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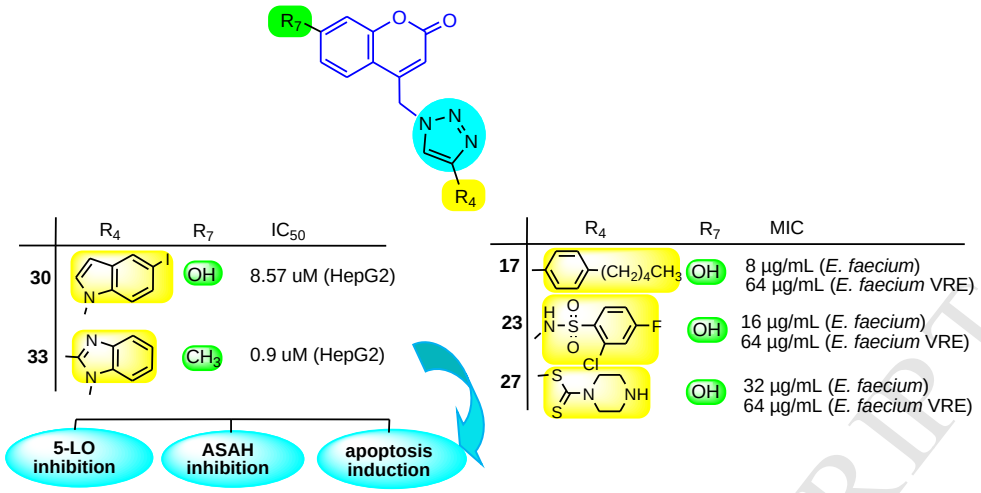


Synthesis, *in vitro* anticancer and antibacterial activities and *in silico* studies of new 4-substituted 1,2,3-triazole–coumarin hybrids

Tatjana Gazivoda Kraljević,[a] Anja Harej,[b,c] Mirela Sedić,[b,c] Sandra Kraljević Pavelić,[b,c] Višnja Stepanić,[d] Domagoj Drenjančević,[e,f] Jasminka Talapko[f] and Silvana Raić-Malić[a*]

[a] University of Zagreb, Faculty of Chemical Engineering and Technology, Department of Organic Chemistry, Marulićev trg 20, HR-10000 Zagreb, Croatia

[b] University of Rijeka, Department of Biotechnology, Radmile Matejčić 2, HR-51000 Rijeka, Croatia

[c] University of Rijeka, Centre for high-throughput technologies, Radmile Matejčić 2, HR-51000 Rijeka, Croatia

[d] Division of Molecular Medicine, Ruđer Bošković Institute, Bijenička 54, HR-10002 Zagreb, Croatia

[e] Osijek University Hospital, Department of Transfusion Medicine, Josipa Huttlera 4, HR-31000 Osijek, Croatia

[f] University of Osijek, Faculty of Medicine, Department of Microbiology and Parasitology, Cara Hadrijana 10/E, HR-31000 Osijek, Croatia

Abstract. The 4-substituted 1,2,3-triazole core in designed coumarin hybrids (**4**–**35**) with diverse physicochemical properties was introduced by eco-friendly copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition under microwave irradiation. Coumarin–1,2,3-triazole–benzofused heterocycle hybrids emerged as the class of compounds exhibiting the highest antiproliferative activity. The strong relationship between lipophilicity and antiproliferative activities was observed indicating that lipophilic 1,2,3-triazole–coumarin hybrids containing phenylethyl (**13**), 3,5-difluorophenyl (**14**), 5-iodoindole (**30**) and benzimidazole (**33** and **35**) subunits showed the most potent cytostatic effects. The 7-methylcoumarin–1,2,3-triazole–2-methylbenzimidazole hybrid **33** can be highlighted as a lead that exerted the highest cytotoxicity against hepatocellular carcinoma HepG2 cells with IC_{50} value of 0.9 μM and high selectivity (SI = 50). This compound induced cell death, mainly due to early apoptosis. Strong antiproliferative effect of **33** could be associated with its inhibition of 5-lipoxygenase (5-LO) activity and perturbation of sphingolipid signaling by interfering with intracellular acid ceramidase (ASAH) activity. Outlined considerable effect of lipophilicity on antiproliferative activity was not observed for antibacterial activity. The compounds with *p*-pentylphenyl (**17**), 2-chloro-4-fluorobenzenesulfonamide (**23**) and dithiocarbamate (**27**) moiety were endowed with high selectivity against *Enterococcus* species. Moreover, these compounds found superior in inhibiting the growth of clinically isolated vancomycin resistant *Enterococcus faecium*, while the reference antibiotics exhibited the lack of activity. Our findings indicate that coumarin–1,2,3-triazole could be used as a model skeleton for structural optimization to develop more potent and selective anticancer agents and encourage further development of novel structurally related analogs of **33** as more effective 5-LO inhibitors.

Keywords: 1,2,3-triazole–coumarin hybrids, click chemistry, cytostatic activity, antibacterial activity, 5-lipoxygenase (5-LO), acid ceramidase (ASAH)

1. Introduction

The exploration of new heterocycles that can accommodate potency to multiple biological targets remains an intriguing scientific endeavour. Among the oxygen heterocycles, coumarins are privileged structural motifs widely found in many natural products that have attracted intense interest in recent years because of their diverse biological and pharmacological properties [1,2]. Beside clinically proven anticoagulant and antithrombotic actions, various coumarins have exhibited anticancer [3–6] and antimicrobial [7–11] activities that have been the prime reason for their inclusion in the hybrids framework. Thus, hybridizing the coumarin nucleus with other moieties has afforded new molecules with improved anticancer and antimicrobial activity profiles [2–4]. It was found that coumarin derivatives target a number of pathways in cancer such as kinase inhibition, cell cycle arrest, angiogenesis inhibition, heat shock protein (HSP90) inhibition, telomerase inhibition, antimitotic activity, carbonic anhydrase inhibition, monocarboxylate transporters inhibition, aromatase inhibition and sulfatase inhibition [12–14]. Some plant-derived or synthetic coumarins have been also reported to interfere with 5-lipoxygenase (5-LO) metabolic pathways that are identified to be involved in cancer progression [15,16]. It is demonstrated that the inhibition of the 5-LO pathway may be useful for cancer therapy through antiproliferative, pro-apoptotic, pro-differentiation and antiangiogenic effects in cell lines and animal models [17–19]. Although the 5-LO is recognized as a potential target in prevention and/or treatment of cancer, to the best of our knowledge reports describing potent and selective 5-LO inhibitors based on the coumarin core have been limited [20–22].

On the other hand, life-threatening infections caused by multi-drug resistant (MDR) pathogens continue to present serious public health issues worldwide. A few antibiotics with coumarin skeleton have been found that are active against MDR bacteria [23]. Moreover, for many years *Enterococcus* species were believed to be harmless to humans and, therefore, considered unimportant medically. During the past few decades, enterococci have emerged as the only Gram-positive pathogens associated with a high risk of death [24]. In-hospital mortality associated with enterococcal blood stream infections (BSI) has been estimated between 25-50%, leading to the recognition of the *Enterococcus* species as formidable pathogens [25]. Bacteremia and endocarditis [26,27] are the more common manifestations of infections due to enterococci. The treatment of enterococcal BSI is complicated by high rates of antimicrobial resistance. The rapid spread of enterococci with resistance to vancomycin

(VRE) has been of particular concern. To address this problem, the development of new antimicrobials remains a high priority for the continued effective treatment of infections caused by resistant strains.

1,2,3-Triazoles occupy a prominent place in drug discovery due to their facile synthesis through click chemistry [28] and wide biological profiles, which include antibacterial [29–34] and anticancer activities [35–39]. Potential structural features of bioactive triazoles include stability to metabolic degradation, high selectivity and capability of hydrogen bonding that could be favorable in the binding of biomolecular targets [40]. It was demonstrated that different types of chemical bridges at C-4 of the 1,2,3-triazole core eliminated the planarity, thereby could ameliorate the druggability and facilitate the compounds binding to their possible receptor through induced fit [36]. In recent years, a library of coumarin derivatives conjugated with 1,2,3-triazole were synthesized and proved to possess significant anticancer and antimicrobial activities. Thus, novobiocin analogue **I** with 1,2,3-triazole at the C-3 position of coumarin displayed potent cytotoxic activity against breast cancer cell lines (Fig. 1) [41].

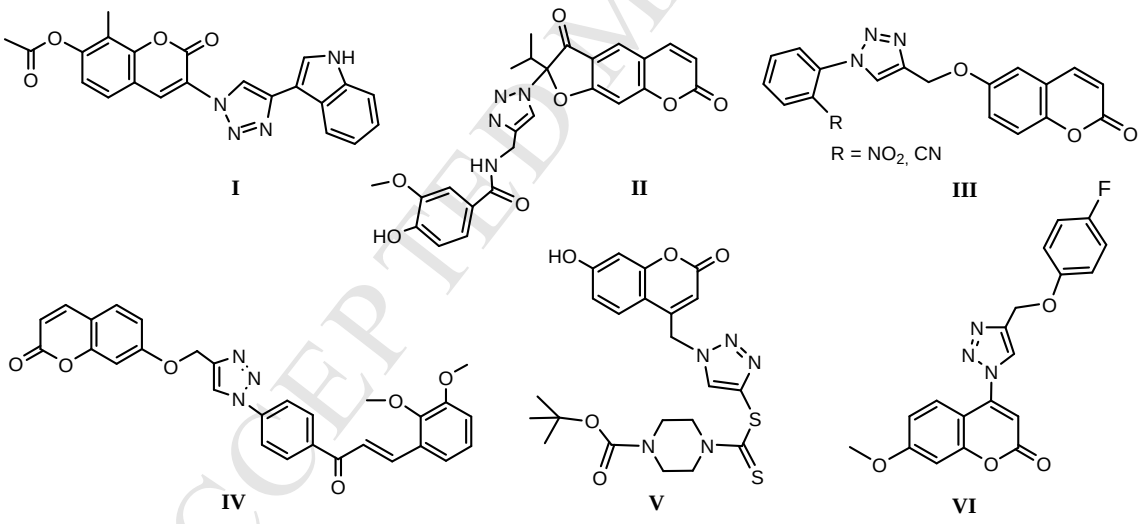
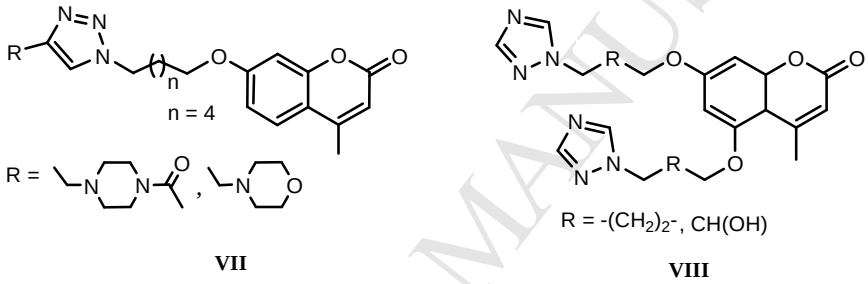


Fig. 1. Representatives of 1,2,3-triazoles–coumarin hybrids as potent anticancer agents.

1*H*-1,2,3-triazole linked (arylamidomethyl)-dihydrofurocoumarin hybrid **II** with the highest cytotoxicity was studied as inhibitors of phosphodiesterase [42]. Triazoles linked 6-hydroxycoumarin **III** with ortho substituted phenyl moiety showed to be selectively cytotoxic against lung cancer cell line [43]. Chalcone–coumarin hybrid **IV** with a triazole linker was of interest as potential anticancer and antimycobacterial agent [44]. Coumarin–1,2,3-triazole–

dithiocarbamate hybrid **V** [45,46] and 4-(1,2,3-triazole-1-yl)coumarin **VI** with *p*-fluorophenoxy)methyl substituent exhibited anticancer activity through inducing apoptosis of the cell lines. Furthermore, among the triazolyl cumarin derivatives, it was observed that substituent, length and position of alkyl spacer had profound effect on the antimicrobial potency. Thus, triazolyl 4-methylcumarins **VII** containing morpholine and piperazine moiety showed noticeable activity against various bacterial pathogens, particularly against *Pseudomonas aeruginosa* (Fig. 2) [29]. Bis-1,2,4-triazole coumarin derivative and its hydrochloride **VIII** containing a butylene linker displayed much stronger antibacterial and antifungal efficacy than corresponding mono-triazole derivative [11]. Moreover, bis-triazole coumarin alcohol **VIII** gave better anti-MRSA (methicillin-resistant *Staphylococcus aureus*) activity than its mono-triazole and standard drug (Fig. 2) [47].



VII **VIII**

Fig. 2. Representatives of triazoles–coumarin hybrids **VII** and **VIII** as potent antimicrobial agents.

Inspired by multifarious bioactivity of coumarin and 1,2,3-triazole heterocycles, and dramatic increase in multidrug resistance to clinically available drugs, we conceptualized the hybridization of 7-hydroxycoumarin (umbelliferone) or its 7-methyl analog and 1,2,3-triazole entity with the aim to develop a new lead possessing efficient and selective pharmacological activities. Taking into consideration the aforementioned and as a continuation of our project on searching for new biologically active molecules possessing 1,2,3-triazole moiety [48–50], we envisaged that coumarin and 1,2,3-triazole pharmacophores if linked together through rotatable bond would generate novel hybrid molecules with promising anticancer and/or antibacterial activities (Fig. 3).

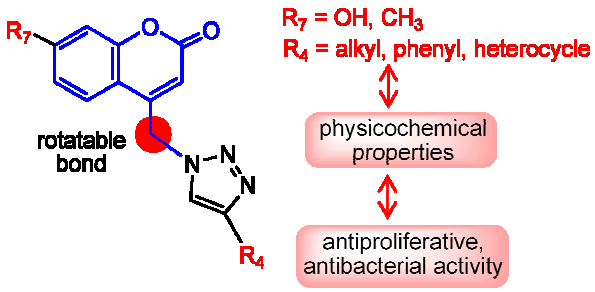


Fig. 3. Conformationally unrestricted 4-substituted 1,2,3-triazole–coumarin hybrids (**4**–**35**).

