0.35

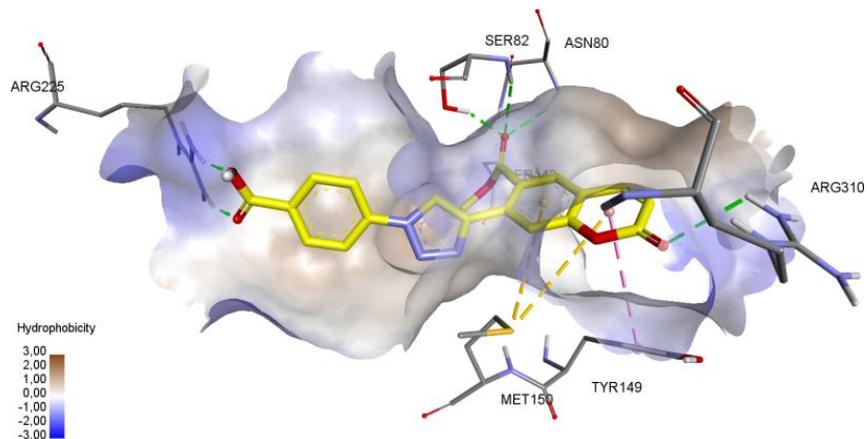
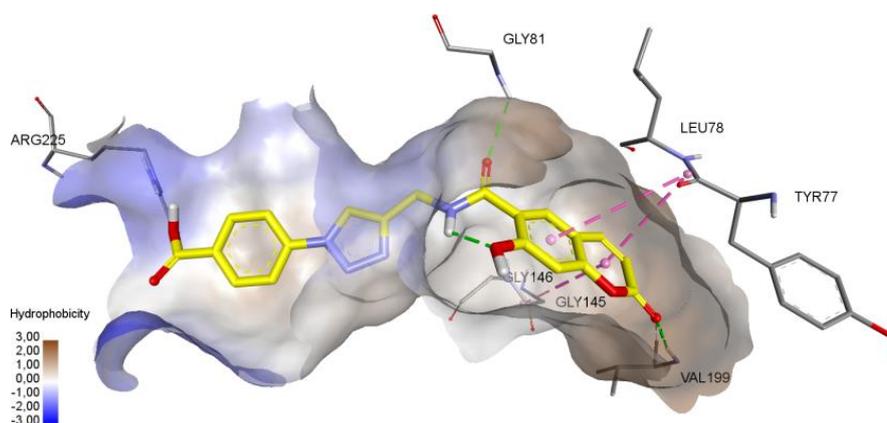
### Molecular Docking

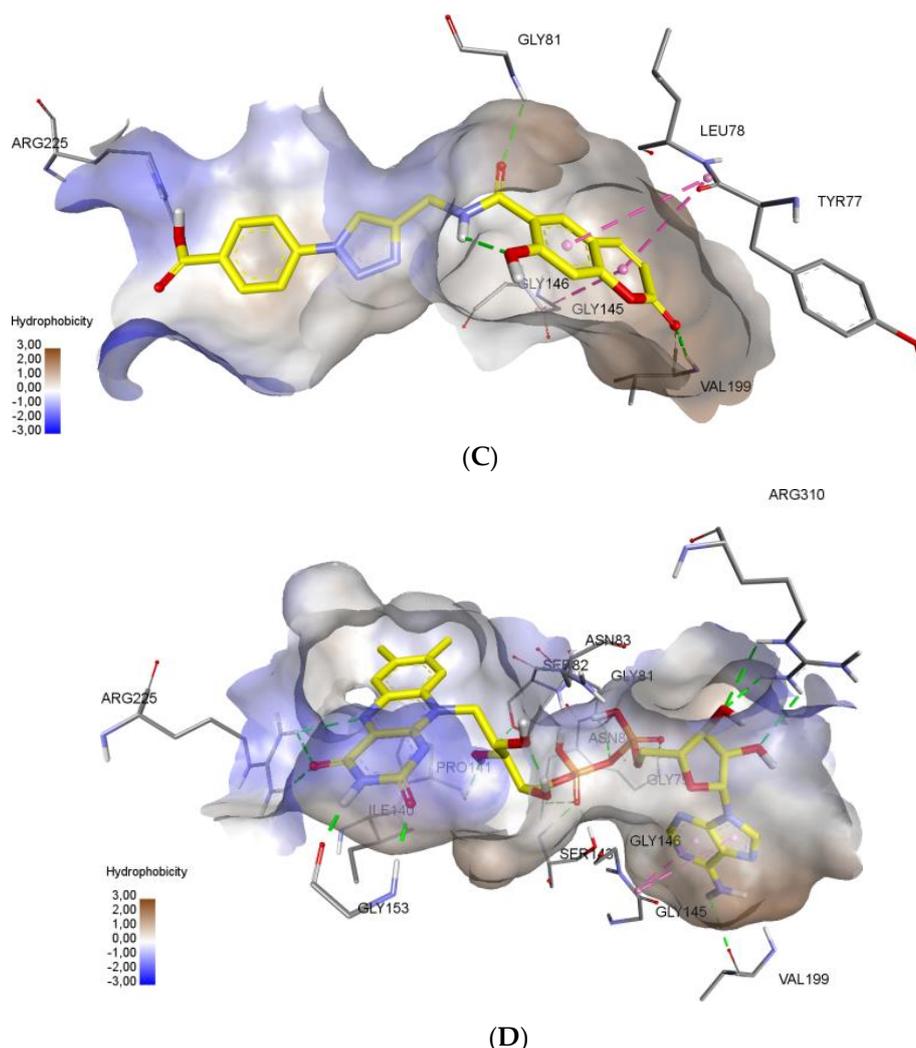
Molecular docking protocol is an essential tool to mimic the natural course of interaction of the ligand with active sites of receptors through lowest binding energies in which two molecules fit together in 3D space. Here, we discuss the mechanism of interaction between coumarinotriazole derivatives **3**, **4** and **5** with the MurB protein (PDB ID: 1HSK). MurB is an attractive target protein in bacterial infection diseases and it is an essential enzyme which takes part in amino sugar metabolism which reduces the enolpyruvyluridine diphosphate N-acetyl glucosamine (EP-UNAG) as an intermediate in the assembly of the UNAM-pentapeptide (m-A2pm) protein to uridine diphosphate N-acetylmuamic acid (UNAM) of cell wall precursor [27]. For the calculations, the XRD model of *S. aureus* N-acetylenolpyruvylglucosamine reductase (MurB) with PDB ID 1HSK was chosen (resolution 2.3 Å) [28]. To model a possible mechanism of MurB inhibition, molecular docking of new coumarins was performed at the binding site of flavin adenine dinucleotide (FAD) in the Glide application [29]. We have screened the coumarinotriazoles **4a–c**, **8**, **9**, and **42a–c** and also coumarin-furocoumarin hybrids **36**, **37b**, and **37c**. The molecules **4a–c**, **37c** and **42a,c** strongly approach the MurB protein receptor as shown by their minimum binding energies  $-8.416$ – $-8.983$  Kcal/mol (Table 4). The docking results were found to be in good agreement with in vitro antibacterial experimental MIC values (Tables 1–3). The FAD binding site is saturated with polar amino acids. This facilitates formation of a large number of hydrogen bonds due to the large number of polar groups in the FAD molecule. Additional stabilization in the binding site provided stacking interactions of aromatic cycles of adenine. Inspection of the binding mode demonstrated, that compounds **4c**, **37c** and **42c** successfully combine in their structure a large number of polar groups and  $\pi$ -systems (Figure 3). The presence of the carboxy function in compounds **4c**, **42c** allowed the formation of hydrogen bond with amino acid ARG225 residue (Figure 3A–C). As shown in Figure 3C, the inhibitor **37c** formed additional hydrogen bonding interactions with the active site residues: the furan ring C=O with SER 238 (2.92 Å) and the coumarin ring C=O with TYR 155. All those interactions contribute to a spatial orientation close to FAD and the formation of hydrogen bonds with the same amino acid residues as FAD (Figure 3D). We found that the MurB protein receptor amino acids ASN80, SER82, SER238, GLY81 are the most active sites responsible for interactions with the ligand. Important interaction centers at the binding site are arginines 225 and 310, protein chain section 79–83, glycines 145, 146 and 153, isoleucine 140 and proline 141, valine 199. New coumarins interact with almost all these centers, except for 140–141, and also form bonds characteristic only for these molecules. The formation of interactions with aromatic amino acid residues increases the stability of the conformations of new coumarins at the binding site.

**Table 4.** Values of the minimum binding energy for new compounds to MurB protein.

Ligand	Binding Energy *, kcal/mol	Ligand	Binding Energy *, kcal/mol
FAD	-12.081	2	-7.884
42c	-8.983	37b	-7.844
37c	-8.977	42b	-7.441
42a	-8.898	8	-6.578
4c	-8.473	1	-6.509
4b	-8.416	4a	-6.300
9	-8.171	36	-5.399

\* Value is not genuine binding energy but estimated docking score.

**Figure 3.** *Cont.*



**Figure 3.** Noncovalent interactions of compounds (3A—4c, 3B—42c, 3C—37c, 3D—FAD) are shown by dotted lines: green—hydrogen bonds, purple—stacking interactions, orange—electrostatic interactions. Hydrophobic interactions and nonpolar hydrogens are omitted. (A) 3D interactions of ligand 4c at the FAD binding site of MurB Protein. (B) 3D interactions of ligand 42c at the FAD binding site of MurB Protein. (C) 3D interactions of ligand 37c at the FAD binding site of MurB Protein. (D) 3D interactions of FAD molecule at the binding site of MurB Protein.