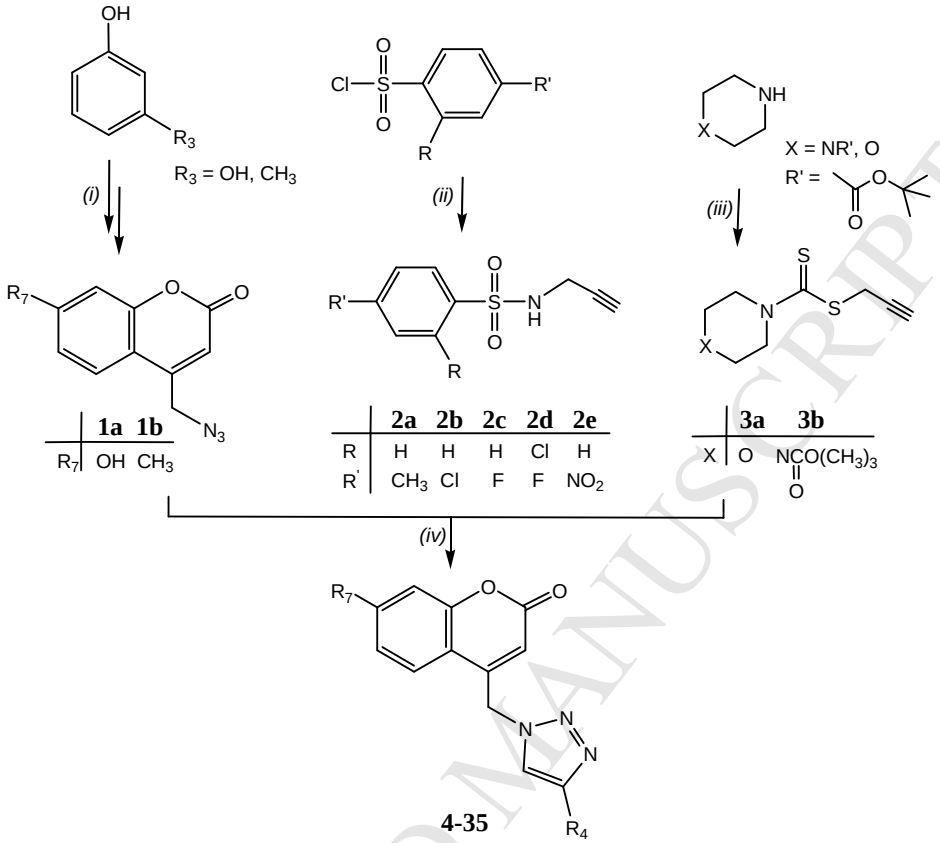
Moreover, varied alkyl, phenyl and heterocycle moieties introduced to C-4 of 1,2,3-triazole were taken into account in order to evaluate their contribution to the antiproliferative activity. Therefore, herein we have reported the synthesis of a diverse conformationally unrestricted hybrids (**4**–**35**) of 7-substituted coumarin and 4-substituted 1,2,3-triazole linked through single methylene unit in order to assess the influence of obtained structural modifications on their physicochemical properties and consequently on antiproliferative and/or antibacterial activities. The effect of selected 1,2,3-triazole–coumarin hybrids on the regulation of specific lipid metabolic pathways mediated by enzymes 5-LO, sphingosine kinase 1 and acid ceramidase that control major aspects of cancer cell behavior has been also investigated for the first time.

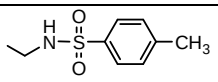
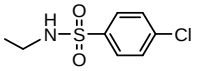
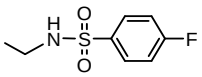
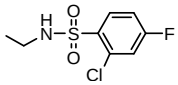
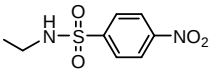
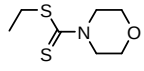
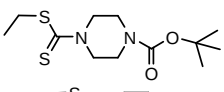
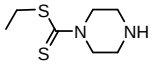
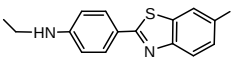
2. Results and discussion

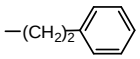
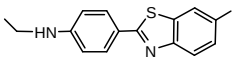
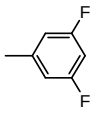
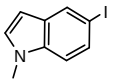
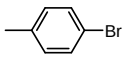
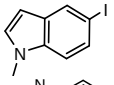
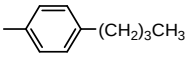
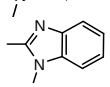
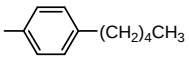
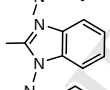
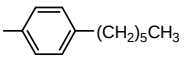
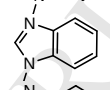
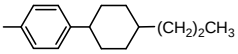
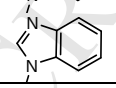
2.1. Chemistry

Adopting the hybridization approach [1], a series of 1,2,3-triazole–coumarin hybrids (**4**–**35**) was synthesized using copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition reaction of a terminal alkyne with an azide (Scheme 1). 4-Azidomethyl coumarin derivatives **1** and **2** were obtained in the reaction of sodium azide with 4-chloromethyl-7-hydroxycoumarin and 4-chloromethyl-7-methylcoumarin, which were synthesized in the reaction of *m*-hydroxyphenol and *m*-cresol with 4-chloroacetoacetate ethyl ester under Pechmann cyclization condition using sulphuric acid to give 7-hydroxy and 7-methylcoumarin derivatives, respectively [46,51]. 1,2,3-Triazole–coumarin hybrids **4**–**19** comprising various 4-alkyl- and substituted 4-aryl-1,2,3-triazole subunits were subsequently prepared by click chemistry of 4-azidomethyl coumarin derivative and corresponding alkyne in the presence of Cu(I), as the catalyst, [52]

(Scheme 1) which was generated *in situ* by reaction of copper(II) sulfate and metallic copper, as reducing agent.



Compd	R_7	R_4	Yield	Compd	R_7	R_4	Yield
4	OH		81	**20**	OH		60
5	OH	$(CH_2)_3Cl$	92	**21**	OH		84
6	OH	$(CH_2)_2OH$	39	**22**	OH		14
7	OH		83	**23**	OH		6
8	OH	$(CH_2)_3OH$	79	**24**	OH		77
9	OH	$(CH_2)_4OH$	43	**25**	OH		76
10	OH		51	**26**	OH		51
11	OH	$(CH_2)_5CH_3$	51	**27**	OH		41
12	OH	$(CH_2)_7CH_3$	45	**28**	OH		68

13	OH		77	**29**	CH_3		71
14	OH		88	**30**	OH		85
15	OH		54	**31**	CH_3		78
16	OH		95	**32**	OH		83
17	OH		52	**33**	CH_3		81
18	OH		56	**34**	OH		76
19	OH		81	**35**	CH_3		67

Scheme 1. *Reagents and conditions*: (*i*) a) ethyl 4-chloroacetoacetate, conc. H_2SO_4, –5 °C; b) NaN_3, CH_3CN, reflux; (*ii*) propargyl amine, pyridine, r.t., 24 h; (*iii*) CS_2, Na_3PO_4 x 12 H_2O, propargyl bromide, acetone, Ar, r.t.; (*iv*) corresponding azide, Cu, 1M $CuSO_4$ solution, *tert*-butanol : H_2O = 1 : 1, DMF, 80 °C, 1 h.

The reactions were performed under microwave irradiation in a mixture of water and organic solvent to give compounds **4**–**19** with a good yields ranging from 43–95%. The versatility of target hybrids was further extended to the synthesis of coumarin–triazole–arylsulfonamide hybrids **20**–**24** from coumarin azide derivative **1a** and corresponding *N*-propargylated aryl sulfonamides **2a**–**2e** prepared by reaction of propargyl amine and arylsulfonyl chloride in the presence of pyridine, as a base (Scheme 1). Inspired by potent anticancer activity of 1,2,3-triazole–dithiocarbamate hybrids [46], we prepared 1,2,3-triazole–dithiocarbamate hybrids **25** and **27** containing unprotected morpholine and piperazine subunit. Cu(I)-catalyzed click reaction of **20**–**26** were performed in a very good yield, except for 4-(2-fluorosulfonamide)-(**22**) and 4-(2-chloro-4-fluorophenylsulfonamide) (**23**) substituted 1,2,3-triazole with a yield of 14% and 6%, respectively. The structural diversity was further explored with the synthesis of triazole-linked nitrogen/sulfur heterocycles to coumarin [48]. Firstly, propargylated derivatives of benzothiazole, 5-iodoindole, benzimidazole and 2-methylbenzimidazole were prepared that were subsequently submitted to Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction with azidomethyl coumarin derivatives **1a** and **1b** under microwave irradiation to give **28**–**35** [48].

2.2. In vitro biological evaluations and in silico physicochemical profile of a coumarin–1,2,3-triazole library

2.2.1. Antiproliferative evaluations

Results of antiproliferative evaluations of compounds **4**–**35** on human tumor cell lines: lung adenocarcinoma (A549), hepatocellular carcinoma (HepG2), ductal pancreatic adenocarcinoma (CFPAC-1), cervical carcinoma (HeLa) and colorectal adenocarcinoma, metastatic (SW620), as well as normal human lung fibroblast (WI38) and mouse embryonic fibroblast (3T3) are presented in Table 1. It can be noted that among the compounds exhibiting the highest activity ($IC_{50} \leq 30$ μM) most of the compounds showed inhibitory effect against A549 and HeLa cell lines (Table S1, Supplementary data). However, none of the compounds exhibited inhibitory effect with $IC_{50} \leq 30$ μM against SW620 cells. From the alkyl and phenyl C-4 substituted coumarin–1,2,3-triazole hybrids **4**–**19**, the compounds **4**–**12** bearing the 4-alkyl and 4-(hydroxyalkyl) chains showed poor, if any antiproliferative activities. Introducing of the phenyl- and 4-(*p*-alkylphenyl)-substituted 1,2,3-triazoles (**13**–**18**) increased the activity, particularly against lung adenocarcinoma (A549; IC_{50} = 8.87–32.34 μM) with **13** and **14** showing the selectivity index (SI) above 4. Whereas the compounds with benzenesulfonamide (**20**–**24**) and dithiocarbamate (**25**–**27**) units showed no appreciable inhibitory effects, the incorporation of benzofused heterocycles, such as benzothiazole (**28** and **29**), 5-iodoindole (**30** and **31**) and benzimidazole (**32**–**35**) led to improvements in activities. Thus, 7-hydroxycoumarin–1,2,3-triazole–2-arylbenzothiazole **28** demonstrated excellent activity against cervical carcinoma (HeLa, IC_{50} = 7.26 μM). Additionally, 7-hydroxycoumarin–1,2,3-triazole–5-iodoindole hybrid **30** showed high potency against hepatocellular carcinoma (HepG2, IC_{50} = 8.57 μM) with selectivity index of 3. The substitution at C-7 of the coumarin ring seemed to have considerable impact on the antiproliferative activity. Thus, inhibitory activities of 7-hydroxycoumarins **28** and **30** were to 7-fold higher than those of their 7-methylcoumarin congeners **29** and **31**. In contrast, among the coumarin–1,2,3-triazole–benzimidazole conjugates, the activities of 7-hydroxycoumarin derivatives **32** and **34** were 2–4-fold lower than those of corresponding 7-methylcoumarin analogs **33** and **35**. The 7-methylcoumarin–1,2,3-triazole–2-methylbenzimidazole hybrid **33** exerted the cytotoxicity against HepG2 cells in nM range with the highest selectivity index of 50. The hybrids **13**, **14**, **30** and **35** bearing phenylethyl, 3,5-difluorophenyl, 5-iodoindole and

benzimidazole subunits, respectively, showed also selective inhibitory activity, particularly against A549 and HepG2 cell lines. Unfortunately, most of the coumarin–1,2,3-triazole hybrids have been also cytotoxic against normal fibroblasts WI38 and 3T3.

Table 1.

The growth-inhibition effects *in vitro* of compounds **4**–**35** on selected tumor cell lines and normal fibroblasts.