### 3. Conclusions

In summary, we have synthesized new series of coumarinotriazole compounds using [2 + 3]-cycloaddition reactions or Sonogashira cross-coupling reactions as the main approaches. The synthesized coumarino-triazole type derivatives were screened for their *in vitro* antimicrobial activity. Compounds 4c and 42c, having the 4-(carboxyphenyl)triazolyl substituent in the 6 or 7 position of the coumarin ring showed excellent antibacterial activity against *S. aureus* strains, with MIC values of 0.16–3.75  $\mu\text{g}/\text{mL}$  and 0.21–6.28  $\mu\text{g}/\text{mL}$  respectively. Most of the compounds of series 11, 29 and 30 with bromine, and aryl(hetaryl)substituents in the 3 position revealed significant MIC values (between 0.41 to 2.0  $\mu\text{g}/\text{mL}$  for compound 29), indicating that 3-substituted coumarin analogues show promising antibacterial activity and are key compounds for further development as antibacterial agents. Coumarin-2,3-dihydrofurocoumarin hybrid compound 37c was found to be selective against *Bacillus subtilis* and *E. coli*, with MIC values of 0.02–0.15  $\mu\text{g}/\text{mL}$ . A molecular docking study was performed for the most active compounds against the MurB protein. Molecular docking results were well corroborated with the *in vitro* antibacterial activity findings. Finally, the successful synthesis and

antimicrobial evaluation along with docking study of new coumarino-triazole scaffolds obtained on the base of accessible plant coumarins peucedanin and peuruthenicin will provide further advantages to design and develop triazole derivatives with selective antibacterial activity.

## 4. Experimental Section

### 4.1. General Information

NMR spectra were acquired on Bruker AV-400 ( $^1\text{H}$ : 400.13 MHz,  $^{13}\text{C}$ : 100.78 MHz) or Bruker AV-600 ( $^1\text{H}$ : 600.30 MHz,  $^{13}\text{C}$ : 150.95 MHz) instruments (Bruker BioSpin GmbH, Rheinstetten, Germany), using tetramethylsilane (TMS) as an internal standard. In the description of the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra, the furocoumarin and coumarin skeleton atoms numeration system given in structures **1** and **2** was used. Chemical shifts are reported in parts per million (ppm). The IR spectra were recorded by means of the KBr pellet technique on a Bruker Vector-22 spectrometer. The UV spectrum were obtained on an HP 8453 UV Vis spectrometer (Hewlett-Packard, Waldbronn, Germany). HRMS spectra were recorded on a DFS mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), evaporator temperature 180–220 °C, EI ionization at 70 eV. The specific rotation values  $[\alpha]_D$  were determined on a PolAAR 3005 polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA), and expressed in (deg·mL)/(g·dm), while concentration was expressed in g per 100 mL of solution. Melting points were determined using Stuart SMP30 melting point apparatus (Bibby Scientific, Staffordshire, UK). The microwave irradiation reaction was performed in a Microwave 50 reactor (Anton Paar, Graz, Austria). Elemental analysis was carried out on an 1106 Elemental analysis instrument (Carlo-Erba, Milan, Italy). The molecular weights of compounds **37a–c**, **38c** were determined using a Knauer vapor pressure osmometer (Knauer, Berlin, Germany). Spectral and analytical investigations were carried out at Collective Chemical Service center of Siberian Branch of the Russian Academy of Sciences.

Reaction products were isolated by column chromatography on silica gel 60 (0.063–0.200 mm, Merck KGaA, Darmstadt, Germany) eluting with chloroform and chloroform-ethanol (100:1; to 25:1). The reaction progress and the purity of the obtained compounds were monitored by TLC on Silufol UV-254 plates ( $\text{CHCl}_3$ -EtOH, 9:1; detection under UV light or by treatment with iodine vapor).

The chemicals used: dioxane dibromide, trifluoromethanesulfonic anhydride,  $\text{NaN}_3$ ,  $\text{CuSO}_4$ , sodium ascorbate,  $\text{CuSO}_4$ , CuI, N,N-dicyclohexylcarbodiimide (DCC), propargyl amine hydrochloride (**5**), propargyl bromide (80% in toluene solution), aryl acetylenes **17**, **19–2**, **24**, 2-methylbut-3-yn-2-ol (**31**), trimethylsilylacetylene (**33**), 1,6-heptadiyne (**39**), 1,7-octadiyne (**40**) and 1,9-decadiyne (**41**) were purchased from Aldrich (St. Louis, MO, USA) or Alfa Aesar (GmbH, Karlsruhe, Germany). Dichlorobis(triphenylphosphine)palladium(II) was obtained as described in [30]. Peucedanin (**1**) was isolated from *Peucedanum morisonii* according to [31]. Peuruthenicin (**2**) [32], umbelliferone carboxylic acid **3** [19], azidobenzoic acids **7a–c** [33], methyl ether of 9-aminopelargonic acid hydrochloride [34], D,L-2-aminobutyric acid and L-phenylalanine hydrochlorides [35], methyl 2-(prop-2-ynylamino)butanoate (**14**), and (S)-methyl 3-phenyl-2-(prop-2-ynylamino)propanoate (**15**) [36], methyl ethynylantranilate (**23**) [37] and 2-azidooreoselone (**35**) [14] were synthesized according to published procedures. Solvents ( $\text{CH}_2\text{Cl}_2$ ,  $\text{CHCl}_3$ , MeCN, DMF, THF, benzene, dioxane) and  $\text{Et}_3\text{N}$  were purified by standard methods and distilled under a stream of argon just before use. Copies of NMR spectra ( $^1\text{H}$  &  $^{13}\text{C}$ ) are given in Supplementary Materials.

### 4.2. Syntheses and Spectral Data

*7-Hydroxy-2-oxo-N-(prop-2-ynyl)-2H-chromene-6-carboxamide* (**6**) To a stirred solution of umbelliferone-6-carboxylic acid (**3**, 500 mg, 2 mmol) and hydroxybenzotriazole (135 mg, 1 mmol) in DMF (5 mL) was added propargylamine hydrochloride (**5**, 120 mg, 2.1 mmol) and  $\text{Et}_3\text{N}$  (0.27 mL, 2 mmol) and the reaction mixture was left for 5 min. Then diisopropylcarbodiimide (300 mg, 2.4 mmol) was added and the mixture was stirred for 2 days. The reaction mixture was poured into a Petri dish to evaporate in air, the residue was dissolved in  $\text{CH}_2\text{Cl}_2$  (10 mL) and acetone was added

(5 mL). The precipitate was filtered, the solution was evaporated and residue was dried. The product was isolated by fractional precipitation from acetone. Compound **6** was obtained in 67% yield (320 mg). Colorless powder. M.p. 188 °C (dec.). IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3388, 3286, 3243, 3059, 2921, 2852, 2100, 1737, 1708, 1649, 1620, 1598, 1570, 1500, 1392, 1344, 1310, 1234, 1213, 1144, 911, 854, 837, 735, 688, 639, 628. UV (EtOH)  $\lambda_{\text{max}}$ , (lg $\epsilon$ ): 222(3.22), 240 (3.35), 329 (2.89), 372 (2.88) nm.  $^1\text{H-NMR}$  ( $\text{CDCl}_3 + \text{CD}_3\text{OD}$ , 400 MHz,  $\delta_{\text{H}}$ ): 3.16 (s, 1H,  $\equiv\text{CH}$ ), 4.20 (s, 2H,  $\text{CH}_2$ ), 6.26 (d,  $J = 9.6$  Hz, 1H, H-4), 6.83 (s, 1H, H-8), 7.76 (d,  $J = 9.6$  Hz, 1H, H-3), 8.10 (s, 1H, H-5).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3 + \text{CD}_3\text{OD}$ , 100 MHz,  $\delta_{\text{C}}$ ): 45.8, 71.0, 78.8, 103.9, 111.4, 112.6, 113.8, 129.3, 144.0, 157.1, 161.2, 162.4, 167.1 (C=O). Anal. calcd for  $\text{C}_{13}\text{H}_9\text{NO}_4$ : C, 64.20; H, 3.73; N, 5.76; found: C 64.33; H 3.77; N 5.65.

#### 4.2.1. General Method for the Synthesis of Compounds **4a–c**

A solution of the appropriate azidobenzoic acid **7a–c** (35 mg, 0.14 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) was mixed with a solution of sodium ascorbate (42 mg, 15 mol%, 0.021 mmol) and  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (1.2 mg, 5 mol%, 0.007 mmol) in  $\text{H}_2\text{O}$  (5 mL) and 6-(N-propynyl)carboxamidocoumarin (**6**, 20 mg, 0.14 mmol) was added while stirring. The reaction mixture was stirred for 3 h at room temperature and additional for 1 h at 40 °C. After the completion of the reaction, the mixture was diluted with  $\text{H}_2\text{O}$  (10 mL), the product was extracted with  $\text{CH}_2\text{Cl}_2$  ( $4 \times 10$  mL), the combined extracts were dried over anhydrous  $\text{MgSO}_4$ , and the solvent was removed at reduced pressure. The product was purified by column chromatography (eluent  $\text{CHCl}_3$ ).

*2-(4-((7-Hydroxy-2-oxo-2H-chromene-6-carboxamido)methyl)-1H-1,2,3-triazol-1-yl)benzoic acid (4a)*. Colorless powder, m.p. 201–202 °C (ethanol). Yield 50%. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3367, 3257, 3066, 2922, 2852, 2666, 1740, 1691, 1596, 1575, 1485, 1446, 1392, 1304, 1265, 1148, 1078, 1020, 908, 829, 796, 752, 687, 640. UV (EtOH)  $\lambda_{\text{max}}$ , (lg $\epsilon$ ): 246 (3.45), 301 (2.76), 331 (3.51), 357 (3.23) nm.  $^1\text{H-NMR}$  ( $\text{CDCl}_3 + \text{CD}_3\text{OD}$ , 400 MHz,  $\delta_{\text{H}}$ ): 4.93 (s, 2H,  $\text{CH}_2$ ), 6.22 (d,  $J = 9.8$  Hz, 1H, H-3), 6.82 (s, 1H, H-8), 7.13 (dd,  $J = 7.6, 7.2$  Hz, 1H, H-4''), 7.18 (1H, d,  $J = 8.0$  Hz, 1H, H-6''), 7.48 (dd,  $J = 8.0, 7.2$  Hz, 1H, H-5''), 7.63 (d,  $J = 9.8$  Hz, 1H, H-4), 7.78 (s, 1H, H-5), 7.88 (d,  $J = 7.6$  Hz, 1H, H-3''), 7.98 (s, 1H, H-5').  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3 + \text{CD}_3\text{OD}$ , 100 MHz,  $\delta_{\text{C}}$ ): 44.5, 104.3, 111.8, 113.4, 119.8, 121.3, 123.0, 124.1, 125.9, 129.5, 130.7, 132.1, 135.9, 142.3, 143.8, 158.5, 160.5, 163.8, 167.6, 169.2. Anal. calcd for  $\text{C}_{20}\text{H}_{14}\text{N}_4\text{O}_6$ : C, 59.12; H, 3.47; N, 13.79; found: C, 58.88; H, 3.77; N, 14.14.

*3-(4-((7-Hydroxy-2-oxo-2H-chromene-6-carboxamido)methyl)-1H-1,2,3-triazol-1-yl)benzoic acid (4b)*. Yellowish powder, m.p. 186–187 °C (hexane). Yield 64%. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3427, 3095, 3062, 2923, 2852, 2665, 1738, 1687, 1631, 1600, 1581, 1456, 1305, 1236, 1153, 1113, 1097, 1056, 1008, 931, 912, 893, 800, 795, 754, 698, 673. UV (EtOH)  $\lambda_{\text{max}}$ , (lg $\epsilon$ ): 225 (3.66), 246 (3.53), 301 (3.23), 336 (3.22), 347 (3.18), 357 (3.23) nm.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz,  $\delta_{\text{H}}$ ): 4.97 (s, 2H,  $\text{CH}_2$ ), 6.30 (d,  $J = 9.8$  Hz, 1H, H-3), 6.87 (s, 1H, H-8), 7.45 (d,  $J = 8.2$  Hz, 1H, H-6''), 7.47 (dd,  $J = 8.2, 7.6$  Hz, 1H, H-5''), 7.62 (d,  $J = 9.8$  Hz, 1H, H-4), 7.77 (s, 1H, H-5), 7.88 (d,  $J = 7.6$  Hz, 1H, H-4''), 8.01 (s, 2H, H-5', 2''), 11.19 (br.s, 3H, OH, NH).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz,  $\delta_{\text{C}}$ ): 44.2, 104.9, 111.9, 114.2, 120.4, 121.7, 123.4, 124.3, 126.9, 127.1, 130.0, 130.7, 135.7, 140.8, 143.1, 158.9, 160.2, 161.0 (C-7), 164.2, 170.8. Anal. calcd for  $\text{C}_{20}\text{H}_{14}\text{N}_4\text{O}_6$ : C, 59.12; H, 3.47; N, 13.79; found: C, 59.55; H, 3.49; N, 13.43.

*4-(4-((7-Hydroxy-2-oxo-2H-chromene-6-carboxamido)methyl)-1H-1,2,3-triazol-1-yl)benzoic acid (4c)*. Grayish powder, m.p. 203–204 °C (hexane). Yield 60%. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3433, 3062, 2958, 2924, 2852, 2673, 1734, 1676, 1630, 1600, 1579, 1448, 1427, 1392, 1288, 1236, 1215, 1143, 1113, 1076, 946, 904, 858, 827, 796, 768, 732. UV (EtOH)  $\lambda_{\text{max}}$ , (lg $\epsilon$ ): 225 (3.66), 246 (3.78), 270 (3.65), 293 (3.53), 324 (3.36), 351 (3.21) nm.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz,  $\delta_{\text{H}}$ ): 4.95 (s, 2H,  $\text{CH}_2$ ), 6.28 (d,  $J = 9.8$  Hz, 1H, H-3), 6.87 (s, 1H, H-8), 7.10 (d,  $J = 8.4$  Hz, 2H, H-2'', 6''), 7.61 (d,  $J = 9.8$  Hz, 1H, H-4), 7.98 (s, 1H, H-5), 8.02 (s, 1H, H-5'), 8.03 (br.s, 1H, NH), 8.10 (d,  $J = 8.4$  Hz, 2H, H-3'', 5''), 11.19 (br.s, 2H, OH).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz,  $\delta_{\text{C}}$ ): 42.5, 104.9, 111.9, 114.1, 118.9 (2C), 121.8, 125.3, 125.7, 127.6, 130.7, 132.1 (2C), 143.0, 145.7, 158.9, 160.1, 164.2, 169.5, 170.7. Anal. calcd for  $\text{C}_{20}\text{H}_{14}\text{N}_4\text{O}_6$ : C, 59.12; H, 3.47; N, 13.79; found: C, 59.16; H, 3.14; N, 13.42.

**3-Bromopeuruthenicin (11).** A solution of 1.0 g (4.5 mmol) of peuruthenicin (**2**) and 2.48 g (9 mmol) of dioxane dibromide in  $\text{CH}_2\text{Cl}_2$  (10 mL) was stirred at rt for 16 h. Then the reaction mixture was filtered, and the filtrate was evaporated. 3-Bromopeuruthenicin (**11**, yield 86%, 1.14 g) was finally purified by crystallization from chloroform. White solid; m.p. 187–188 °C (ether). IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3480, 3350, 3116, 3050, 2956, 2853, 1720, 1675, 1621, 1616, 1598, 1440, 1311, 1293, 1231, 1163, 1124, 1089, 1030, 989, 964, 918, 845, 783, 750, 729, 719. UV (EtOH)  $\lambda_{\text{max}}$ , ( $\lg \epsilon$ ): 244 (4.19), 314 (4.02), 334 (4.10), 400 (3.39) nm.  $^1\text{H-NMR}$  (600 MHz,  $\text{CDCl}_3$ ,  $\delta_{\text{H}}$ ): 3.99 (s, 3H,  $\text{OCH}_3$ ), 6.85 (s, 1H, H-8), 7.97 (s, 2H, H-4,5), 11.21 (s, 1H, OH).  $^{13}\text{C-NMR}$  (150 MHz,  $\text{CDCl}_3$ ,  $\delta_{\text{C}}$ ): 52.9, 104.8, 108.8, 110.5, 112.5, 129.9, 143.8, 156.4, 157.9, 164.3, 169.2. HRMS (ESI),  $m/z$  ( $I_{\text{rel}}$ , %): 298(66), 269(15.9), 240(24), 220(18), 188(31), 160(16), 131(17), 103(19), 79(12); calcd for  $\text{C}_{11}\text{H}_7\text{BrO}_5$  [ $M$ ]: 297.9471; found: 297.9468; Anal. Calcd. for  $\text{C}_{11}\text{H}_7\text{BrO}_5$ : C, 44.18; H, 2.36; Br, 26.72. Found: C, 44.08; H, 2.16; Br, 26.70.

**3-Azidopeuruthenicin (12).** To a stirred solution of 25 mg (0.84 mmol) of 3-bromopeuruthenicin (**11**) in DMF at room temperature was added  $\text{NaN}_3$  (82 mg, 1.26 mmol). The reaction mixture was heated at 40 °C for 14h. After consumption of the bromocoumarin, the mixture was cooled to room temperature, quenched with eq.  $\text{NaCl}$  (10 mL), and the products and were extracted with  $\text{CH}_2\text{Cl}_2$  (5 × 5 mL). The combined organic phases were dried over anhydrous  $\text{MgSO}_4$ , after filtration, the solvent was removed under reduced pressure and was further dried in a freeze drier at oil pump vacuum. ( $P = 1$  mm). The product (0.15 g, 68%) was obtained as a yellow solid, m.p. 161–162 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3455, 3390, 3357, 3299, 3004, 2971, 2923, 2858, 2121, 1727, 1621, 1467, 1434, 1375, 1309, 1290, 1263, 1193, 975, 869, 728. UV (EtOH)  $\lambda_{\text{max}}$ , ( $\lg \epsilon$ ): 223(4.51), 243(4.4), 329(4.2), 373(3.97).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz,  $\delta_{\text{H}}$ ): 3.99 (s, 3H,  $\text{OCH}_3$ ), 6.83 (s, 1H, H-8), 7.58 (s, 1H, H-4), 8.00 (s, 1H, H-5), 11.19 (s, 1H, OH).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz,  $\delta_{\text{C}}$ ): 52.7, 104.8, 109.9, 111.9, 114.0, 120.6, 142.9, 158.8, 159.9, 164.2, 169.4. Anal. Calcd for  $\text{C}_{11}\text{H}_7\text{N}_3\text{O}_5$ : C, 50.58; H, 2.70; N, 16.09; found C, 51.04; H, 3.12; N, 15.74.

#### 4.2.2. General Method for the N-Propargylation of Amino Acid Methyl Esters

Propargyl bromide (10.5 mmol, 80% in toluene solution) was added to a solution of the appropriate amino acid ether hydrochlorides (10 mmol) and potassium carbonate (21 mmol) in dry DMF at room temperature under argon. The mixture was stirred for 1–3 days (TLC control), the solvent was evaporated under reduced pressure and the residue partitioned between water (30 mL) and  $\text{CH}_2\text{Cl}_2$  (30 mL). The organic phase was separated and the aqueous phase was extracted with  $\text{CH}_2\text{Cl}_2$  (3 × 10 mL). The organic phases were combined, dried ( $\text{MgSO}_4$ ), filtered and the solvent evaporated under reduced pressure. The residue was purified by column chromatography to give secondary amines **13**, **14**, **15**.

**Methyl 9-(prop-2-ynylamino)nonanoate (13).** Yellowish oil.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz,  $\delta_{\text{H}}$ ): 1.22 (m, 4H,  $\text{CH}_2$ -11',12'), 1.39 (2H,  $\text{CH}_2$ -13'), 1.52 (2H,  $\text{CH}_2$ -10'), 2.12 (4H,  $\text{CH}_2$ -9',14'), 2.21 (m, 2H,  $\text{CH}_2$ -15'), 2.58 (2H,  $\text{CH}_2$ -8'), 3.32 (s, 2H,  $\text{CH}_2$ -6'), 3.57 (s, 3H,  $\text{OCH}_3$ ).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz,  $\delta_{\text{C}}$ ): 25.2, 27.4, 29.3, 29.5, 30.0, 34.32, 38.40, 48.90 ( $\text{CH}_2$ -12',9',5'), 51.62 ( $\text{OCH}_3$ ), 71.38, 73.02 ( $\text{C}\equiv\text{CH}$ ). Anal. calcd for  $\text{C}_{13}\text{H}_{23}\text{NO}_2$ : C, 69.29; H, 10.29; N, 6.22; found C, 68.74; H, 10.10; N, 5.94.