Compd	IC_{50} (μM)[a]					
	Cell lines					
	A549	**HepG2**	**CFPAC-1**	**HeLa**	**SW620**	**3T3/ WI38[b]**
4	91.71	>100	>100	>100	>100	37.98
5	30.07	>100	75.81	49.59	59.76	65.56
6	90.33	>100	>100	>100	>100	>100
7	>100	>100	>100	>100	>100	57.70
8	96.22	>100	>100	90.20	>100	40.83
9	>100	>100	>100	>100	>100	>100
10	67.59	>100	>100	81.08	>100	38.48
11	66.15	61.56	>100	59.01	>100	58.50[b]
12	46.03	34.22	62.65	35.14	50.87	43.39[b]
13	24.78	50.46	>100	35.05	84.38	>100
14	21.06	38.91	>100	27.61	66.73	>100
15	32.34	61.11	52.92	40.17	97.66	0.05
16	13.83	48.40	69.77	38.99	49.24	0.35
17	13.65	27.34	50.40	24.63	39.64	0.71
18	8.87	9.16	>100	8.73	36.79	13.96[b]
19	41.03	30.41	52.79	34.26	37.52	7.91[b]
20	46.40	29.84	68.86	55.71	92.19	0.03[b]
21	47.93	31.40	81.91	58.46	>100	0.82[b]
22	88.46	76.70	>100	>100	>100	24.95[b]
23	91.86	74.82	>100	74.88	>100	1.45[b]
24	> 100	> 100	> 100	> 100	> 100	<0.01[b]

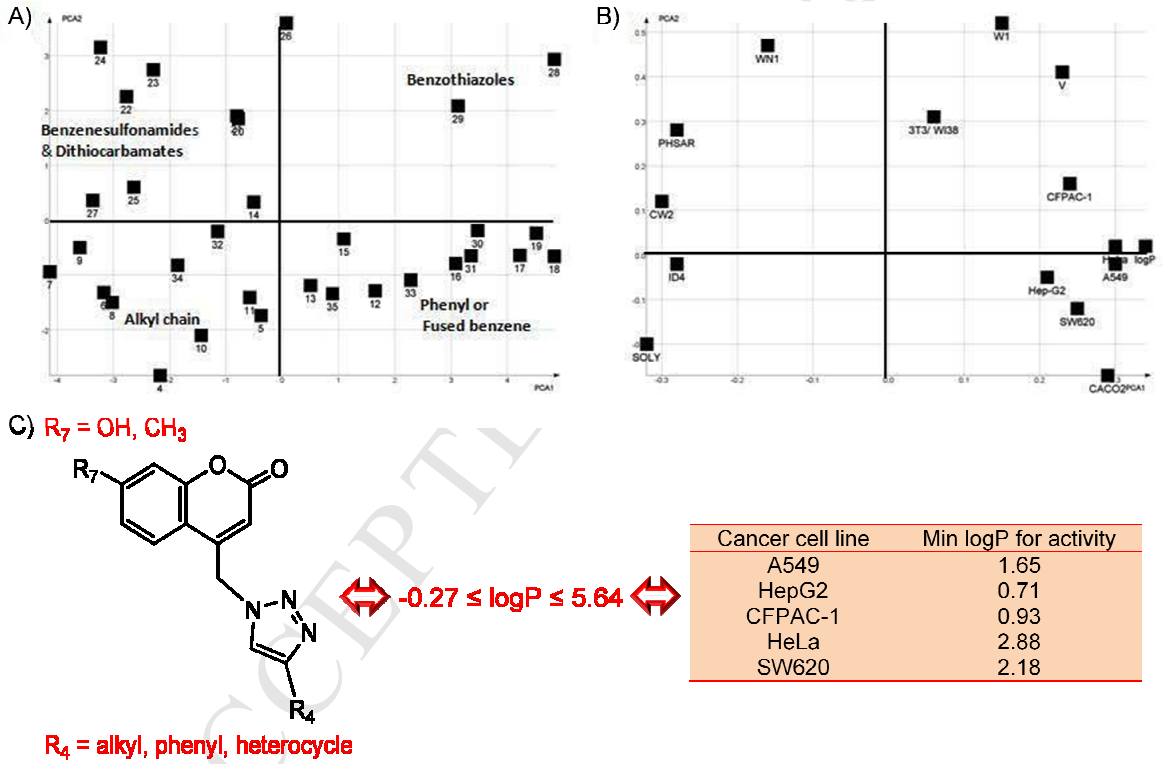
25	70.17	99.35	56.30	40.80	≥100	59.15[b]
26	35.41	56.94	88.52	47.95	>100	2.82[b]
27	96.04	>100	>100	>100	>100	>100[b]
28	16.16	52.82	10.54	7.26	89.95	5.01
29	46.24	94.74	34.89	51.42	≥100	18.24
30	28.63	8.57	29.89	14.16	34.34	25.84
31	34.09	38.06	41.71	34.43	70.02	8.31
32	>100	70.69	76.51	49.15	>100	97.46
33	16.85	0.90	59.41	17.48	>100	45.33
34	>100	78.06	>100	>100	>100	>100
35	40.13	22.52	80.55	23.91	>100	96.44

[a]Compound concentration required to inhibit tumor cell proliferation by 50%; [b]Compounds **11**, **12**, **18–27** were tested in WI38 cell line.

2.2.2. Principle component analysis in terms of the measured IC50 values and calculated VolSurf+ descriptors

The set of 32 synthetized coumarin–1,2,3-triazole hybrids has been characterized in terms of molecular interaction field descriptors calculated by VolSurf+ [53] and principle component analysis (PCA). According to the PCA model, the nine descriptors account for 83.3% of the variance within the set of compounds differing primarily by substituent at the C-4 of the 1,2,3-triazole ring (Fig. 4). Following descriptors have been used: molecular volume (V), hydrophilic volume accounting for polarizability and dispersion forces (W1), the ratio of the hydrophilic volume over the total molecular surface (CW2), hydrogen bond acceptor volume (WN1), the ratio between the polar and the hydrophobic surface areas (PHSAR), a vector integy moment pointing from the centre of mass to the centre of the hydrophobic regions (ID4) indicating concentration of nonpolar regions in only one part of the molecular surface, intrinsic aqueous solubility (SOLY), membrane permeability (CACO2) and the logarithm of the partition coefficient between 1-octanol and water (logP). According to the PCA and our main rationale in selection of C-4 substituents, the synthetized compounds were divided into subgroups depending on their physicochemical properties (Fig. 4). Substituents at position C-4 of triazole in described hybrids had considerable impact on their physicochemical

characteristics and hence on the observed antiproliferative activities. The subgroup containing derivatives with saturated alkyl side-chains (**4**–**12**) was the most soluble (SOLY) and the least lipophilic within the set. A subgroup of soluble derivatives with arylsulfonamide and dithiocarbamate fragments (**20**–**27**) were characterized by a relatively large hydrophilic surface (W1), concentration of hydrophobic regions in a part of the molecular surface (ID4), hydrogen bond acceptor volume (WN1) and the least potential within the compound set to cross membranes. The two benzothiazole derivatives (**28** and **29**), forming a separate subgroup, were the most lipophilic and the least soluble compounds with the largest volumes in the set (Fig. S1, Supplementary data). The fourth group comprises compounds with phenyl-containing side-chains (**13**–**19**), indole (**30** and **31**) and benzimidazole (**32**–**35**) subunits, which were all lipophilic molecules with a capacity for passive transport across membranes.



Cancer cell line	Min logP for activity
A549	1.65
HepG2	0.71
CFPAC-1	0.93
HeLa	2.88
SW620	2.18

Fig. 4. The A) score and B) loadings plots for the two-component PCA model (R^2(cum) = 71.0 %, Q2(cum) = 54%, Multibase 2015) built for 32 C-4-substituted coumarin–1,2,3-triazole hybrids (Table 1) in terms of nine preselected VolSurf+ descriptors (Table S2, Supplementary data). C) The significant effect of substituents on lipophilicity and cytotoxicity for the tested set of novel hybrids.

Considering the antiproliferative activities, the compounds from the most lipophilic group were the most active ones having the lowest IC_{50} values (Fig. 4, Table 1). Furthermore, simple two-class decision tree models (active/inactive) based on logP values confirmed that lipophilicity was a quite good parameter for distinguishing active from inactive compounds, particularly in A549, HepG2 and HeLa cell lines with the highest number of active compounds (IC_{50} < 90 μM) (Table 2). Thus, 93.7% of the compound set, in which inactive compounds were defined to have $IC_{50} \geq 90.0$ μM, was predicted correctly. Moreover, the simple two-class model correctly classified 84.4% and 87.5% of compounds based on their activities in A549 and HepG2 cancer cell lines, respectively. The prediction with the lowest accuracy of 75% in CPFAC-1 cell line (with seven misclassified compounds as false positives) was obtained demonstrating satisfactory accuracy of the applied logP model.

Table 2.

The two-class model for cytotoxicity in terms of lipophilicity (logP) against cancer cell lines.[a]

Cancer cell line	Minimum logP for activity	# of predicted inactives/ # false negatives	# of predicted actives/ # false positives
A549	1.65	15/5	17/0
HepG2	0.71	7/0	25/4
HeLa	0.93	9/1	23/1
CPFAC-1	2.88	9/1	23/7
SW620	2.18	18/1	14/5

[a]IC_{50} < 90 μM for active compounds. The values of 1-octanol/water partition coefficient logP were calculated by VolSurf+.

2.2.3. The prediction of activity spectra for substances (PASS) analysis

The Prediction of Activity Spectra for Substances (PASS) has been applied in the present study to explore the biological potentials of selected compounds and to prioritize them for further *in vitro* studies. The PASS is an *in silico* tool used for predicting biological activity spectra for natural and synthetic substances, which is based on the structure-activity relationships knowledgebase for more than 260,000 compounds with known biological activities including drugs, drug candidates, pharmaceutical leads and toxic compounds [54]. The PASS predicts the tentative biological potential of the compound based on its structure and reveals the predicted activities as the probability of activity (Pa) and inacitivity (Pi). The higher Pa value, the lower is the predicted probability of obtaining false positives in biological

testing. The PASS analysis revealed that compounds **30** and **33** have high activity score for leukotriene synthesis inhibitory effect with Pa values of 0.47 and 0.49, respectively (Table S3, Supplementary data). The obtained *in silico* findings were further validated *in vitro* by Western blot method as described below.

2.2.4. Western blot analysis of predicted protein target

The results of PASS analysis indicated that compounds **30** and **33** could be potential inhibitors of 5-lipoxygenase (5-LO), an enzyme that catalyzes two steps in biosynthesis of leukotrienes, a group of lipid mediators of inflammation derived from arachidonic acid. Mounting evidence suggested that 5-LO and 5-LO-catalyzed products were implicated in the development and progression of different cancer types including lung, colon, pancreatic and liver cancer [55–57]. Therefore, drugs that directly interfere with the production of 5-LO metabolites or antagonize the signaling functions of 5-LO products may be effective in preventing cancer [58]. It was also demonstrated that leukotrienes worked in concert with other bioactive lipids such as sphingolipids in complex network of cellular signaling [59]. Simple sphingolipids such as ceramide and sphingosine are antiproliferative or pro-apoptotic, whereas their phosphorylated derivatives ceramide-1-phosphate and sphingosine-1-phosphate (S1P) have growth-promoting and anti-apoptotic properties. Overexpression of sphingosine kinase 1 (SK1) and acid ceramidase (ASAH) resulting in an increased production of S1P and degradation of ceramide, respectively, tips the balance towards survival, growth, and proliferation, which makes these two enzymes attractive drug targets for cancer therapy.

Results of our study revealed that compound **30** neither inhibits 5-LO nor suppresses the activities of the two major regulators of sphingolipid metabolism, namely SK1 and ASAH in hepatocellular carcinoma cells (Fig. 5), in which this compound exerted the most potent cytostatic effects. Thus, one can conclude that induced growth inhibition of HepG2 cells by compound **30** is not associated with down-regulation of the key enzymes regulating leukotriene and sphingolipid biosynthesis. On the contrary, compound **33** proved to be putative 5-LO inhibitor and negative regulator of sphingolipid metabolic conversion with selective inhibitory effect towards the ASAH activity (Fig. 5). Interestingly, this compound did not impose inhibitory effect on the SK1 enzyme activity. Previous study demonstrated that combination of drugs targeting the key enzymes involved in arachidonic acid metabolism,

namely cyclooxygenase-2 and 5-LO, determined a significant increase in ceramide levels in colon cancer cells [60]. Additional study in human leukemia-derived HL-60 cells showed that TNF-α-stimulated release of arachidonic acid stimulated hydrolysis of sphingomyelin by sphingomyelinase and concomitant ceramide generation [61]. Altogether, the growth-suppressive properties of compound **33** could be associated with its ability to inhibit arachidonic acid metabolism resulting in the accumulation of arachidonic acid that could, in turn, regulate cellular ceramide levels probably through the negative regulation of ASAH activity.

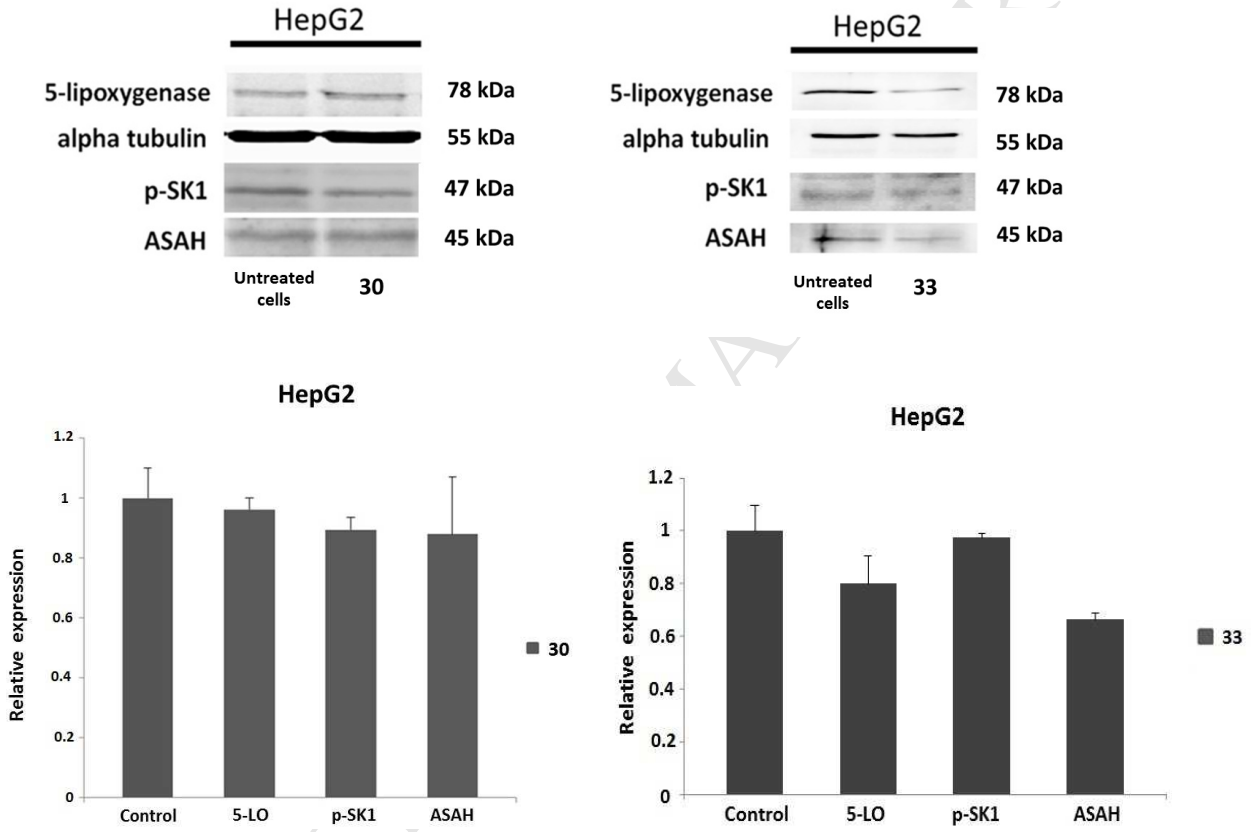


Fig. 5. Western blot analysis of potential protein targets of compounds **30** and **33** in HepG2 cells. Cells were treated with tested compounds at their 2 x IC_{50} values for 48 h. Samples were normalized for protein loading (50 μg) by reblotting the membrane-bound protein with an anti-actin antibody. 5-LO, 5-lipoxygenase; p-SK1, phospho-sphingosine kinase 1; ASAH, acid ceramidase.