**Methyl 7-hydroxy-3-(4-((9-methoxy-9-oxononylamino)methyl)-1H-1,2,3-triazol-1-yl)-2-oxo-2H-chromene-6-carboxylate (8).** A solution of 3-azidopeuruthenicin (**12**, 50 mg, 0.19 mmol) in methylene chloride (10 mL) and a solution of sodium ascorbate (15 mol%, 0.255 mmol) and  $\text{CuSO}_4 \times 5\text{H}_2\text{O}$  (5 mol%, 0.085 mmol) in water (10 mL) were mixed and the terminal alkyne **13** (63 mg, 0.28 mmol) was added with stirring. The reaction mixture was stirred for 3 h at room temperature and then for 1 h at 40 °C. The cooled mixture was diluted with water (10 mL) and the product was extracted with methylene chloride (4 × 10 mL). The combined extracts were dried over  $\text{MgSO}_4$ , and the solvent was removed in vacuo. The residue was purified by column chromatography to give 73 mg (67%) of compound **8** as a yellow oil. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3465, 3396, 3118, 3047, 2954, 2923, 2852, 1751, 1735, 1673, 1623, 1602, 1573, 1469, 1438, 1367, 1288, 1230, 1162, 1124, 1089, 1031, 962, 943, 865, 792. UV (EtOH)  $\lambda_{\text{max}}$ , ( $\lg \epsilon$ ): 242 (4.49), 299 (4.17), 313 (4.26), 334 (4.34), 390 (3.39) nm.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz,  $\delta_{\text{H}}$ ): 1.25 (m, 4H,  $\text{CH}_2$ -11',12'), 1.66 (2H,  $\text{CH}_2$ -13'), 2.18 (2H,  $\text{CH}_2$ -10'), 2.40 (4H,  $\text{CH}_2$ -9',14'), 2.47 (m, 2H,  $\text{CH}_2$ -15'),

2.72 (2H, CH<sub>2</sub>-8'), 3.65 (s, 3H, OCH<sub>3</sub>), 3.99 (s, 3H, OCH<sub>3</sub>), 4.02 (s, 2H, CH<sub>2</sub>-6'), 6.87 (s, 1H, H-8), 7.97 (s, 1H, H-4), 7.98 (s, 1H, H-5), 8.01 (s, 1H, H-5'), 11.25 (br.s, 2H, OH, NH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz, δ<sub>C</sub>): 29.7, 30.8, 31.9, 35.5, 39.6, 40.1, 40.5, 45.1, 52.9, 61.9, 104.7, 108.7, 110.5, 112.5, 121.4, 124.9, 142.2, 143.8, 156.4, 157.8, 164.3, 169.2, 174.4. Anal. calcd. for C<sub>24</sub>H<sub>30</sub>N<sub>4</sub>O<sub>7</sub>: C, 59.25; H, 6.22; N, 11.52; found: C, 59.02; H, 6.12; N, 11.27.

#### 4.2.3. Preparation of 3-(Triazolyl)coumarins **9**, **10**

A stirred solution of 3-azidopeuruthenicin (**12**, 90 mg, 0.35 mmol) in MeCN (15 mL) was successively treated with alkyne **14** or **15** (1.2 equiv.), CuI (0.1 equiv.) and Et<sub>3</sub>N (1.1 equiv.) and the reaction mixture was stirred at room temperature until complete consumption of the starting material. Then, water (5 mL) was added and the products were extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 10 mL). The combined organic phases were dried (MgSO<sub>4</sub>), filtered and concentrated in vacuo. The residue was purified by column chromatography, eluting with chloroform, chloroform:ethanol 100:1.

*Methyl 7-hydroxy-3-(4-((1-methoxy-1-oxobutan-2-ylamino)methyl)-1H-1,2,3-triazol-1-yl)-2-oxo-2H-chromene-6-carboxylate (9)*. Yield 56%. Yellow oil. IR (KBr, ν, cm<sup>-1</sup>): 3463, 3398, 3307, 3050, 2954, 2923, 2852, 1733, 1677, 1625, 1575, 1465, 1440, 1367, 1309, 1292, 1232, 1220, 1164, 1124, 1089, 1012, 968, 865, 794, 948. UV (EtOH) λ<sub>max</sub>, (lgε): 207 (4.69), 244 (4.29), 300 (3.95), 315 (4.02), 336 (4.1), 387 (3.41) nm. <sup>1</sup>H-NMR (CDCl<sub>3</sub>+CD<sub>3</sub>OD, 400 MHz, δ<sub>H</sub>): 0.81 (t, 3H, J = 7.0 Hz, CH<sub>3</sub>-10'), 3.15 (m, 2H, CH<sub>2</sub>-9'), 3.27 (m, 1H, H-8'), 3.62 (s, 3H, OCH<sub>3</sub>), 3.98 (s, 3H, OCH<sub>3</sub>), 3.90 (m, 2H, H-6'), 6.76 (s, 1H, H-8), 7.90 (s, 1H, H-4), 7.91 (s, 1H, H-5), 8.09 (s, 1H, H-5'). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz, δ<sub>C</sub>): 9.5, 25.8, 36.3, 51.6, 51.8, 60.7, 104.4, 108.2, 110.3, 112.3, 121.3, 124.8, 141.0, 144.1, 156.5, 157.5, 164.0, 168.9, 174.8. Anal. calcd. for C<sub>19</sub>H<sub>20</sub>N<sub>4</sub>O<sub>7</sub>: C, 54.81; H, 4.84; N, 13.46; found, %: C, 55.13; H, 5.02; N, 13.59.

*(S)-Methyl 7-hydroxy-3-(4-((1-methoxy-1-oxo-3-phenylpropan-2-ylamino)methyl)-1H-1,2,3-triazol-1-yl)-2-oxo-2H-chromene-6-carboxylate (10)*. Yield 62% (by method for synthesis of compound **8**–34%). Yellow oil. [α]<sub>D</sub> +12.04 (c 1.00, CHCl<sub>3</sub>). IR (ν, cm<sup>-1</sup>): 3459, 3395, 3290, 3049, 2954, 2925, 2854, 1737, 1673, 1623, 1602, 1573, 1537, 1490, 1438, 1367, 1288, 1232, 1162, 1124, 1087, 962, 943, 865, 792. UV (EtOH) λ<sub>max</sub>, (lgε): 244 (4.22), 312 (3.94), 334 (4.02), 399 (3.26) nm. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz, δ<sub>H</sub>): 2.90–3.03 (m, 2H, H-9'), 3.36 (dd, J = 16.3, 1.8 Hz, 2H, H-6'), 3.65 s (3H, OCH<sub>3</sub>), 3.73 (dd, J = 6.4, 7.0 Hz, 1H, H-8'), 3.98 (s, 3H, OCH<sub>3</sub>), 6.84 s (1H, H-8), 7.16–7.18 (m, 2H, *o*-Ph), 7.19–7.22 (m, 1H, *p*-Ph), 7.26–7.32 (m, 2H, *m*-Ph), 7.93 s (1H, H-4), 7.95 (s, 1H, H-5), 8.08 (s, 1H, H-5'), 11.13 (br s, 2H, OH, NH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz, δ<sub>C</sub>): 36.7, 39.3, 51.8, 52.8, 61.0, 104.9, 108.4, 110.3, 112.4, 120.5 (C-4), 125.1, 126.9, 129.1 (2C), 129.5 (2C), 132.5, 141.3, 143.8, 156.1, 157.7, 164.2, 169.2, 174.2. Anal. calcd. for C<sub>24</sub>H<sub>22</sub>N<sub>4</sub>O<sub>7</sub>: C, 60.25; H, 4.63; N, 11.71; found: C, 60.16; H, 4.96; N, 11.55.

#### 4.2.4. General Procedure for the Sonogashira Cross-coupling Reactions of 3-Bromopeuruthenicin **11** with Aryl Alkynes **17**, **19–24** or 2-methylbut-3-yn-2-ol **31**

To a solution of 3-bromopeuruthenicin (**11**, 100 mg, 0.34 mmol) and terminal alkynes **17**, **19–24** or **31** (0.54 mmol) in benzene (5 mL) was added CuI (6 mg, 10 mol%), Pd(PPh<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub> (12 mg, 5 mol%), and Et<sub>3</sub>N (0.076 mL, 0.44 mmol; 1.3 eq. to **11**) under argon. The reaction mixture was stirred at 80 °C for 12–14 h (TLC). The mixture was cooled and 5 mL of water was added. The separated water layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (5 × 4 mL). The combined organic extracts were washed with water, dried over MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel. Eluting with chloroform and crystallization from ether afforded the corresponding 3-(*R*)-alkynylcoumarins **18**, **25–30**, **32**.

*Methyl 7-hydroxy-2-oxo-3-(phenylethynyl)-2H-chromene-6-carboxylate (18)*. Yield 65% (70 mg). Yellow oil. IR (ν, cm<sup>-1</sup>): 3430, 3350, 3052, 2954, 2925, 2852, 2112 (C≡C), 1736, 1682, 1616, 1571, 1442, 1367, 1290, 1224, 1160, 1128, 1091, 966, 873, 792, 750. UV (EtOH) λ<sub>max</sub>, (lgε): 237 (4.14), 272 (3.86), 280 (3.88), 315 (3.78), 335 (3.83) nm. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz, δ<sub>H</sub>): 3.85 (s, 3H, OCH<sub>3</sub>), 6.79 (s, 1H, H-8), 7.48–7.56 (m, 5H, H-Ph), 7.92 (s, 1H, H-4), 7.93 (s, 1H, H-5), 11.21 (1H, OH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz, δ<sub>C</sub>): 52.1, 83.3,

95.2, 108.1, 112.0, 113.1, 114.7, 129.8 (2C), 130.0, 130.8, 132.8, 133.6 (2C), 144.4, 157.6, 158.4, 164.6, 169.7. Anal. Calcd for C<sub>19</sub>H<sub>12</sub>O<sub>5</sub>: 320.0745; found: 320.0743.