2.2.5. Apoptosis detection

In order to determine whether antiproliferative effects of compounds **30** and **33** that similarly showed strong and selective cytostatic activity in HepG2 cells could be ascribed to induction of apoptosis, Annexin V assay was carried out as previously described [62]. We found that compound **30** resulted in a slight decline in the viable cell population by 9.2% with concomitant increase in early apoptotic and late apoptotic/primary necrotic cells by 5.2% and 9.2%, respectively (Table 3, Fig. 6B). Compound **33** induced marked reductions in the viable cell population by 32.9% accompanied by dramatic increase in early apoptotic cells by 35.3% in comparison with untreated cells (Table 3, Fig. 6C). Different extent of cell death response triggered by tested compounds could be associated with their 5-iodoindole or 2-methylbenzimidazole heterocycle and hydroxyl or methyl substituent at C-7 of the coumarin moiety.

Table 3.

Results of Annexin V assay for apoptosis detection in HepG2 cells.[a]

	HepG2 cells (%)			
	Control	**30**	Control	**33**
Late apoptotic/primary necrotic cells	0	9.2	9.6	5.6
Viable cells	94.2	85	38.46	5.6
Early apoptotic cells	0.6	5.8	51.9	87.2
Secondary necrotic cells	5.2	0	9.6	7.2

[a]Shown are the percentages of viable cells (PI-/Ann V-), early apoptotic cells (PI-/Ann V+), late apoptotic/primary necrotic cells (PI+/Ann V+), and secondary necrotic cells (PI+) after 48 h treatment with compounds **30** and **33** at their 2 x IC_{50} values.

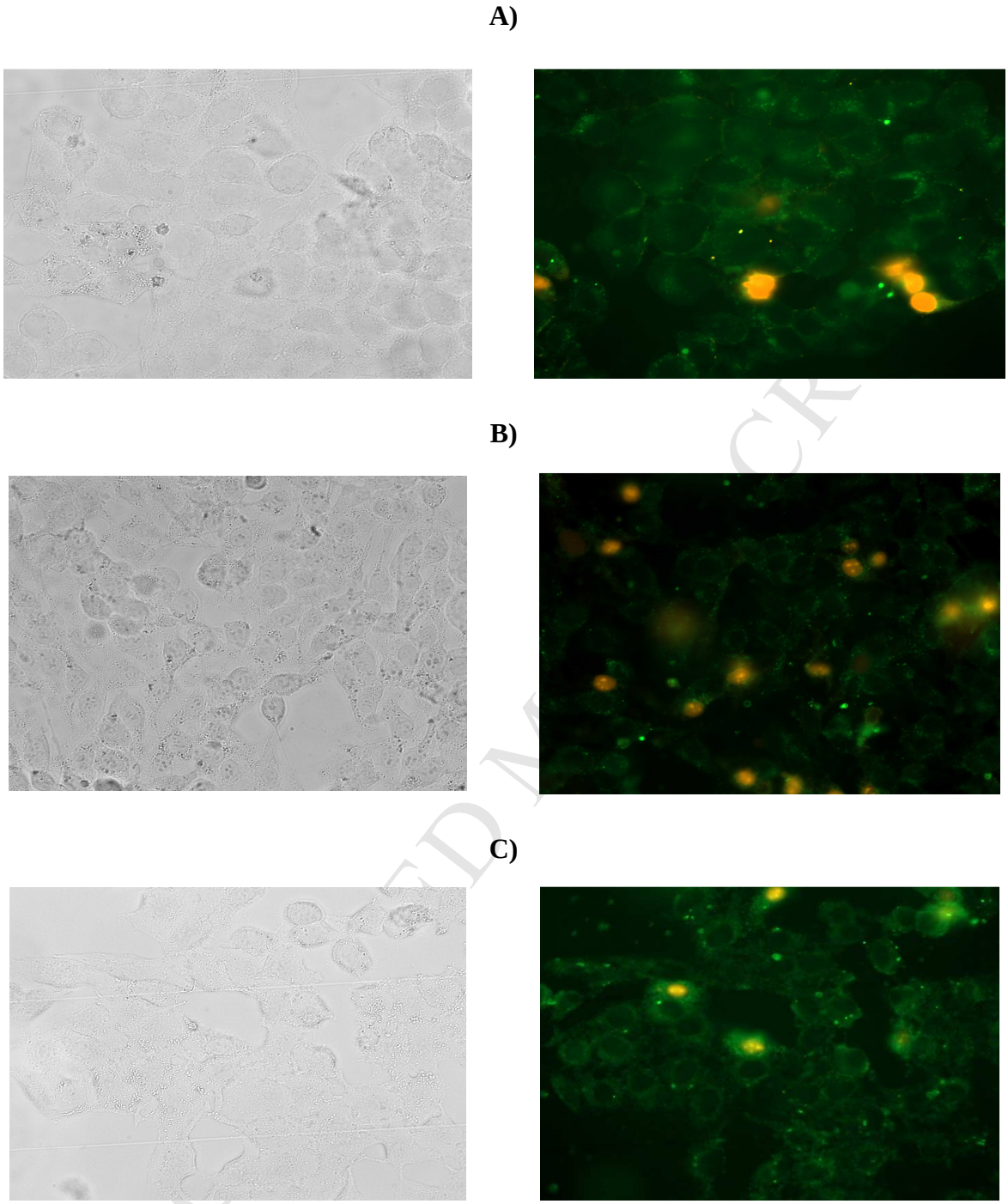


Fig. 6. Visualization of apoptotic cells stained with annexin V-FITC using fluorescence microscopy at 40x magnification. PI staining was used as nuclear marker. Shown here are bright-field images (left) and late apoptotic/primary necrotic cells (PI+/Ann V+, right). A) Untreated HepG2 cells; B) HepG2 cells treated with 2 x IC_{50} of compound **30** for 48 h; and C) HepG2 cells treated with 2 x IC_{50} of compound **33** for 48 h.

2.2.6. Antibacterial evaluations

The *in vitro* antibacterial activity of novel 1,2,3-triazole–coumarin conjugates **4**–**35** was tested against Gram-positive bacteria including *Staphylococcus* (ATCC 25923), *Enterococcus faecalis*, vancomycin-resistant *Enterococcus faecium* (VRE) and Gram-negative bacteria including *Pseudomonas aeurigonsa* (ATCC 27853), *Escherichia coli* (ATCC 25925), *Acinetobacter baumannii* (ATCC 19606), extended-spectrum β-lactamase (ESBL)-producing *Klebisiella pneumoniae.* The obtained results were compared with known antibiotics ceftazidime (CAZ) and ciprofloxacin (CIP). To evaluate the activity of synthesized compounds against bacteria minimum inhibitory concentrations (MICs) were determined and given in Table 5 for selected compounds. Compounds having MIC values higher than 256 µg/mL against all tested bacterial strains were considered inactive and were not included in the Table 5. Generally, results of antibacterial evaluations revealed that compounds **4**–**35** did not exhibit considerable antibacterial activities against tested Gram-positive and Gram-negative bacteria, with the exception of some selective activities against *Enterococcus* species. Compounds showing antibacterial activity exhibited a wide range of logP values (-0.20–5.61) suggesting that the effect of lipophilic property of 1,2,3-triazole–coumarin conjugates on their inhibitory activity against selected seven Gram-positive and Gram-negative bacteria was not found (Table 5). It can be observed that from the series of coumarin–1,2,3-triazole hybrids containing substituted phenyl unit in **13**–**19**, compounds with *p*-alkylphenyl (**16**–**18**) and (*p*-alkylcyclohexyl)phenyl (**19**) 4-substituted 1,2,3-triazole moiety displayed promising activity against *Enterococcus faecalis* at MICs ranging from 8 to 64 µg/mL. On the contrary to this, variously hydroxyalkyl and alkyl substituted 1,2,3-triazole moiety in **4**–**12** were deprived of anti-*Enterococcus faecalis* activities (MICs were > 256 µg/mL). Among the 4-(benzenesulfonamide)methyl-1,2,3-triazole–coumarin conjugates **20**–**24**, compounds with *p*-methyl- (**20**) and *p*-fluorobenzenesulfonamide (**22** and **23**) subunits demonstrated inhibitory effect against *E. faecalis* at 64 and 32 µg/mL of MICs. In addition, coumarin–1,2,3-triazole hybrids with 4-(methylthiocarbonothioyl)morpholine (**25**) and -piperazine (**27**) units were found to exhibit good potency at 32 µg/mL and 16 µg/mL of MICs, respectively. It is interesting to note that representatives of afore-mentioned classes, namely *p*-pentylphenyl (**17**), 2-chloro-4-fluorobenzenesulfonamide (**23**) and (methylthiocarbonothioyl)piperazine (**27**) 4-substituted 1,2,3-triazole–coumarin hybrids revealed the inhibitory activities against clinically isolated vancomycin resistant *Enterococcus*

faecium, while the reference antibiotics ceftazidime and ciprofloxacin exhibited the lack of activity (MICs were > 256 μg/mL).

Table 5.

Antimicrobial activities of selected compounds **10**, **12**, **15–20**, **22–25** and **27** against Gram-positive bacteria *S aureus*, *E. faecalis*, vancomycin-resistant *E. faecium* (VRE)[a] and Gram-negative bacteria including *P. aeurigonsa*, *E. coli*, *A. baumannii* and ESBL[b]-producing *K. pneumoniae*.

Compd	MIC (μg/mL)						
	S. aureus	*E. faecalis*	*E. faecium* VRE	*P. aeurigonsa*	*E. coli*	*A. baumannii*	*K. pneumoniae ESBL*
10	> 256	128	> 256	> 256	> 256	> 256	> 256
12	> 256	256	> 256	> 256	> 256	> 256	> 256
15	> 256	256	> 256	> 256	> 256	> 256	> 256
16	> 256	64	256	> 256	> 256	> 256	> 256
17	> 256	8	64	> 256	> 256	> 256	> 256
18	> 256	16	> 256	> 256	> 256	> 256	> 256
19	> 256	64	> 256	> 256	> 256	> 256	> 256
20	256	64	128	> 256	> 256	> 256	> 256
22	> 256	64	128	> 256	> 256	> 256	> 256
23	> 256	32	64	> 256	> 256	> 256	> 256
24	> 256	256	> 256	> 256	> 256	> 256	> 256
25	> 256	32	> 256	> 256	> 256	> 256	> 256
27	> 256	16	64	> 256	> 256	> 256	> 256
CAZ	64	256	> 256	4	0.5	16	> 256
CIP	0.25	0.5	> 256	< 0.125	< 0.125	< 0.125	> 256

[a]Vancomycin-resistant *E. faecium*; [b]Extended-spectrum β-lactamases (resistant to most β-lactam antibiotics).

3. Conclusions

This work focused on the development of new potentially active cytostatic and antibacterial agents based on coumarin–1,2,3-triazole pharmacophoric system. A variety of 1,2,3-triazole tethered coumarins (**4**–**35**) containing 4-substituted alkyl, phenyl, alkylphenyl, dithiocarbamate, benzenesulfonamide and benzofused heterocycle subunit were designed and synthesized by regioselective copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition. A physicochemical rationale in selection of C-4 substituents has been applied for designed coumarin–1,2,3-triazole hybrid library. While the strong relationship between lipophilicity of the coumarin–1,2,3-triazole library and antiproliferative activities was indicated, such effect of lipophilicity was not observed on antibacterial activities. The two-class models based on logP values confirmed that lipophilicity was a quite good parameter for distinguishing active from inactive compounds for all tested cancer cells lines. *In vitro* antiproliferative activity of compounds evaluated against five human cancer cell lines (A549, HepG2, CFPAC-1, HeLa and SW620) revealed that five of the compounds comprising phenylethyl (**13**), 3,5-difluorophenyl (**14**), 5-iodoindole (**30**), 2-methylbenzimidazole (**33**) and benzimidazole (**35**) exhibited high antiproliferative activities. We may conclude that benzofused heterocycle unit proved to be effective in increasing the cytostatic activity with exceptional profile of compound **33** in HepG2 cells displaying an IC_{50} value of 0.9 µM and selectivity index of 50. Similarly, 5-iodoindole heterocycle proved to confer selectivity of compound **30** for HepG2 cells with IC_{50} of 8.57 µM and selectivity index of 3. Due to their highly selective cytostatic activity in HepG2 cells, compounds **30** and **33** were selected for further biological studies. The underlying mechanism of cytostatic effects of compound **30** included induction of apoptosis and primary necrosis, whereas compound **33** triggered mainly early apoptosis. Importantly, the growth-suppressive properties of compound **33** in HepG2 cells could be associated with its ability to inhibit 5-lipoxygenase (5-LO) and acid ceramidase activities that may, in turn, lead to accumulation of pro-apoptotic lipids arachidonic acid and ceramide, respectively. On the contrary, growth inhibition of HepG2 cells by compound **30** was not associated with down-regulation of the key enzymes regulating leukotriene synthesis from arachidonic acid and ceramide turnover, which clearly demonstrated that heterocycle subunit and 5-substituent at coumarin ring in **30** and **33** were crucial for their impact on the regulation of lipid metabolism in HepG2 cells. Results of antibacterial evaluations revealed a coumarin–1,2,3-triazole hybrids comprising *p*-pentylphenyl (**17**), 2-chloro-4-fluorobenzenesulfonamide

(**23**) and dithiocarbamate (**27**) subunit with selective anti-*Enterococcus* species activities. More importantly, whereas the reference antibiotics ceftazidime and ciprofloxacin exhibited the lack of activity against clinically isolated vancomycin resistant *Enterococcus faecium* (MICs were > 256 μg/mL), compounds **17**, **23** and **27** demonstrated the inhibitory effect against *E. faecium* (VRE).

4. Experimental part

4.1. Materials and methods

Thin layer chromatography (TLC) was performed on pre-coated *Merck* silica gel 60F-254 plates using an appropriate solvent system and the spots were detected under UV light (254 nm). For column chromatography silica gel (*Fluka*, 0.063–0.2 mm) was employed, glass column was slurry-packed under gravity. Melting points (uncorrected) were determined with *Kofler* micro hot-stage (*Reichert*, Wien). Microwave-assisted syntheses were performed in a Milestone start S microwave oven using glass cuvettes at 80 ^{o}C and 300 W under the pressure of 1 bar. Elemental analyses were performed in the Central Analytic Service, Ruđer Bošković Institute, Zagreb. All elemental compositions were within the 0.4% of the calculated values. ^{1}H and ^{13}C NMR spectra were acquired on a Bruker 300 and 600 MHz NMR spectrometer. All data were recorded in DMSO-d_6 at 298 K. Chemical shifts were referenced to the residual solvent signal of DMSO at δ 2.50 ppm for ^{1}H and δ 39.50 ppm for ^{13}C. Individual resonances were assigned on the basis of their chemical shifts, signal intensities, multiplicity of resonances and H–H coupling constants. Mass spectra were recorded on an Agilent 6410 instrument equipped with electrospray interface and triple quadrupole analyzer (LC/MS/MS). High performance liquid chromatography was performed on an Agilent 1100 series system with UV detection (photodiode array detector) using Zorbax C18 reverse-phase analytical column (2.1 x 30 mm, 3.5 μm).

4.2. Procedures for the preparation of compounds

4-(Azidomethyl)-7-hydroxy-2*H*-chromen-2-one (**1a**) [46], 4-(azidomethyl)-7-methyl-2*H*-chromen-2-one (**1b**) [51], *tert*-butyl 4-[(prop-2-ynylthio)carbonothioyl]piperazine-1-carboxylate (**3b**) [46], *tert*-butyl 4-{[(1-(4-(7-hydroxy-2*H*-chromen-2-on-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methylthio]carbonothioyl}piperazine-1-carboxylate (**26**) [46] and compounds **28**–**35** [48] were synthesized in accord with procedures given in the literature.

*4.2.1. General procedure for the synthesis of **2a-e***

Pyridine (6.5 eq) was added dropwise in the propargyl amine (1.9 eq) and the solution was stirred at 0 °C under an argon atmosphere. Corresponding sulfonyl chloride (1 eq) was added in small portions, and the reaction mixture was stirred overnight at room temperature. The solvent was evaporated and ethyl acetate (100 mL) was added, followed by 1M HCl (50 ml). Organic layer was separated, dried over $MgSO_4$ and evaporated to dryness to give crude product which was then purified by column chromatography.

*4.2.1.1. 4-Methyl-N-(prop-2-ynyl)benzenesulfonamide (**2a**).* Compound **2a** was synthesized according to the general procedure using pyridine (12.3 mL), propargyl amine (2.43 g, 44 mmol) and *p*-toluenesulfonyl chloride (2.5 g, 22.8 mmol). After purification by column chromatography (CH_2Cl_2) compound **2a** was isolated as white powder (2.2 g, 47%, m.p. = 81−83 °C). ^{1}H-NMR (300 MHz, DMSO) δ 8.03 (1H, s, NH), 7.69 (2H, d, J = 8.3 Hz, H-7), 7.39 (2H, d, *J* = 8.0 Hz, H-8), 3.65 (2H, d, *J* = 2.3 Hz, H-3), 3.05 (1H, t, *J* = 2.5 Hz, H-1), 2.38 (3H, s, CH_3) ppm. ^{13}C-NMR (75 MHz, DMSO) δ 142.77 (C-9), 137.56 (C-6), 129.50 (C-8), 128.71 (C-7), 79.39 (C-2), 74.60 (C-1), 31.88 (C-3), 20.99 (CH_3) ppm.

*4.2.1.2. 4-Chloro-N-(prop-2-ynyl)benzenesulfonamide (**2b**).* Compound **2b** was synthesized according to the general procedure using pyridine (5.1 mL), propargyl amine (1.01 g, 18 mmol) and 4-chlorobenzenesulfonyl chloride (2 g, 9.5 mmol). After purification by column chromatography (CH_2Cl_2) compound **2b** was isolated as white powder (1.6 g, 73%, m.p. = 106−108 °C). ^{1}H-NMR (300 MHz, DMSO) δ 8.25 (1H, s, NH), 7.85–7.75 (2H, m, H-7), 7.73–7.56 (2H, m, H-8), 3.73 (2H, d, *J* = 2.5 Hz, H-3), 3.05 (1H, t, *J* = 2.5 Hz, H-1) ppm. ^{13}C-NMR (75 MHz, DMSO) δ 139.93 (C-6), 137.84 (C-9), 129.65 (C-8), 129.17 (C-7), 79.58 (C-2), 75.32 (C-1), 32.32 (C-3) ppm.

*4-Fluoro-N-(prop-2-ynyl)benzenesulfonamide (**2c**).* Compound **2c** was synthesized according to the general procedure using pyridine (6.9 mL), propargyl amine (1.37 g, 24 mmol) and 4-fluorobenzenesulfonyl chloride (2.5 g, 12.8 mmol). After purification by column chromatography (CH_2Cl_2) compound **2c** was isolated as white powder (2 g, 73%, m.p. = 88−90 °C). ^{1}H-NMR (600 MHz, DMSO) δ 8.16 (1H, s, NH), 7.89–7.85 (2H, m, H-7), 7.43 (2H, t, *J* = 6.0 Hz, H-8), 3.72 (2H, d, *J* = 2.4 Hz, H-3), 3.02 (1H, t, *J* = 2.4 Hz, H-1) ppm. ^{13}C-NMR (151 MHz, DMSO) δ 164.19 (d, $J_{C\text{-}F}$ = 250.7 Hz, C-9), 136.93 (d, $J_{C\text{-}F}$ = 3.0 Hz, C-6), 129.74 (d, $J_{C\text{-}F}$ = 9.1 Hz, C-7), 116.1 (d, $J_{C\text{-}F}$ = 22.7 Hz, C-8), 79.14 (C-2), 74.40 (C-1), 31.83 (C-3) ppm.

*4.2.1.3. 2-Chloro-4-fluoro-N-(prop-2-ynyl)benzenesulfonamide (**2d**).* Compound **2d** was synthesized according to the general procedure using pyridine (1.4 mL), propargyl amine (0.26 g, 4.6 mmol) and 2-chloro-4-fluorobenzenesulfonyl chloride (0.6 g, 2.5 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 100 : 1), compound **2d** was isolated as white powder (406.7 mg, 66%, m.p. = 97−100 °C). ^{1}H-NMR (300 MHz, DMSO) δ 8.38 (1H, s, NH), 8.04 (1H, dd, *J* = 8.9, 6.0 Hz, H-8), 7.70 (1H, dd, *J* = 8.8, 2.6 Hz, H-7'), 7.41 (1H, td, *J* = 8.6, 2.6 Hz, H-8'), 3.78 (2H, d, *J* = 2.4 Hz, H-3), 2.99 (1H, t, *J* = 2.4 Hz, H-1) ppm. ^{13}C-NMR (75 MHz, DMSO) δ 163.96 (d, J_{C-F} = 252.8 Hz, C-9), 134.79 (d, J_{C-F} = 5.2 Hz, C-6), 133.06 (d, J_{C-F} = 9.8 Hz, C-7'), 132.97 (d, J_{C-F} = 11.6 Hz, C-7), 119.14 (d, J_{C-F} = 25.5 Hz, C-8), 114.75 (d, J_{C-F} = 21.8 Hz, C-8'), 78.96 (C-2), 74.41 (C-1), 31.75 (C-3) ppm.

*4.2.1.4. 4-Nitro-N-(prop-2-ynyl)benzenesulfonamide (**2e**).* Compound **2e** was synthesized according to the general procedure using pyridine (12.3 mL), propargyl amine (0.2 g, 3 mmol) and 4-nitrobenzenesulfonyl chloride (0.4 g, 1.9 mmol). After purification by column chromatography (CH_2Cl_2) compound **2e** was isolated as white powder (2 g, 77%, m.p. = 165−167 °C). ^{1}H-NMR (300 MHz, DMSO) δ 8.52 (1H, s, NH), 8.45–8.39 (2H, m, H-8), 8.10–8.04 (2H, m, H-7), 3.79 (2H, d, *J* = 2.5 Hz, H-3), 3.01 (1H, t, *J* = 2.5 Hz, H-1) ppm. ^{13}C-NMR (75 MHz, DMSO) δ 150.09 (C-9), 146.73 (C-6), 128.89 (C-7), 124.86 (C-8), 79.27 (C-2), 75.66 (C-1), 32.35 (C-3) ppm.

*4.2.2. Prop-2-ynyl morpholine-4-carbodithioate (**3a**).* CS_2 (0.62 mL, 10.3 mmol) was added dropwise to the solution of morpholine (300 mg, 3.4 mmol) and Na_3PO_4 x 12 H_2O (785 mg, 2.1 mmol) in acetone (20 mL). The reaction mixture was stirred at room temperature for 0.5 h. Then propargyl bromide (0.32 mL, 3.8 mmol) was added and the reaction mixture was stirred at room temperature for 30 min. Upon completion, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in EtOAc (350 mL), washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford compound **3a** as a yellow oil (213 mg, 31%). ^{1}H-NMR (300 MHz, DMSO) δ 4.14 (4H, d, *J* = 2.6 Hz, H-7), 3.69–3.66 (6H, m, H-3, H-8), 3.18 (1H, t, *J* = 2.6 Hz, H-1). ^{13}C-NMR (75 MHz, DMSO) δ 194.03 (C=S), 79.53 (C-2), 74.69 (C-8), 66.02 (C-1), 40.04 (C-7), 25.42 (C-3).

*4.2.3. General procedure for the synthesis of **4–25***

A mixture of 4-(azidomethyl)-7-hydroxycoumarin, Cu(0) (1.2 eq) and terminal alkyne (1.2 eq), 1M $CuSO_4$ (0.06 mL) in *t*-BuOH/H_2O (1 : 1) (3 mL) and DMF (0.5 mL) was added into

glass tube with a magnetic stirring bar and sealed with a plastic cap. The synthesis was carried out at 80 °C for 1h under microwave irradiation (300 W). Completion of the reaction was monitored by TLC. After the reaction was completed, the solvent was evaporated under reduced pressure and the residue was chromatographed on silica column.

*4.2.3.1. [4-(4-Cyclopropyl-1H-1,2,3-triazol-1-yl)methyl]-7-hydroxy-2H-chromen-2-one (**4**).* Compound **4** was synthesized according to the general procedure using compound **1a** (60 mg, 0.28 mmol) and ethynyl cyclopropane (0.019 mL, 0.23 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 20 : 1), compound **4** (21 mg, 81%, m.p. = 154–156 °C) was isolated as light yellow powder. ^{1}H-NMR (600 MHz, DMSO) δ: 10.62 (1H, s, OH), 7.95 (1H, s, H-3'), 7.66 (1H, d, *J* = 8.8 Hz, H-5), 6.82 (1H, dd, *J* = 8.7, 2.3 Hz, H-6), 6.76 (1H, d, *J* = 2.3 Hz, H-8), 5.80 (2H, s, H-1'), 5.58 (1H, s, H-3), 1.98–1.95 (1H, m, H-5'), 0.96–0.87 (2H, m, H-6'), 0.76 – 0.68 (2H, m, H-7'). ^{13}C-NMR (151 MHz, DMSO): 161.59 (C-2), 159.93 (C-7), 155.08 (C-4), 150.47 (C-8a), 149.45 (C-4'), 126.01 (C-5), 121.76 (C-3'), 113.14 (C-6), 109.40 (C-4a), 109.39 (C-3), 102.49 (C-8), 49.06 (C-1'), 7.65 (C-6', C-7'), 6.45 (C-5'). MS (ESI): *m*/*z* = 284.3 ([*M* + H]$^+$). Anal. calcd. for $C_{15}H_{13}N_3O_3$: C, 63.60; H, 4.63; N, 14.83. Found: C, 63.38; H, 4.64; N, 14.80.

*4.2.3.2. {4-[4-(3-Chloropropyl)-1H-1,2,3-triazol-1-yl]methyl}-7-hydroxy-2H-chromen-2-one (**5**).* Compound **5** was synthesized according to the general procedure using compound **1a** (60 mg, 0.28 mmol) and 5-chloropentyne (0.024 mL, 0.23 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 20 : 1), compound **5** (68 mg, 92%, m.p. = 201–203 °C) was isolated as light yellow powder. ^{1}H-NMR (600 MHz, DMSO) δ: 10.67 (1H, s, OH), 8.04 (1H, s, H-3'), 7.67 (1H, d, *J* = 8.8 Hz, H-5), 6.83 (1H, dd, *J* = 8.7, 2.3 Hz, H-6), 6.76 (1H, d, *J* = 2.3 Hz, H-8), 5.85 (2H, s, H-1'), 5.58 (1H, s, H-3), 3.68 (2H, t, *J* = 6.5 Hz, H-7'), 2.79 (2H, t, *J* = 7.5 Hz, H-5'), 2.16–1.98 (2H, m, H-6'). ^{13}C-NMR (75 MHz, DMSO): 163.07 (C-2), 160.42 (C-7), 155.57 (C-4), 151.17 (C-8a), 146.59 (C-4'), 126.52 (C-5), 123.74 (C-3'), 113.62 (C-6), 109.89 (C-4a), 109.80 (C-3), 102.99 (C-8), 49.56 (C-1'), 45.13 (C-7'), 32.13 (C-5'), 22.76 (C-6'). MS (ESI): *m*/*z* = 320.7 ([*M* + H]$^+$). Anal. calcd. for $C_{15}H_{14}ClN_3O_3$: C, 56.35; H, 4.41; N, 13.14. Found: C, 56.51; H, 4.42; N, 13.15.