*Methyl 3-((4-fluorophenyl)ethynyl)-7-hydroxy-2-oxo-2H-chromene-6-carboxylate (25)*. Yield 68% (78 mg), yellowish powder, m.p. 167–168 °C (ether). IR (KBr,  $\nu$ , cm<sup>-1</sup>): 3425, 2933, 2836, 2145, 1733, 1673, 1608, 1517, 1463, 1450, 1373, 1346, 1257, 1228, 1124, 1012, 939, 873, 852, 840, 792, 753. UV (EtOH)  $\lambda_{\max}$ , (lg $\epsilon$ ): 218 (4.56), 225 (4.54), 245 (4.64), 305 (4.3), 329 (4.41) nm. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz,  $\delta_{\text{H}}$ ): 3.91 (s, 3H, OCH<sub>3</sub>), 6.77 (s, 1H, H-8), 6.93 (dd,  $J$  = 8.2, 7.2 Hz, 2H, H-3',5'), 7.40 (dd,  $J$  = 8.2, 3.4 Hz, 2H, H-2',6'), 7.74 (s, 1H, H-4), 7.92 (s, 1H, H-5), 11.26 (1H, OH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz,  $\delta_{\text{C}}$ ): 53.3, 82.0, 95.7, 104.7, 110.7, 111.2, 112.1, 118.9 (2C), 128.6, 130.5, 131.8 (2C), 143.4, 158.0, 160.1(C-4', J<sub>C-F</sub> 259.1 Hz), 161.3, 164.4, 169.4. Anal. calcd for C<sub>19</sub>H<sub>11</sub>FO<sub>5</sub>: C, 67.46; H, 3.28; F, 5.62; found: C, 68.08; H, 3.50; F, 5.12.

*Methyl 7-hydroxy-2-oxo-3-(p-tolylolethynyl)-2H-chromene-6-carboxylate (26)*. Yield 61% (70 mg). brownish powder, m.p. 132–133 °C (ether). IR (KBr,  $\nu$ , cm<sup>-1</sup>): 3430, 3299, 3259, 3049, 2956, 2852, 2121, 1753, 1735, 1697, 1677, 1594, 1516, 1436, 1367, 1321, 1294, 1234, 1203, 1162, 1087, 960, 848, 794, 748, 734. UV (EtOH)  $\lambda_{\max}$ , (lg $\epsilon$ ): 237 (4.41), 251 (4.21), 272 (4.13), 281 (4.16), 314 (4.04), 338 (4.11) nm. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz,  $\delta_{\text{H}}$ ): 2.36 (s, 3H, CH<sub>3</sub>), 4.00 (s, 3H, OCH<sub>3</sub>), 6.88 (s, 1H, H-8), 7.15 (d,  $J$  = 8.2 Hz, 2H, H3',5'), 7.45 (d,  $J$  = 8.2 Hz, 2H, H-2',6'), 7.81 (s, 1H, H-4), 8.00 (s, 1H, H-5), 11.24 (1H, OH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz,  $\delta_{\text{C}}$ ): 21.7, 52.9, 82.4, 95.9, 104.8, 110.5, 111.2, 112.1, 119.00, 129.2 (2C), 130.5, 131.8 (2C), 139.3, 143.9, 158.0, 158.8, 164.4, 169.5. Anal. calcd for C<sub>20</sub>H<sub>14</sub>O<sub>5</sub>: C 71.85, H4.22, found: C 71.36, H3.94.

*Methyl 3-((4-ethylphenyl)ethynyl)-7-hydroxy-2-oxo-2H-chromene-6-carboxylate (27)*. Yield 52% (61 mg) brownish powder, m.p. 130–131 °C (ether). IR (KBr,  $\nu$ , cm<sup>-1</sup>): 3380, 3139, 3049, 2929, 2856, 2185, 1736, 1673, 1625, 1579, 1481, 1439, 1389, 1351, 1288, 1230, 1162, 1126, 1091, 1037, 962, 946, 865, 825, 750, 736. UV (EtOH)  $\lambda_{\max}$ , (lg $\epsilon$ ): 225 (4.1), 249 (3.67), 259 (3.59), 335 (4.18) nm. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz,  $\delta_{\text{H}}$ ): 1.21 (t,  $J$  = 6.8 Hz, 3H, Et), 2.62 (q,  $J$  = 6.8 Hz, 2H, Et), 3.97 (s, 3H, OCH<sub>3</sub>), 6.85 (s, 1H, H-8), 7.13 (dd,  $J$  = 8.4 Hz, 2H, H-3',5'), 7.40 (d,  $J$  = 8.4 Hz, 2H, H-2',6'), 7.96 (s, 1H, H4), 7.97 (s, 1H, H5), 11.21 (1H, OH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz,  $\delta_{\text{C}}$ ): 17.3, 28.2, 53.5, 81.9, 97.3, 105.6, 112.1, 113.5, 114.8, 122.9, 129.2, 129.9 (2C), 132.6 (2C), 144.2, 144.5, 158.8, 159.5, 165.2, 170.2. Anal. calcd for C<sub>21</sub>H<sub>16</sub>O<sub>5</sub>: C, 72.41; H, 4.63; found: C, 72.18; H, 4.90.

*Methyl 7-hydroxy-3-((4-methoxyphenyl)ethynyl)-2-oxo-2H-chromene-6-carboxylate (28)*. Yield 58% (70 mg) brownish powder, m.p. 134–135 °C (ether). IR (KBr,  $\nu$ , cm<sup>-1</sup>): 3430, 3303, 3216, 3049, 3014, 2956, 2129, 1732, 1677, 1594, 1516, 1491, 1436, 1367, 1321, 1294, 1267, 1234, 1203, 1163, 1124, 1088, 960, 865, 849, 794, 748, 734. UV (EtOH)  $\lambda_{\max}$ , (lg $\epsilon$ ): 237 (4.18), 265 (3.78), 277 (3.91), 280 (3.92), 324 (3.84), 335 (3.88) nm. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz,  $\delta_{\text{H}}$ ): 3.91 (s, 3H, OCH<sub>3</sub>), 3.96 (s, 3H, OCH<sub>3</sub>), 6.86 (s, 1H, H-8), 7.13 (d,  $J$  = 8.6 Hz, 2H, H-3',5'), 7.41 (d,  $J$  = 8.6 Hz, 2H, H-2',6'), 7.97 (s, 1H, H-4), 7.98 (s, 1H, H-5), 11.18 (1H, OH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz,  $\delta_{\text{C}}$ ): 53.2, 53.6, 81.6, 95.4, 105.1, 111.1, 111.7, 112.8, 118.7, 119.4 (2C), 129.5, 133.3 (2C), 144.1, 157.9, 158.7, 161.6, 164.7, 169.8. Anal. calcd for C<sub>20</sub>H<sub>14</sub>O<sub>6</sub>: C, 68.57; H, 4.03; found: C, 68.88; H, 3.90.

*Methyl 3-((4-acetylamino-3-(methoxycarbonyl)phenyl)ethynyl)-7-hydroxy-2-oxo-2H-chromene-6-carboxylate (29)*. Yield 45% (66 mg) yellowish powder, m.p. 154–155 °C (ethanol). IR (KBr,  $\nu$ , cm<sup>-1</sup>): 3430, 3299, 3259, 3216, 3049, 2956, 2925, 2852, 2208, 1753, 1735, 1697, 1677, 1620, 1594, 1515, 1491, 1437, 1367, 1321, 1294, 1234, 1203, 1162, 1124, 1088, 960, 920, 866, 849, 795, 748, 735. UV (EtOH)  $\lambda_{\max}$ , (lg $\epsilon$ ): 237 (4.28), 272 (4.00), 280 (4.01), 316 (3.92), 335 (3.96) nm. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz,  $\delta_{\text{H}}$ ): 2.19 (s, 3H, CH<sub>3</sub>-CO), 3.88 (s, 3H, OCH<sub>3</sub>), 3.95 (s, 3H, OCH<sub>3</sub>), 6.82 (s, 1H, H-8), 7.55 (dd,  $J$  = 8.8, 2.0 Hz, 1H, H-6'), 7.94 (s, 1H, H-4), 7.95 (s, 1H, H-5), 8.09 (d,  $J$  = 2.0 Hz, 1H, H-2'), 8.58 (d,  $J$  = 8.8 Hz, 1H, H-5'), 11.16 (br.s, 2H, NH, OH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>+CD<sub>3</sub>OD, 100 MHz,  $\delta_{\text{C}}$ ): 25.2, 52.5, 52.8, 71.3, 82.2, 104.6, 108.5, 112.4, 116.2, 120.0, 130.0, 130.5, 133.7, 134.6, 137.7, 141.2, 143.9, 156.5, 157.7, 166.4, 167.9, 169.1, 169.3. Anal. calcd for C<sub>23</sub>H<sub>17</sub>NO<sub>8</sub>: C, 63.45; H, 3.94; N, 3.22; found: C, 63.18; H, 3.92; N, 3.12.

*Methyl 7-hydroxy-2-oxo-3-(pyridin-2-ylethynyl)-2H-chromene-6-carboxylate (30)*. Yield 55% (60 mg) brown oil. IR ( $\nu$ , cm<sup>-1</sup>): 3444, 2971, 2929, 2856, 2133, 1735, 1674, 1626, 1579, 1481, 1438, 1388, 1352, 1300, 1230,

1163, 1126, 1092, 1038, 962, 947, 865, 825, 750, 736, 723. UV (EtOH)  $\lambda_{\max}$ , (lg $\epsilon$ ): 227 (4.25), 238 (4.15), 294 (3.93), 306 (3.92), 351 (4.13) nm.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz,  $\delta_{\text{H}}$ ): 3.76 (s, 3H,  $\text{OCH}_3$ ), 6.72 (s, 1H, H-8), 7.18 (ddd,  $J = 7.4, 5.2, 1.4$  Hz, 1H, H-4'), 7.31 (dd,  $J = 6.6, 1.4$  Hz, 1H, H-6'), 7.54 (ddd,  $J = 7.4, 6.6, 1.8$  Hz, 1H, H-5'), 7.69 (s, 1H, H-4), 7.77 (s, 1H, H-5), 8.49 (ddd,  $J = 7.2, 1.8$  Hz, 1H, H-3'), 11.20 (1H, OH).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz,  $\delta_{\text{C}}$ ): 52.9, 82.6, 94.8, 105.0, 112.8, 115.1, 116.6, 123.5, 129.4, 130.4, 134.1, 138.1, 141.6, 144.4, 156.9, 158.1, 164.4, 169.7. Anal. calcd for  $\text{C}_{18}\text{H}_{11}\text{NO}_5$ : C, 67.29; H, 3.45; N, 4.36; found: C, 67.08; H, 3.38; N, 4.22.

*Methyl 7-hydroxy-3-(3-hydroxy-3-methylbut-1-ynyl)-2-oxo-2H-chromene-6-carboxylate (32)*. Yield 66% (68 mg), m.p. 114–115 °C (ethanol). IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3282, 3221, 2981, 2932, 2868, 2250, 1741, 1679, 1612, 1600, 1584, 1462, 1446, 1363, 1296, 1269, 1210, 1170, 1120, 1096, 1058, 998, 964, 954, 900, 889, 842, 792, 735, 723. UV (EtOH)  $\lambda_{\max}$ , (lg $\epsilon$ ): 223 (4.04), 246 (3.84), 338 (3.67), 365 (3.57) nm.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz,  $\delta_{\text{H}}$ ): 1.34 (s, 6H, 2  $\times$   $\text{CH}_3$ ), 3.95 (s, 3H,  $\text{OCH}_3$ ), 6.78 (s, 1H, H-8), 7.76 (br. s, 1H, H-4), 7.93 (s, 1H, H-5).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz,  $\delta_{\text{C}}$ ): 30.9, 31.0, 52.7, 65.3, 66.2, 84.1, 104.8, 109.9, 110.5, 114.0, 130.6, 143.1, 157.8, 160.0, 164.1, 169.4. Anal. calcd for  $\text{C}_{16}\text{H}_{14}\text{O}_6$ : C, 63.57; H, 4.67; found: C, 63.22; H, 4.44. HRMS,  $m/z$  calcd.: 302.0785; found 302.0783 [M].

*Methyl 7-hydroxy-2-oxo-3-((trimethylsilyl)ethynyl)-2H-chromene-6-carboxylate (34)*. A sealed 10 mL glass tube containing 3-bromopeuruthenicin (**11**, 100 mg (0.34 mmol), toluene (3 mL), trimethylsilylacetylene (**33**, 0.1 mL, 0.68 mmol), CuI (3 mg, 5 mol %), Pd(PPh<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub> (23 mg, 10 mol. %), and Et<sub>3</sub>N (0.061 mL, 1.3 eq.) was placed in the cavity of a microwave reactor and irradiated for 2 h at 100 °C and power 50 W in an Anton Paar Microwave 50 reactor. After cooling to 25 °C, the tube was removed from the reactor. Then the mixture was cooled and 5 mL of water was added. The separated water layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (5  $\times$  4 mL). The combined organic extracts was washed with water, dried over MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was crystallized from ether, yield 64% (68 mg), m.p. 104–107 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3437, 2924, 2852, 2223, 1735, 1672, 1314, 1597, 1508, 1425, 1388, 1328, 1236, 1222, 1159, 1107, 908, 839, 752, 694, 680. UV (EtOH)  $\lambda_{\max}$ , (lg $\epsilon$ ): 325 (3.97), 304 (3.85), 241 (4.01) nm.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz,  $\delta_{\text{H}}$ ) 0.20 (s, 9H, SiMe<sub>3</sub>), 3.93 (s, 3H,  $\text{OCH}_3$ ), 6.76 (s, 1H, H-8), 7.74 (s, 1H, H-4), 7.91 (s, 1H, H-5), 10.97 (s, 1H, OH).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz,  $\delta_{\text{C}}$ ): 8.6, 52.7, 73.8, 81.5, 101.6, 110.2, 110.4, 111.7, 128.4, 145.1, 158.0, 158.3, 164.45, 169.2. Anal. calcd for  $\text{C}_{16}\text{H}_{16}\text{O}_5\text{Si}$ : C, 60.74; H, 5.10; Si, 8.88; found: C, 60.55; H, 5.18; Si, 8.54.

*Methyl 3-ethynyl-7-hydroxy-2-oxo-2H-chromene-6-carboxylate (16)*. To a solution of compound **34** (60 mg, 0.00019 mol) in methanol (3 mL) were added CsF (14 mg, 0.00095 mol) and TEBA (5 mg). The mixture was stirred at rt for 10 h in an argon flow (TLC). Then 10 mL of water was added and the mixture was extracted with methylene chloride (5  $\times$  4 mL). The combined extract was washed with water, dried over MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel. Eluting with chloroform and crystallization from ether gave compound **16**, yield 85%, m.p. 84–88 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3463, 2956, 2923, 2854, 2102, 1741, 1677, 1621, 1581, 1494, 1444, 1416, 1367, 1295, 1234, 1186, 1157, 1120, 1099, 1024, 952, 914, 856, 721. UV (EtOH)  $\lambda_{\max}$ , (lg $\epsilon$ ): 225 (4.1), 246 (3.96), 321 (3.84), 344 (3.92).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz) 2.16 (s, 1H, CH), 3.99 (s, 3H,  $\text{OCH}_3$ ), 6.86 (s, 1H, H-8), 7.83 (s, 1H, H-4), 8.00 (s, 1H, H-5), 11.29 (s, 1H, OH).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz,  $\delta_{\text{C}}$ ): 52.3, 75.4, 82.2, 105.1, 112.6, 114.0, 114.8, 128.5, 143.3, 159.4, 159.7, 164.7, 168.9. Anal. calcd for  $\text{C}_{13}\text{H}_8\text{O}_5$  C 63.94, H3.30 found: C 63.65, H3.84.

*Methyl 7-hydroxy-3-[1-(2-isopropyl-3,7-dioxo-2,3-dihydro-7H-furo[3,2-g]chromen-2-yl)-1H-1,2,3-triazol-4-yl]-2-oxo-2H-chromene-6-carboxylate (36)*. A solution of 2-azidooreoselone (**35**, 100 mg, 0.35 mmol) and 3-ethynylpeuruthenicin (**16**, 85 mg, 0.35 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was mixed with a solution of sodium ascorbate (10 mg, 15 mol%) and CuSO<sub>4</sub>·5H<sub>2</sub>O (4 mg, 5 mol%) in H<sub>2</sub>O (5 mL). The reaction mixture was stirred for 3 h at rt and additional for 1 h at 40 °C. After the completion of the reaction, the mixture was diluted with H<sub>2</sub>O (10 mL), the product was extracted with CH<sub>2</sub>Cl<sub>2</sub> (4  $\times$  10 mL), the combined extracts were dried over anhydrous MgSO<sub>4</sub>, filtered, and the solvent was removed at reduced pressure.

Yield 70% (0.130 g), yellowish solid. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3469, 3049, 2983, 2956, 2883, 2850, 1739, 1675, 1629, 1556, 1467, 1438, 1390, 1353, 1284, 1132, 1162, 1128, 1093, 1025, 964, 944, 869, 827, 736. UV (EtOH)  $\lambda_{\text{max}}$ , ( $\lg \epsilon$ ): 217 (4.57), 249 (4.59), 294 (4.26), 307 (4.27), 337 (4.27), 381 (3.76), 393 (3.36) nm.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz,  $\delta_{\text{H}}$ ): 0.89 (d,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ ), 1.11 (d,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ ), 3.30 (m, 1H,  $\text{CH}(\text{Me})_2$ ), 3.99 (s, 3H,  $\text{OCH}_3$ ), 6.41 (d,  $J = 9.6$  Hz, 1H, H-6), 6.88 (s, 1H, H-8'), 7.24 (s, 1H, H-9), 7.71 (d,  $J = 9.6$  Hz, 1H, H-5), 7.87 (s, 1H, H-4), 7.97 (s, 1H, H-4'), 7.98 (s, 1H, H-5'), 8.13 (s, 1H, H-1''), 11.29 (s, 1H, OH).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz,  $\delta_{\text{C}}$ ): 16.2, 16.3, 30.1, 53.3, 96.5, 101.4, 105.2, 110.7, 111.8, 112.9, 115.7, 117.3, 117.5, 125.7, 128.0, 130.3, 142.9, 143.5, 144.3, 158.5, 159.3, 162.1, 164.7, 165.8, 169.6, 172.4, 194.7. Anal. calcd for  $\text{C}_{27}\text{H}_{19}\text{N}_3\text{O}_9$ : C, 61.25; H, 3.62; N, 7.94; found: C, 61.01; H, 3.54; N, 7.62.

#### 4.2.5. General Method for the Preparation of Monoalkynes 38a–c

A solution of 2-azidooreoselone (**35**, 1.7 mmol) in methylene chloride (10 mL) and a solution of sodium ascorbate (15 mol%, 0.255 mmol) and  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (5 mol%, 0.085 mmol) in water (10 mL) were mixed and the appropriate terminal alkyne **39–41** (0.85 mmol) was added with stirring. The reaction mixture was stirred for 5 h at 40 °C. The cooled mixture was diluted with water (10 mL) and the product was extracted with methylene chloride ( $4 \times 10$  mL). The combined extracts were dried over  $\text{MgSO}_4$ , filtered, the solvent was removed to give the desired products **38a–c**.