*4.2.3.3. 7-Hydroxy-{4-[4-(2-hydroxyethyl)-1H-1,2,3-triazol-1-yl]methyl}-2H-chromen-2-one (**6**).* Compound **6** was synthesized according to the general procedure using compound **1a** (60 mg, 0.28 mmol) and 3-butyn-1-ol (0.017 mL, 0.23 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 10 : 1) compound **6** (25 mg, 39%, m.p. = 219–221°C)

was isolated as light yellow powder. ^{1}H-NMR (600 MHz, DMSO) δ: 10.66 (1H, s, OH), 7.98 (1H, s, H-3'), 7.68 (1H, d, *J* = 8.8 Hz, H-5), 6.81 (1H, dd, *J* = 8.7, 2.3 Hz, H-6), 6.74 (1H, d, *J* = 2.3 Hz, H-8), 5.83 (2H, s, H-1'), 5.55 (1H, s, H-3), 4.67 (1H, s, OH), 3.63 (2H, dd, *J* = 11.1, 6.5 Hz, H-6'), 2.78 (2H, t, *J* = 6.8 Hz, H-5'). ^{13}C-NMR (75 MHz, DMSO): 162.06 (C-2), 160.45 (C-7), 155.57 (C-4), 151.17 (C-8a), 145.04 (C-4'), 126.55 (C-5), 124.07 (C-3'), 113.63 (C-6), 109.92 (C-4a), 109.71 (C-3), 102.99 (C-8), 60.69 (C-1'), 49.47 (C-6'), 29.56 (C-5'). MS (ESI): *m*/*z* = 288.3 ([*M* + H]$^{+}$). Anal. calcd. for $C_{14}H_{13}N_3O_4$: C, 58.53; H, 4.56; N, 14.63. Found: C, 58.41; H, 4.57; N, 14.59.

*4.2.3.4. 7-Hydroxy-{4-[4-(1-hydroxyethyl)-1H-1,2,3-triazol-1-yl]methyl}-2H-chromen-2-one (**7**).* Compound **7** was synthesized according to the general procedure using compound **1a** (60 mg, 0.28 mmol) and 3-butyn-2-ol (0.018 mL, 0.23 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 30 : 1) compound **7** (75 mg, 81%, m.p. > 250 °C) was isolated as light brown powder. ^{1}H-NMR (600 MHz, DMSO) δ: 10.64 (1H, s, OH), 8.05 (1H, s, H-3'), 7.72 (1H, d, *J* = 8.7 Hz, H-5), 6.84 (1H, dd, *J* = 8.7, 2.3 Hz, H-6), 6.76 (1H, d, *J* = 2.4 Hz, H-8), 5.86 (2H, s, H-1'), 5.57 (1H, s, H-3), 5.25 (1H, d, *J* = 4.9 Hz, OH), 4.04 (1H, q, *J* = 5.3 Hz, H-5'), 1.42 (3H, d, *J* = 6.5 Hz, CH_3). ^{13}C-NMR (151 MHz, DMSO): 161.58 (C-2), 159.94 (C-7), 156.07 (C-4), 153.17 (C-8a), 150.69 (C-4'), 126.06 (C-5), 122.42 (C-3'), 113.13 (C-6), 109.40 (C-4a), 109.16 (C-3), 102.49 (C-8), 61.54 (C-5'), 49.03 (C-1'), 23.65 (CH_3). MS (ESI): *m*/*z* = 288.3 ([*M* + H]$^{+}$). Anal. calcd. for $C_{14}H_{13}N_3O_4$: C, 58.53; H, 4.56; N, 14.63. Found: C, 58.72; H, 4.55; N, 14.60.

*4.2.3.5. 7-Hydroxy-{4-[4-(3-hydroxypropyl)-1H-1,2,3-triazol-1-yl]methyl}-2H-chromen-2 one (**8**).* Compound **8** was synthesized according to the general procedure using compound **1a** (60 mg, 0.28 mmol) and 4-pentyn-1-ol (0.021 mL, 0.23 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 10 : 1), compound **8** (55 mg, 79%, m.p. = 98–100 °C) was isolated as light yellow powder. ^{1}H-NMR (300 MHz, DMSO) δ: 10.69 (1H, s, OH), 7.99 (1H, s, H-3'), 7.68 (1H, d, *J* = 8.7 Hz, H-5), 6.93–6.68 (2H, m, H-6, H-8), 5.84 (2H, s, H-1'), 5.54 (1H, s, H-3), 4.49 (1H, t, *J* = 5.1 Hz, OH), 3.43 (2H, dd, *J* = 11.5, 6.2 Hz, H-7'), 2.68 (2H, t, *J* = 7.6 Hz, H-5'), 1.76 (2H, dd, *J* = 14.6, 6.8 Hz, H-6'). ^{13}C-NMR (75 MHz, DMSO): 162.07 (C-2), 160.43 (C-7), 155.57 (C-4), 151.17 (C-8a), 147.99 (C-4'), 126.51 (C-5), 123.43 (C-3'), 113.63 (C-6), 109.91 (C-4a), 109.71 (C-3), 102.99 (C-8), 60.44 (C-1'), 49.52 (C-7'), 32.64 (C-5'), 22.07 (C-6'). MS (ESI): *m*/*z* = 302.3 ([*M* + H]$^{+}$). Anal. calcd. for $C_{15}H_{15}N_3O_4$: C, 59.79; H, 5.02; N, 13.95. Found: C, 59.60; H, 5.01; N, 13.92.

*4.2.3.6. 7-Hydroxy-{4-[4-(4-hydroxybutyl)-1H-1,2,3-triazol-1-yl]methyl}-2H-chromen-2-one (**9**).* Compound **9** was synthesized according to the general procedure using compound **1a** (60 mg, 0.28 mmol) and 5-hexyn-1-ol (0.025 mL, 0.23 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 10 : 1) compound **9** (31 mg, 43%, m.p. = 152–154 °C) was isolated as light yellow powder. ^{1}H-NMR (300 MHz, DMSO) δ: 10.69 (1H, s, OH), 7.99 (1H, s, H-3'), 7.68 (1H, d, *J* = 8.8 Hz, H-5), 6.83 (1H, dd, *J* = 8.7, 2.3 Hz, H-6), 6.76 (1H, d, *J* = 2.3 Hz, H-8), 5.84 (2H, s, H-1'), 5.53 (1H, s, H-3), 4.38 (1H, t, *J* = 5.1 Hz, OH), 3.41 (2H, dd, *J* = 11.5, 6.3 Hz, H-8'), 2.64 (2H, t, *J* = 7.5 Hz, H-5'), 1.63–1.60 (2H, m, H-7'), 1.45 (2H, dt, *J* = 10.3, 6.7 Hz, H-6'). ^{13}C-NMR (75 MHz, DMSO): 162.07 (C-2), 160.43 (C-7), 155.57 (C-4), 151.18 (C-8a), 148.04 (C-4'), 126.51 (C-5), 123.38 (C-3'), 113.63 (C-6), 109.91 (C-4a), 109.71 (C-3), 102.99 (C-8), 60.85 (C-1'), 49.52 (C-8'), 32.43 (C-5'), 25.96 (C-7'), 25.28 (C-6'). MS (ESI): *m*/*z* = 316.3 ([*M* + H]$^+$). Anal. calcd. for $C_{16}H_{17}N_3O_4$: C, 60.94; H, 5.43; N, 13.33. Found: C, 61.05; H, 5.44; N, 13.30.

*4.2.3.7. 4-[(4-Tert-butyl-1H-1,2,3-triazol-1-yl)methyl]-7-hydroxy-2H-chromen-2-one (**10**).* Compound **10** was synthesized according to the general procedure using compound **1a** (60 mg, 0.28 mmol) and 3,3-dimethylbutyne (0.028 mL, 0.23 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 30 : 1), compound **10** (35 mg, 51%, m.p. > 250 °C) was isolated as light yellow powder. ^{1}H-NMR (300 MHz, DMSO) δ: 10.68 (1H, s, OH), 8.00 (1H, s, H-3'), 7.70 (1H, d, *J* = 8.7 Hz, H-5), 6.88–6.72 (2H, m, H-6, H-8), 5.82 (2H, s, H-1'), 5.56 (1H, s, H-3), 1.29 (9H, s, CH_3). ^{13}C-NMR (151 MHz, DMSO): 161.62 (C-2), 159.96 (C-7), 156.78 (C-4), 155.08 (C-8a), 150.62 (C-4'), 126.03 (C-5), 120.86 (C-3'), 113.15 (C-6), 109.41 (C-4a), 109.27 (C-3), 102.50 (C-8), 48.98 (C-1'), 39.51 (C-5'), 30.19 (CH_3). MS (ESI): *m*/*z* = 300.3 ([*M* + H]$^+$). Anal. calcd. for $C_{16}H_{17}N_3O_3$: C, 64.20; H, 5.72; N, 14.04. Found: C, 63.95; H, 5.73; N, 14.01.

*4.2.3.8. 4-[(4-Hexyl-1H-1,2,3-triazol-1-yl)methyl]-7-hydroxy-2H-chromen-2-one (**11**).* Compound **11** was synthesized according to the general procedure using compound **1a** (30 mg, 0.14 mmol) and octyne (0.03 mL, 0.17 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 100 : 1), compound **11** (22.5 mg, 51%, m.p. = 200–203 °C) was isolated as yellow powder. ^{1}H-NMR (DMSO) δ: 10.74 (1H, s, OH), 8.63 (1H, s, H-3'), 7.64 (1H, d, *J* = 8.8 Hz, H-5), 6.80 (1H, dd, *J* = 8.8, 2.2 Hz, H-6), 6.73 (1H, d, *J* = 2.1 Hz, H-8), 5.82 (2H, s, H-1'), 5.50 (1H, s, H-3), 2.61 (2H, t, *J* = 7.5 Hz, H-5'), 1.57 (2H, dt, *J* = 7.7 Hz, H-6'), 1.25–1.22 (6H, m, H-7', H-8', H-9'), 0.83 (3H, t, *J* = 6.4 Hz, CH_3). ^{13}C-NMR (75

MHz, DMSO): 161.81 (C-2), 160.12 (C-7), 154.67 (C-4), 150.84 (C-8a), 147.63 (C-4'), 126.05 (C-5), 123.05 (C-3'), 113.32 (C-6), 109.44 (C-4a), 109.21 (C-3), 102.60 (C-8), 49.15 (C-1'), 30.99 (C-5'), 28.84 (C-6'), 28.16 (C-7'), 24.95 (C-9'), 22.05 (C-8'), 13.94 (CH_3). MS (ESI): *m*/*z* = 328.4 ([*M* + H]$^+$). Anal. calcd. for $C_{18}H_{21}N_3O_3$: C, 66.04; H, 6.47; N, 12.84. Found: C, 65.89; H, 6.45; N, 12.80.

4.2.3.9. 7-Hydroxy-[4-(4-octyl-1H-1,2,3-triazol-1-yl)methyl]-2H-chromen-2-one ***(12)***. Compound **12** was synthesized according to the general procedure using compound **1a** (30 mg, 0.14 mmol) and decyne (0.03 mL, 0.16 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 100 : 1) compound **12** (22.5 mg, 45%, m.p. = 109–111 °C) was isolated as yellow powder. ^{1}H-NMR (DMSO) δ: 10.86 (1H, s, OH), 7.97 (1H, s, H-3'), 7.65 (1H, d, *J* = 8.7 Hz, H-5), 6.81 (1H, dd, *J* = 8.8, 1.1 Hz, H-6), 6.75 (1H, d, *J* = 2.1 Hz, H-8), 5.82 (2H, s, H-1'), 5.50 (1H, s, H-3), 2.61 (2H, t, *J* = 7.5 Hz, H-5'), 1.59–1.55 (2H, m, H-6'), 1.26–1.19 (10H, H-7'–11'), 0.83 (3H, t, *J* = 7.0 Hz, CH_3). ^{13}C-NMR (75 MHz, DMSO): 161.72 (C-2), 160.16 (C-7), 155.02 (C-4), 150.87 (C-8a), 147.68 (C-4'), 126.15 (C-5), 123.10 (C-3'), 113.32 (C-6), 109.53 (C-4a), 109.31 (C-3), 102.63 (C-8), 49.18 (C-1'), 31.30 (C-5'), 28.90 (C-6'), 28.75 (C-7'), 28.53 (C-9'), 28.67 (C-8'), 24.96 (C-10'), 22.15 (C-11'), 14.02 (CH_3). MS (ESI): *m*/*z* = 356.4 ([*M* + H]$^+$). Anal. calcd. for $C_{20}H_{25}N_3O_3$: C, 67.58; H, 7.09; N, 11.82. Found: C, 67.47; H, 7.11; N, 11.79.

4.2.3.10. 7-Hydroxy-[4-(4-phenylethyl-1H-1,2,3-triazol-1-yl)methyl]-2H-chromen-2-one ***(13)***. Compound **13** was synthesized according to the general procedure using compound **1a** (60 mg, 0.28 mmol) and 4-phenyl-1-butyne (0.032 mL, 0.23 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 30 : 1) compound **13** (61 mg, 77%, m.p. = 150–152°C) was isolated as white powder. ^{1}H-NMR (600 MHz, DMSO) δ: 10.66 (1H, s, OH), 7.88 (1H, s, H-3'), 7.64 (1H, d, *J* = 8.8 Hz, H-5), 7.22 (2H, dd, *J* = 10.3, 4.6 Hz, H-9'), 7.18–7.12 (3H, m, H-8', H-10'), 6.81 (1H, dd, *J* = 8.7, 2.4 Hz, H-6), 6.75 (1H, d, *J* = 2.3 Hz, H-8), 5.81 (2H, d, *J* = 0.9 Hz, H-1'), 5.48 (1H, s, H-3), 2.99–2.81 (4H, m, H-5', H-6'). ^{13}C-NMR (151 MHz, DMSO): 161.57 (C-2), 159.93 (C-7), 155.05 (C-4), 150.72 (C-8a), 146.65 (C-4'), 140.98 (C-7'), 128.33 (C-9'), 128.18 (C-8'), 126.00 (C-5), 125.87 (C-10'), 123.20 (C-3'), 113.14 (C-6), 109.36 (C-4a), 109.05 (C-3), 102.47 (C-8), 49.02 (C-1'), 34.70 (C-6'), 26.87 (C-5'). MS (ESI): *m*/*z* = 348.4 ([*M* + H]$^+$). Anal. calcd. for $C_{20}H_{17}N_3O_3$: C, 69.15; H, 4.93; N, 12.10. Found: C, 69.28; H, 4.92; N, 12.06.

4.2.3.11.{4-[4-(3,5-difluorophenyl)-1H-1,2,3-triazol-1-yl]methyl}-7-hydroxy-2H-chromen-2-

*one (**14**)*. Compound **14** was synthesized according to the general procedure using compound **1a** (50 mg, 0.23 mmol) and 1-ethynyl-3,5-difluorobenzene (0.022 mL, 0.19 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 50 : 1) compound **14** (59 mg, 88%, m.p. > 250 °C) was isolated as yellow powder. ^{1}H-NMR (600 MHz, DMSO) δ: 10.82 (1H, s, OH), 8.80 (1H, s, H-3'), 7.63 (1H, d, *J* = 8.7 Hz, H-5), 7.57 (2H, d, *J* = 6.0 Hz, H-6'), 7.20 (1H, ddd, *J* = 9.3, 5.8, 2.3 Hz, H-8'), 6.80 (1H, d, *J* = 8.7 Hz, H-6), 6.75 (1H, s, H-8), 5.95 (2H, s, H-1'), 5.81 (1H, s, H-3). ^{13}C-NMR (75 MHz, DMSO): 163.45 (d, J_{C-F} = 243.8, 13.5 Hz, C-7'), 162.29 (C-2), 160.41 (C-7), 155.72 (C-4), 150.06 (C-8a), 145.33 (C-4'), 134.35 (C-5'), 126.48 (C-3'), 124.13 (C-5), 113.78 (C-6), 110.76 (C-3), 109.78 (C-4a), 108.55 (d, J_{C-F} = 26.25 Hz, C-6'), 103.75 (t, J_{C-F} = 26.25 Hz, C-8'), 103.08 (C-8), 50.03 (C-1'). MS (ESI): *m*/*z* = 356.1 ([*M* + H]$^+$). Anal. calcd. for $C_{18}H_{11}F_2N_3O_3$: C, 60.85; H, 3.12; F, 10.69; N, 11.83. Found: C, 61.02; H, 3.13; N, 10.66.

*4.2.3.12.{4-[4-(4-Bromophenyl)-1H-1,2,3-triazol-1-yl]methyl}-7-hydroxy-2H-chromen-2-one (**15**)*. Compound **15** was synthesized according to the general procedure using compound **1a** (60 mg, 0.28 mmol) and 1-bromo-4-ethynylbenzene (41.6 mg, 0.23 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 30 : 1) compound **15** (25 mg, 54%, m.p. > 250 °C) was isolated as light brown powder. ^{1}H-NMR (300 MHz, DMSO) δ: 10.68 (1H, s, OH), 8.76 (1H, s, H-3'), 7.82 (2H, d, *J* = 8.5 Hz, H-6'), 7.72−7.65 (3H, m, H-5, H-7'), 6.85 (1H, d, *J* = 8.7 Hz, H-6), 6.81 (1H, s, H-8), 5.95 (2H, s, H-1'), 5.77 (1H, s, H-3'). ^{13}C-NMR (75 MHz, DMSO): 162.29 (C-2), 160.43 (C-7), 155.65 (C-4), 150.38 (C-8a), 146.21 (C-4'), 132.35 (C-7'), 130.13 (C-5'), 127.74 (C-6'), 126.46 (C-5), 123.22 (C-3'), 121.51 (C-8'), 113.76 (C-6), 110.46 (C-3), 109.81 (C-4a), 103.07 (C-8), 49.93 (C-1'). MS (ESI): *m*/*z* = 398.0 ([*M* + H]$^+$). Anal. calcd. for $C_{18}H_{12}BrN_3O_3$: C, 54.29; H, 3.04; N, 10.55. Found: C, 54.44; H, 3.03; N, 10.57.

*4.2.3.13.{4-[4-(4-Butylphenyl)-1H-1,2,3-triazol-1-yl]methyl}-7-hydroxy-2H-chromen-2-one (**16**)*. Compound **16** was synthesized according to the general procedure using compound **1a** (60 mg, 0.28 mmol) and 1-butyl-4-ethynylbenzene (0.04 mL, 0.23 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 30 : 1) compound **16** (82 mg, 95%, m.p. = 221–223 °C) was isolated as white powder. ^{1}H-NMR (600 MHz, DMSO) δ: 10.64 (1H, s, OH), 8.62 (1H, s, H-3'), 7.76 (2H, d, *J*=8.2 Hz, H-6'), 7.69 (1H, d, *J*=8.8 Hz, H-5), 7.27 (2H, d, *J* = 8.1 Hz, H-7'), 6.83 (1H, dd, *J* = 8.7, 2.4 Hz, H-6), 6.77 (1H, d, *J* = 2.3 Hz, H-8), 5.93 (2H, s, H-1'), 5.77 (1H, s, H-3), 2.60 (2H, t, *J* = 7.6 Hz, H-9'), 1.57 (2H, p, *J* = 7.6 Hz, H-10'),

1.32 (2H, hex, *J* = 7.4 Hz, H-11'), 0.90 (3H, t, *J* = 7.4 Hz, CH_3). ^{13}C-NMR (75 MHz, DMSO): 161.59 (C-2), 159.91 (C-7), 155.16 (C-4), 150.00 (C-8a), 146.86 (C-4'), 142.30 (C-8'), 128.77 (C-7'), 127.82 (C-5'), 126.01 (C-5), 125.20 (C-6'), 121.91 (C-3'), 113.17 (C-6), 109.97 (C-3), 109.43 (C-4a), 102.53 (C-8), 49.36 (C-1'), 34.51 (C-9'), 32.95 (C-11'), 21.65 (C-10'), 13.71 (CH_3). MS (ESI): *m*/*z* = 377.2 ([*M* + H]$^+$). Anal. calcd. for $C_{22}H_{21}N_3O_3$: C, 70.38; H, 5.64; N, 11.19. Found: C, 70.27; H, 5.63; N, 11.22.

*4.2.3.14. 7-Hydroxy-{4-[4-(4-pentylphenyl)-1H-1,2,3-triazol-1-yl]methyl}-2H-chromen-2-one (**17**).* Compound **17** was synthesized according to the general procedure using compound **1a** (50 mg, 0.23 mmol) and 1-ethynyl-4-pentylbenzene (0.037 mL, 0.19 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 80 : 1) compound **17** (38 mg, 52%, m.p. = 231–233 °C) was isolated as yellow powder. ^{1}H-NMR (600 MHz, DMSO) δ: 10.63 (1H, s, OH), 8.61 (1H, s, H-3'), 7.74 (2H, d, *J* = 8.2 Hz, H-6'), 7.67 (1H, d, *J* = 8.8 Hz, H-5), 7.25 (2H, d, *J* = 8.2 Hz, H-7'), 6.81 (1H, dd, *J* = 8.7, 2.3 Hz, H-6), 6.75 (1H, d, *J* = 2.4 Hz, H-8), 5.91 (2H, s, H-1'), 5.73 (1H, s, H-3), 2.57 (2H, t, *J* = 7.6 Hz, H-9'), 1.57 (2H, p, *J* = 7.5 Hz, H-10'), 1.29–1.25 (4H, m, H-11', H-12'), 0.84 (3H, t, *J* = 7.1 Hz, CH_3). ^{13}C-NMR (75 MHz, DMSO): 162.14 (C-2), 160.43 (C-7), 155.66 (C-4), 150.52 (C-8a), 147.36 (C-4'), 142.83 (C-8'), 129.27 (C-7'), 128.32 (C-5'), 126.51 (C-5), 125.70 (C-6'), 122.42 (C-3'), 113.70 (C-6), 110.43 (C-3), 109.91 (C-4a), 103.04 (C-8), 49.87 (C-1'), 35.29 (C-9'), 31.28 (C-11'), 30.95 (C-10'), 22.39 (C-12'), 14.36 (CH_3). MS (ESI): *m*/*z* = 390.1 ([*M* + H]$^+$). Anal. calcd. for $C_{23}H_{23}N_3O_3$: C, 70.93; H, 5.95; N, 10.79. Found: C, 71.06; H, 5.93; N, 10.82.

*4.2.3.15.{4-[4-(4-Hexylphenyl)-1H-1,2,3-triazol-1-yl]methyl}-7-hydroxy-2H-chromen-2-one (**18**).* Compound **18** was synthesized according to the general procedure using compound **1a** (40 mg, 0.18 mmol) and 1-ethynyl-4-hexylbenzene (0.05 mL, 0.22 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 70 : 1) compound **18** (41 mg, 56%, m.p. = 214–216 °C) was isolated as light yellow powder. ^{1}H-NMR (600 MHz, DMSO) δ: 10.73 (1H, s, OH), 8.63 (1H, s, H-3'), 7.76 (2H, d, *J* = 8.1 Hz, H-6'), 7.68 (1H, d, *J* = 8.8 Hz, H-5), 7.26 (2H, d, *J* = 8.2 Hz, H-7'), 6.82 (1H, dd, *J* = 8.7, 2.2 Hz, H-6), 6.76 (1H, d, *J* = 2.2 Hz, H-8), 5.93 (2H, s, H-1'), 5.75 (1H, s, H-3), 2.59 (2H, t, *J* = 7.6 Hz, H-9'), 1.59–1.56 (2H, m, H-10'), 1.28–1.24 (6H, m, H-11'–H-13'), 0.85 (3H, t, *J* = 6.7 Hz, CH_3). ^{13}C-NMR (75 MHz, DMSO): 161.74 (C-2), 160.15 (C-7), 155.28 (C-4), 150.23 (C-8a), 147.02 (C-4'), 142.55 (C-8'), 128.97 (C-7'), 127.88 (C-5'), 126.18 (C-5), 125.34 (C-6'), 122.10 (C-3'), 113.36 (C-6), 110.02 (C-3), 109.55 (C-4a), 102.67 (C-8), 49.51 (C-1'), 34.97 (C-9'), 31.18 (C-11'), 30.91 (C-10'), 28.34

(C-12'), 22.15 (C-13'), 14.05 (CH_3). MS (ESI): *m*/*z* = 404.2 ([*M* + H]$^+$). Anal. calcd. for $C_{24}H_{25}N_3O_3$: C, 71.44; H, 6.25; N, 10.41. Found: C, 71.31; H, 6.27; N, 10.38.

*4.2.3.16.7-Hydroxy-4-{4-[4-(4-propylcyclohexyl)phenyl-1H-1,2,3-triazol-1-yl]methyl}-2H chromen-2-one (**19**).* Compound **19** was synthesized according to the general procedure using compound **1a** (40 mg, 0.18 mmol) and 4-(4-propylcyclohexyl)phenylacetylene (0.05 mL, 0.22 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 70 : 1) compound **19** (66 mg, 81%, m.p. = 228–230 °C) was isolated as yellow powder. ^{1}H-NMR (300 MHz, DMSO) δ: 10.66 (1H, s, OH), 8.63 (1H, d, *J* = 2.0 Hz H-3'), 7.76 (2H, dd, *J* = 8.2, 1.2 Hz, H-6'), 7.68 (1H, d, *J* = 8.7 Hz, H-5), 7.31 (2H, t, *J* = 8.5 Hz, H-7'), 6.83 (1H, dd, *J* = 8.5, 2.2 Hz, H-6), 6.76 (1H, d, *J* = 2.3 Hz, H-8), 5.93 (2H, s, H-1'), 5.75 (1H, s, H-3), 2.59–2.54 (1H, m, H-9'), 1.82 (2H, d, *J* = 11.7 Hz, H-10a'), 1.72–1.54 (4H, m, H-11a', H-14'), 1.44 (2H, d, *J* = 12.6 Hz, H-10b'), 1.37–1.31 (1H, m, H-12'), 1.22 (2H, d, *J* = 10.4 Hz, H-11b'), 1.09–1.02 (2H, m, H-13'), 0.85 (3H, q, *J* = 7.1 Hz, CH_3). ^{13}C-NMR (75 MHz, DMSO): 161.78 (C-2), 160.24 (C-7), 155.32 (C-4), 150.34 (C-8a), 147.66 (C-4'), 147.09 (C-8'), 127.55 (C-7'), 128.08 (C-5'), 126.23 (C-5), 125.47 (C-6'), 122.19 (C-3'), 113.43 (C-6), 110.03 (C-3), 109.61 (C-4a), 102.73 (C-8), 49.57 (C-1'), 43.77 (C-9'), 36.47 (C-12'), 33.89 (C-10'), 33.16 (C-10'), 29.69 (C-11'), 28.57 (C-11'), 20.47 (C-13'), 19.64 (C-14'), 14.39 (CH_3). MS (ESI): *m*/*z* = 444.2 ([*M* + H]$^+$). Anal. calcd. for $C_{27}H_{29}N_3O_3$: C, 73.11; H, 6.59; N, 9.47. Found: C, 72.98; H, 6.61; N, 9.44.