*2-Isopropyl-2-(4-(pent-4-ynyl)-1H-1,2,3-triazol-1-yl)-2H-furo[3,2-g]chromene-3,7-dione (38a)*. Yield 66% (0.420 g), brownish oil. IR ( $\nu$ ,  $\text{cm}^{-1}$ ): 3120, 2981, 2932, 2867, 2140, 1741, 1678, 1600, 1584, 1461, 1446, 1381, 1363, 1295, 1269, 1210, 1169, 1120, 1058, 964, 953, 909, 889, 792, 749. UV (EtOH)  $\lambda_{\text{max}}$ , ( $\lg \epsilon$ ): 231 (3.91), 282 (3.58), 320 (3.52), 348 (3.43) nm.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz,  $\delta_{\text{H}}$ ): 0.89 (d,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ ), 0.95 (d,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ ), 1.40 (m, 2H,  $\text{CH}_2$ -7'), 1.85 (s, 1H, H-10'), 2.06 (m, 2H,  $\text{CH}_2$ -6'), 2.55 (m, 2H,  $\text{CH}_2$ -8'), 3.06 (m, 1H,  $\text{CH}(\text{CH}_3)_2$ ), 6.28 (d,  $J = 9.6$  Hz, 1H, H-6), 6.99 (s, 1H, H-9), 7.39 (s, 1H, H-4), 7.67 (d,  $J = 9.6$  Hz, 1H, H-5), 7.82 (s, 1H, H-5').  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz,  $\delta_{\text{C}}$ ): 15.4, 15.8, 19.5, 28.1, 28.2, 33.7, 69.2, 82.3, 98.4, 100.4, 115.4, 115.8, 117.0, 126.7, 127.5, 137.4, 145.9, 161.2, 164.0, 173.6, 191.5. Anal. calcd for  $\text{C}_{21}\text{H}_{19}\text{N}_3\text{O}_4$ : C, 66.83; H, 5.07; N, 11.13; found: C, 67.01; H, 5.12; N, 10.88.

*2-(4-(Hex-5-ynyl)-1H-1,2,3-triazol-1-yl)-2-isopropyl-2H-furo[3,2-g]chromene-3,7-dione (38b)*. Yield 62% (0.415 g). Yellowish powder, m.p. 111–112 °C (ether). IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 2971, 2828, 2856, 2115, 1735, 1673, 1625, 1579, 1481, 1438, 1388, 1351, 1230, 1126, 1091, 1037, 997, 962, 946, 865, 825, 750. UV (EtOH)  $\lambda_{\text{max}}$ , ( $\lg \epsilon$ ): 221 (4.12), 255 (4.47), 292 (3.98), 308 (3.89), 351 (4.03) nm.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz,  $\delta_{\text{H}}$ ): 0.89 (d,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ ), 1.03 (m, 2H,  $\text{CH}_2$ -8'), 1.12 (d,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ ), 1.24 (m, 2H,  $\text{CH}_2$ -7'), 1.92 (s, 1H, H-11'), 2.14 (m, 2H,  $\text{CH}_2$ -6'), 2.30 (m, 1H,  $\text{CH}(\text{CH}_3)_2$ ), 3.40 (m, 2H,  $\text{CH}_2$ -9'), 6.35 (d,  $J = 9.6$  Hz, 1H, H-6), 7.02 (s, 1H, H-9), 7.43 (s, 1H, H-4), 7.69 (d,  $J = 9.6$  Hz, 1H, H-5), 7.84 (s, 1H, H-5').  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz,  $\delta_{\text{C}}$ ): 14.9, 15.3, 17.8, 27.6, 27.7, 27.73, 33.2, 68.7, 81.8, 97.9, 100.6, 114.9, 115.3, 116.1, 125.4, 126.9, 136.8, 144.7, 162.4, 165.6, 173.1, 191.0. Anal. calcd for  $\text{C}_{22}\text{H}_{21}\text{N}_3\text{O}_4$ : C, 67.51; H, 5.41; N, 10.74; found: C, 67.17; H, 5.21; N, 10.72.

*2-Isopropyl-2-(4-(oct-7-ynyl)-1H-1,2,3-triazol-1-yl)-2H-furo[3,2-g]chromene-3,7-dione (38c)*. Yield 74% (0.527 g). Yellowish powder, m.p. 121–122 °C (ether). IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3143, 3084, 2976, 2935, 2858, 2113, 1735, 1627, 1581, 1481, 1419, 1390, 1352, 1286, 1226, 1128, 1093, 1039, 997, 947, 866, 825, 739. UV (EtOH)  $\lambda_{\text{max}}$ , ( $\lg \epsilon$ ): 221 (4.16), 255 (4.5), 294 (4.00), 307 (3.94), 339 (4.05), 351 (4.06) nm.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz,  $\delta_{\text{H}}$ ): 0.94 (d,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ ), 0.97 (d,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ ), 1.27 (m, 2H,  $\text{CH}_2$ -8'), 1.34 (m, 2H,  $\text{CH}_2$ -9'), 1.46 (m, 2H,  $\text{CH}_2$ -10'), 1.55 (m, 2H, (m, 2H,  $\text{CH}_2$ -7')), 1.89 (s, 1H, H-13'), 2.12 (m, 2H,  $\text{CH}_2$ -6'), 2.60 (m, 2H,  $\text{CH}_2$ -11'), 3.09 (m, 1H,  $\text{CH}(\text{CH}_3)_2$ ), 6.32 (d,  $J = 9.6$  Hz, 1H, H-6), 7.04 (s, 1H, H-9), 7.44 (s, 1H, H-4), 7.72 (d,  $J = 9.6$  Hz, 1H, H-5), 7.87 (s, 1H, H-5').  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz,  $\delta_{\text{C}}$ ): 15.1, 15.5, 18.1, 27.9, 28.0, 28.4, 28.67, 28.73, 33.5, 68.1, 84.4, 98.1, 100.8, 115.1, 115.5, 116.3, 125.7, 128.4, 137.7, 143.1, 158.5, 161.4, 171.4, 191.6. Anal. calcd for  $\text{C}_{24}\text{H}_{25}\text{N}_3\text{O}_4$ : C, 68.72; H, 6.01; N, 10.02; [M] 419; found: C, 69.08; H, 6.06; N, 10.27; M= 419.

#### 4.2.6. Sonogashira Cross-Coupling Reactions of Bromide **11** with Alkynes **38a–c**

To a solution of 3-bromopeurutenicin (**11**, 100 mg, 0.34 mmol) and a terminal alkyne **38a–c** (0.54 mmol) in benzene (5 mL) was added CuI (6 mg, 10 mol%), Pd(PPh<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub> (12 mg, 5 mol%), and Et<sub>3</sub>N (0.076 mL, 0.44 mmol; 1.3 equiv) under argon. The reaction mixture was stirred at 80 °C for 14 h (TLC). The mixture was cooled and 5 mL of water was added. The separated water layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (5 × 4 mL). The combined organic extracts was washed with water, dried over MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel (eluent—chloroform).

*Methyl 7-hydroxy-3-(5-(1-(2-isopropyl-3,7-dioxo-3,7-dihydro-2H-furo[3,2-g]chromen-2-yl)-1H-1,2,3-triazol-4-yl)-pent-1-ynyl)-2-oxo-2H-chromene-6-carboxylate (37a)*. Yield 52% (0.105 g). Brownish oil. IR ( $\nu$ , cm<sup>-1</sup>): 3350, 3200, 2981, 2932, 2867, 2254, 1741, 1678, 1620, 1600, 1580, 1461, 1446, 1381, 1363, 1295, 1269, 1210, 1169, 1120, 1096, 1058, 964, 953, 889, 792, 735, 723. UV (EtOH)  $\lambda_{\max}$ , (lg $\epsilon$ ): 234 (4.41), 256 (4.44), 273 (4.31), 310 (4.09), 339 (4.14), 354 (4.08) nm. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz,  $\delta_{\text{H}}$ ): 0.99 (d,  $J$  = 7.0 Hz, 3H, CH<sub>3</sub>), 1.02 (d,  $J$  = 7.0 Hz, 3H, CH<sub>3</sub>), 1.60 (m, 2H, CH<sub>2</sub>-7'), 2.18 (m, 2H, CH<sub>2</sub>-6'), 2.65 (m, 2H, CH<sub>2</sub>-8'), 3.13 (m, 1H, CH(CH<sub>3</sub>)<sub>2</sub>), 3.97 (s, 3H, OCH<sub>3</sub>), 6.37 (d,  $J$  = 9.8 Hz, 1H, H-6), 6.84 (s, 1H, H-8''), 7.08 (s, 1H, H-9), 7.48 (d, 1H,  $J$  = 9.8 Hz, H-5), 7.76 (s, 1H, H-4), 7.71, 7.74 (both s, 2H, H-4'', 5''), 7.97 (br.s, 2H, H-5', OH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz,  $\delta_{\text{C}}$ ): 15.0, 15.4, 20.0, 23.5, 32.4, 34.7, 55.7, 70.0, 85.7, 98.1, 99.9, 104.3, 107.2, 110.2, 112.3, 115.1, 116.0, 116.3, 125.5, 128.2, 130.7, 139.7, 142.9, 143.8, 156.1, 157.5, 158.5, 161.4, 162.0, 169.0, 171.4, 192.5. Anal. calcd for C<sub>32</sub>H<sub>25</sub>N<sub>3</sub>O<sub>9</sub>: C, 64.54; H, 4.23; N, 7.06; [M] 598; found: C, 64.58, H, 4.36, N, 7.07; M = 595.

*Methyl 7-hydroxy-3-(6-(1-(2-isopropyl-3,7-dioxo-3,7-dihydro-2H-furo[3,2-g]chromen-2-yl)-1H-1,2,3-triazol-1-yl)-hex-1-ynyl)-2-oxo-2H-chromene-6-carboxylate (37b)*. Yield 57% (0.118 g). Yellowish powder, m.p. 126–127 °C (ether). IR (KBr,  $\nu$ , cm<sup>-1</sup>): 3388, 3139, 3049, 2929, 2856, 2235, 1735, 1673, 1626, 1579, 1481, 1439, 1388, 1352, 1288, 1230, 1163, 1126, 1091, 1037, 997, 962, 946, 865, 825, 750, 737. UV (EtOH)  $\lambda_{\max}$ , (lg $\epsilon$ ): 234 (4.39), 256 (4.43), 273 (3.81), 309 (4.09), 339 (4.13), 352 (3.62) nm. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz,  $\delta_{\text{H}}$ ): 0.98 (d,  $J$  = 7.0 Hz, 3H, CH<sub>3</sub>), 1.04 (d,  $J$  = 7.0 Hz, 3H, CH<sub>3</sub>), 1.44–1.53 (m, 4H, CH<sub>2</sub>-7', 8'), 2.14 (m, 2H, CH<sub>2</sub>-6'), 2.64 (m, 2H, CH<sub>2</sub>-9'), 3.13 (m, 1H, CH(CH<sub>3</sub>)<sub>2</sub>), 3.95 (s, 3H, OCH<sub>3</sub>), 6.35 (d,  $J$  = 9.8 Hz, 1H, H-6), 6.92 (s, 1H, H-8''), 7.07 (s, 1H, H-9), 7.64 (d,  $J$  = 9.8 Hz, 1H, H-5), 7.71, 7.74 (both s, 2H, H-4'', 5''), 7.88 (s, 1H, H-4), 7.97 (s, 1H, H-5'). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz,  $\delta_{\text{C}}$ ): 15.6, 16.0, 19.4, 32.5, 33.0, 36.7, 25.7, 54.2, 70.6, 88.5, 98.7, 100.6, 104.9, 108.8, 110.8, 112.8, 115.7, 116.6, 116.9, 126.1, 128.8, 132.3, 141.1, 143.5, 144.4, 156.6, 158.1, 159.1, 162.0, 162.6, 169.5, 172.0, 192.4. Anal. calcd for C<sub>33</sub>H<sub>27</sub>N<sub>3</sub>O<sub>9</sub>: C, 65.02; H, 4.46; N, 6.89; [M] 609; found: C, 65.08; H, 4.06; N, 6.37; M = 605.

*Methyl 7-hydroxy-3-(8-(1-(2-isopropyl-3,7-dioxo-3,7-dihydro-2H-furo[3,2-g]chromen-2-yl)-1H-1,2,3-triazol-4-yl)-oct-1-ynyl)-2-oxo-2H-chromene-6-carboxylate (37c)*. Brownish powder, m.p. 152–153 °C (ether). Yield 55% (0.119 g). IR (KBr,  $\nu$ , cm<sup>-1</sup>): 3440, 3141, 3049, 2971, 2929, 2856, 2213, 1735, 1673, 1625, 1579, 1481, 1388, 1351, 1288, 1230, 1126, 1091, 1037, 962, 946, 865, 825, 750. UV (EtOH)  $\lambda_{\max}$ , (lg $\epsilon$ ): 234 (4.63), 251 (4.67), 256 (4.67), 309 (4.33), 339 (4.37), 350 (4.34) nm. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz,  $\delta_{\text{H}}$ ): 0.91 (d,  $J$  = 7.0 Hz, 3H, CH<sub>3</sub>), 0.94 (d,  $J$  = 7.0 Hz, 3H, CH<sub>3</sub>), 1.23, 1.29 (both m, 4H, CH<sub>2</sub>-8', 9'), 1.42 (m, 2H, CH<sub>2</sub>-10'), 1.51 (m, 2H, CH<sub>2</sub>-7'), 2.07 (m, 2H, CH<sub>2</sub>-6'), 2.56 (m, 2H, CH<sub>2</sub>-11'), 3.07 (m, 1H, CH(CH<sub>3</sub>)<sub>2</sub>), 3.92 (s, 3H, OCH<sub>3</sub>), 6.31 (d,  $J$  = 9.8 Hz, 1H, H-6), 6.78 (s, 1H, H-8''), 7.03 (s, 1H, H-9), 7.46 (s, 1H, H-4), 7.68 (d,  $J$  = 9.8 Hz, 1H, H-5), 7.83 (s, 1H, H-5'). 7.89, 7.90 (both s, 2H, H-4'', 5''). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz,  $\delta_{\text{C}}$ ): 15.2, 15.6, 19.0, 25.2, 29.9, 30.7, 31.2, 33.6, 36.3, 52.7, 70.2, 87.4, 98.2, 101.0, 104.5, 108.4, 110.4, 112.4, 115.2, 115.6, 116.5, 125.7, 128.2, 131.8, 141.3, 143.0, 144.0, 156.2, 157.7, 158.9, 161.6, 162.2, 169.2, 171.5, 191.8. Anal. calcd for C<sub>35</sub>H<sub>31</sub>N<sub>3</sub>O<sub>9</sub>: C, 65.93; H, 4.90; N 6.59; [M] 637; found: C, 65.38; H, 4.66; N, 6.87; M = 635.

### 4.3. Antibacterial Activity Assay

**Method 1.** Compounds **4a–c**, **11**, **26**, **29**, **30**, **37a–c**, **42a–c** and parent compounds **1**, **2**, **3** were tested for their in vitro antibacterial activity against *Staphylococcus aureus* 209pATCC 6538-P and strains from the collection of Department of Microbiology, Immunology and Virology, Novosibirsk State Medical University: *Staphylococcus aureus* C-18 - clinical isolated strain, *Staphylococcus aureus* “Viotko” and *Actinomyces viscosus* U-18. Cultivation of bacterial cultures was carried out on agar and broth media Muller-Hinton in aerobic conditions at a temperature of 37 °C. Cultivation time for *Staphylococcus aureus* was 1–2 days, for *Actinomyces viscosus* U – 2–3 days. Analysis of antibacterial activity was performed using the method of serial macrodilutions in a liquid medium in the total volume of 1.0 mL [24,38]. All compounds were primarily dissolved in 0.05 mL of 96% ethyl alcohol and brought to the desired concentration with 0.9% sodium chloride solution (0.2 mL) and nutrient broth. To assess antibacterial activity, a number of double dilutions of the studied substances starting from 1000 µg/mL were used. Doses of substances above 1000 µg/mL were not considered because of their low solubility. The introduced dose of daily cultures of bacteria was determined using the standard of turbidity according to McFarland and was controlled by seeding on a dense nutrient medium. It gave  $(2.75 \pm 0.85) 10^3$  colony-forming units (CFU). For the minimum inhibitory concentration (MIC), the smallest dose of the substance was taken, completely suppressing the growth of bacteria. The absence of signs of growth in the liquid medium was controlled by seeding to the surface of a agar medium with subsequent incubation under standard conditions. As a negative control, the test culture was introduced into 1 mL of broth and cultivated under the same conditions, followed by sowing on an agar nutrient medium and taking into account the growth of bacteria. Based on the results of triplicate repeated experiments, the mean value of the MIC and the standard error of measurement ( $M \pm SEM$ ) were calculated. The primary data of antibacterial activity are given in Supplementary Materials.

**Method 2.** Compounds **36**, **37a–c**, **1** and **2** were tested for their in vitro antibacterial activity against *Bacillus subtilis* and *Escherichia coli* (JM109). This strain is most similar to wild type of *E. coli* in our bacterial collection. The known tumorigenic compounds 4-nitroquinolin-1-oxide (NQO) was used as the reference compound [39]. This procedure was maintained according to the standard broth microdilution method as recommended in guidelines of Clinical and Laboratory Standards Institute [24,40,41] and the minimum inhibitory concentration (MIC) of compounds was tested. In short, testing was performed in U-bottomed 96-well sterile plastic microdilution trays (Falcon 3077, Becton Dickinson and Co., Cockeysville, MD, USA) in cation ( $Ca^{2+}$  and  $Mg^{2+}$ ) adjusted Mueller-Hinton broth medium (Becton Dickinson and Co., Cockeysville, MD, USA). The concentration range of test compounds was started from 1000 µg/mL by using serial two fold dilution. Standardized initial inoculum was prepared by the direct colony suspension method to the final inoculum to  $5 \times 10^5$  CFU/mL, as described (CLSI M7-A7). After inoculation of previously prepared microdilution trays with tested compounds, trays were incubated at  $35 \pm 2$  °C overnight (18–20 h) in an ambient air incubator. *E. coli* JM109 served as quality control of MIC determination procedure, as well. The MIC was determined as the lowest concentration of tested compound that completely inhibits growth of the organism in the microdilution wells as detected by the unaided eye and comparing the amount of growth in the wells containing the tested agent with the amount of growth in the growth-control wells (no antimicrobial agent). All testing were done in triplicate.

### 4.4. Molecular Docking

Molecular modeling was carried out in the Schrodinger Maestro visualization environment using applications from the Schrodinger Small Molecule Drug Discovery Suite 2016-1 (Python, NY, USA) [42]. Three-dimensional structures of the derivatives were obtained empirically in the *LigPrep* application using the OPLS3 force field [43]. All possible tautomeric forms of compounds, as well as various states of polar protons of molecules in the pH range of  $7.0 \pm 2.0$  were taken into account. The search area for docking was selected automatically, based on the size and physico-chemical properties of FAD. The extra precision (XP) algorithm of docking was applied. Docking was performed in comparison

with FAD. The three-dimensional structures of FAD was obtained in the PubChem database and prepared in the LigPrep application. Non-covalent interactions of compounds in the binding site were visualized using Biovia Discovery Studio Visualizer (Biovia, San Diego, CA, USA) [44].

**Supplementary Materials:** Supplementary data associated with this article can be found in the online version, at Supporting information included the copies of NMR spectra ( $^1\text{H}$  &  $^{13}\text{C}$ ) of new compounds and also the primary data of antibacterial activity.

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**Sample Availability:** Samples of the compounds **1**, **4a–c**, **11**, **16**, **4a–c**, **28**, **29**, **30**, **36**, **37a–c**, **42a–c** are available from the authors.



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