*4.2.3.17. 7-Hydroxy-{4-[4-(4-methylbenzensulfonamide)methyl-1H-1,2,3-triazol-1-yl]methyl} 2H-chromen-2-one (**20**).* Compound **20** was synthesized according to the general procedure using compound **1a** (100 mg, 0.46 mmol) and **2a** (104.4 mg, 0.58 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 100 : 1) compound **20** (147 mg, 60%, m.p. = 201−203 °C) was isolated as yellow powder. ^{1}H-NMR (600 MHz, DMSO) δ 10.72 (1H, s, OH), 8.06 (1H, t, *J* = 5.9 Hz, NH), 8.00 (1H, s, H-3'), 7.70–7.67 (3H, m, H-5, H-9'), 7.36 (2H, d, *J* = 8.0 Hz, H-10'), 6.83 (1H, dd, *J* = 8.7, 2.3 Hz, H-6), 6.76 (1H, d, *J* = 2.3 Hz, H-8), 5.84 (2H, s, H-1'), 5.50 (1H, s, H-3), 4.05 (2H, d, *J* = 5.8 Hz, H-5'), 2.37 (3H, s, CH_3) ppm. ^{13}C-NMR (151 MHz, DMSO) δ 161.65 (C-2), 159.92 (C-7), 155.05 (C-4), 150.55 (C-8a), 143.99 (C-11'), 142.67 (C-4'), 137.40 (C-8'), 129.54 (C-10'), 126.55 (C-9'), 126.06 (C-5), 124.41 (C-3'), 113.16 (C-6), 109.30 (C-4a), 109.01 (C-3), 102.49 (C-8), 49.01 (C-1'), 39.07 (C-5'), 20.89 (CH_3) ppm. MS (ESI): *m*/*z* = 503.1 ([*M* + H]$^+$). Anal. calcd. for $C_{26}H_{22}N_4O_5S$: C, 62.14; H, 4.41; N, 11.15. Found: C, 62.03; H, 4.40; N, 11.11.

4.2.3.18. {4-[4-(4-Chlorobenzenesulfonamide)methyl-1H-1,2,3-triazol-1-yl]methyl}-7

*hydroxy-2H-chromen-2-one (**21**).* Compound **21** was synthesized according to the general procedure using compound **1a** (150 mg, 0.69 mmol) and **2b** (132 mg, 0.58 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 100 :1) compound **21** (207 mg, 84%, 209−211 °C) was isolated as white crystals. ^{1}H-NMR (300 MHz, DMSO) δ 10.71 (1H, s, OH), 8.28 (1H, t, *J* = 5.7 Hz, NH), 8.05 (1H, s, H-3'), 7.79 (2H, d, *J* = 8.6 Hz, H-9'), 7.69 (1H, d, *J* = 8.8 Hz, H-5), 7.63 (2H, d, *J* = 8.6 Hz, H-10'), 6.84 (1H, dd, *J* = 8.7, 2.3 Hz, H-6), 6.77 (1H, d, *J* = 2.3 Hz, H-8), 5.85 (2H, s, H-1'), 5.49 (1H, s, H-3), 4.11 (2H, d, *J* = 5.6 Hz, H-5') ppm. ^{13}C-NMR (75 MHz, DMSO) δ 162.10 (C-2), 160.44 (C-7), 155.53 (C-4), 151.06 (C-8a), 144.21 (C-4'), 139.71 (C-11'), 137.77 (C-8'), 129.73 (C-10'), 128.96 (C-9'), 126.55 (C-5), 125.01 (C-3'), 113.65 (C-6), 109.80 (C-4a), 109.48 (C-3), 102.99 (C-8), 49.52 (C-1'), 38.41 (C-5') ppm. MS (ESI): *m*/*z* = 523.1 ([*M* + H]$^+$). Anal. calcd. for $C_{25}H_{19}ClN_4O_5S$: C, 57.42; H, 3.66; N, 10.71. Found: C, 57.58; H, 3.65; N, 10.74.

*4.2.3.19. {4-[4-(4-Fluorobenzenesulfonamide)methyl-1H-1,2,3-triazol-1-yl]methyl}-7 hydroxy-2H-chromen-2-one (**22**).* Compound **22** was synthesized according to the general procedure using compound **1a** (50 mg, 0.23 mmol) and **2c** (40.9 mg, 0.38 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 50 : 1) oily compound **22** (12 mg, 14%) was isolated. ^{1}H-NMR (300 MHz, DMSO) δ 10.68 (1H, s, OH), 8.46 (1H, s, NH), 7.97 (1H, t, *J* = 8.4 Hz, H-3'), 7.67 (1H, d, *J* = 8.7 Hz, H-5), 7.50 (2H, d, *J* = 8.7, 2.6 Hz, H-9'), 7.33 (2H, td, *J* = 8.5, 2.5 Hz, H-10'), 6.82 (1H, dd, *J* = 8.8, 2.2 Hz, H-6), 6.75 (1H, d, *J* = 2.2 Hz, H-8), 5.81 (2H, s, H-1'), 5.48 (1H, s, H-3), 4.19 (2H, s, H-5') ppm. ^{13}C-NMR (151 MHz, DMSO) δ 163.59 (d, J_{C-F} = 253.7 Hz, H-11'), 161.61 (C-2), 159.94 (C-7), 155.04 (C-4), 150.58 (C-8a), 143.75 (C-4'), 134.67 (d, J_{C-F} = 3.5 Hz, H-8'), 132.75 (d, J_{C-F} = 10.6 Hz, H-9'), 126.07 (C-5), 124.38 (C-3'), 118.91 (d, J_{C-F} = 25.7 Hz, H-10'), 114.61 (d, J_{C-F} = 22.7 Hz, H-10''), 113.15 (C-6), 109.31 (C-4a), 108.95 (C-3), 102.48 (C-8), 48.93 (C-1'), 37.64 (C-5') ppm. MS (ESI): *m*/*z* 507.1 = ([*M* + H]$^+$). Anal. calcd. for $C_{25}H_{19}FN_4O_5S$: C, 59.28; H, 3.78; N, 11.06. Found: C, 59.11; H, 3.79; N, 12.00.

*4.2.3.20. {4-[4-(2-Chloro-4-fluorobenzensulfonamide)methyl-1H-1,2,3-triazol-1-yl]methyl}-7 hydroxy-2H-chromen-2-one (**23**).* Compound **23** was synthesized according to the general procedure using compound **1a** (100 mg, 0.46 mmol) and **2d** (94.9 mg, 0.38 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 50 : 1) compound **23** (11 mg, 6%, m.p. = 152−154 °C) was isolated as white crystals. ^{1}H-NMR (600 MHz, DMSO) δ 10.70 (1H, s, OH), 8.48 (1H, s, NH), 8.00–7.97 (2H, m, H-3', H-10'), 7.69 (1H, d, *J* = 8.8 Hz, H-5),

7.52 (1H, dd, *J* = 8.7, 2.5 Hz, H-13'), 7.34 (1H, td, *J* = 8.7 Hz, *J*= 2.5 Hz, H-12'), 6.84 (1H, dd, *J* = 8.7, 2.4 Hz, H-6), 6.77 (1H, d, *J* = 2.3 Hz, H-8), 5.83 (2H, s, H-1'), 5.50 (1H, s, H-3), 4.20 (2H, s, H-5') ppm. ^{13}C-NMR (151 MHz, DMSO) δ 163.55 (d, J_{C-F} = 264.7 Hz, H-11'), 161.63 (C-2), 159.90 (C-7), 155.16 (C-4), 150.54 (C-8a), 143.78 (C-4'), 134.77 (C-8'), 134.58 (C-9'), 132.70 (C-13'), 126.06 (C-5), 124.36 (C-3'), 118.93 (d, J_{C-F} = 25.7 Hz, H-10'), 114.60 (d, J_{C-F} = 22.7 Hz, H-12'), 113.14 (C-6), 109.33 (C-4a), 109.00 (C-3), 102.50 (C-8), 48.94 (C-1'), 37.69 (C-5') ppm. MS (ESI): *m*/*z* = 541.1 ([*M* + H]$^+$). Anal. calcd. for $C_{25}H_{18}ClFN_4O_5S$: C, 55.51; H, 3.35; N, 10.36. Found: C, 55.63; H, 3.34; N, 10.34.

*4.2.3.21. 7-Hydroxy-{4-[4-(4-nitrobenzensulfonamide)methyl-1H-1,2,3-triazol-1-yl]methyl}-2H-chromen-2-one (**24**).* Compound **14** was synthesized according to the general procedure using compound **1a** (100 mg, 0.46 mmol) and **2e** (92.2 mg, 0.38 mmol). After purification by column chromatography (CH_2Cl_2 : CH_3OH = 50 : 1) oily compound **24** (115 mg, 77%) was isolated. ^{1}H-NMR (300 MHz, DMSO) δ 10.73 (1H, s, OH), 8.58 (1H, s, NH), 8.36 (2H, d, *J* = 8.8 Hz, H-9'), 8.09–7.96 (3H, m, H-3', H-10'), 7.67 (1H, d, *J* = 8.7 Hz, H-5), 6.82 (1H, dd, *J* = 8.7, 2.2 Hz, H-6), 6.75 (1H, d, *J* = 2.2 Hz, H-8), 5.84 (2H, s, H-1'), 5.40 (1H, s, H-3), 4.18 (2H, s, H-5') ppm. ^{13}C-NMR (75 MHz, DMSO) δ 162.11 (C-2), 161.87 (C-7), 155.77 (C-4), 152.32 (C-11'), 151.12 (C-8a), 144.01 (C-4'), 137.83 (C-8'), 128.57 (C-9'), 126.52 (C-5), 125.07 (C-3'), 124.92 (C-10'), 113.62 (C-6), 109.76 (C-4a), 109.29 (C-3), 102.98 (C-8), 49.50 (C-1'), 38.37 (C-5') ppm. MS (ESI): *m*/*z* = 534.2 ([*M* + H]$^+$). Anal. calcd. for $C_{25}H_{19}N_5O_7S$: C, 56.28; H, 3.59; N, 13.13. Found: C, 56.16; H, 3.60; N, 13.00.

*4.2.3.22. {1-[(7-Hydroxy-2H-chromen-2-one-4-yl)methyl]-1H-1,2,3-triazol-4-yl} methylthio)carbonothioyl)morpholine (**25**).* Compound **25** was synthesized according to the general procedure using compound **1a** (91.9 mg, 0.42 mmol) and **3a** (70 mg, 0.35 mmol). The residue was purified by column chromatography (CH_2Cl_2 : CH_3OH = 50 : 1) to afford **25** (90 mg, 62%, m.p. = 241–243 °C) as light yellow powder. ^{1}H-NMR (600 MHz, DMSO) δ: 10.68 (1H, s, OH), 8.23 (1H, s, H-3'), 7.69 (1H, d, *J* = 8.8 Hz, H-5), 6.91–6.68 (2H, m, H-6, H-8), 5.87 (2H, s, H-1'), 5.54 (1H, s, H-3), 4.63 (2H, s, H-5'), 4.22 (2H, s, H-9'), 3.90 (2H, s, H-9'), 3.66 (4H, s, H-10'). ^{13}C-NMR (75 MHz, DMSO): 194.92 (C-7'), 162.13 (C-2), 160.40 (C-7), 155.56 (C-4), 151.03 (C-8a), 143.17 (C-4'), 126.55 (C-5), 125.37 (C-3'), 113.65 (C-6), 109.83 (C-4a), 109.64 (C-3), 102.99 (C-8), 66.03 (C-9'), 55.56 (C-10'), 49.60 (C-1'), 31.65 (C-5'). MS (ESI): *m*/*z* = 495.1 ([*M* + H]$^+$). Anal. calcd. for $C_{24}H_{22}N_4O_4S_2$: C, 58.28; H, 4.48; N, 11.33. Found: C, 58.41; H, 4.47; N, 11.31.

*4.2.4. {1-[(7-Hydroxy-2H-chromen-2-one-4-yl)methyl]-1H-1,2,3-triazol-4-yl} methylthio)carbonothioyl)piperazine (**27**).* To a solution of compound **26** (300 mg, 1.15 mmol) in CH_2Cl_2 (6 mL) cooled to 0 °C trifluoroacetic acid (0.9 mL) was added. Reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated *in vacuo* and residue was dissolved in CH_2Cl_2 (80 mL) and extracted with water (80 mL) and saturated solution of $NaHCO_3$ (80 mL). The water layer was filtered and white crystaline compound **27** was isolated (198 mg, 41%, m.p. = 253–255 °C) from supernatant. ^{1}H-NMR (300 MHz, DMSO) δ 10.62 (1H, s, OH), 8.22 (1H, s, H-3'), 7.68 (1H, d, *J* = 8.8 Hz, H-5), 6.83 (1H, dd, *J* = 8.7, 2.3 Hz, H-6), 6.76 (1H, d, *J* = 2.3 Hz, H-8), 5.87 (1H, s, H-1'), 5.54 (1H, s, H-3), 4.61 (2H, s, H-5'), 4.16–3.87 (4H, m, H-9'), 3.56–3.19 (4H, m, H-10'), 2.80 (1H, t, *J* = 4.8 Hz, NH) ppm. ^{13}C-NMR (151 MHz, DMSO) δ 193.50 (C-7'), 161.63 (C-2), 159.90 (C-7), 155.06 (C-4), 150.52 (C-8a), 142.76 (C-4'), 126.04 (C-5), 124.84 (C-3'), 113.15 (C-6), 109.34 (C-4a), 109.16 (C-3), 102.50 (C-8), 49.10 (C-1'), 31.22 (C-5') ppm. MS (ESI): *m*/*z* = 494.1 ([*M* + H]$^+$). Anal. calcd. For $C_{24}H_{23}N_5O_3S_2$: C, 58.40; H, 4.70; N, 14.19. Found: C, 58.27; H, 4.71; N, 14.22.

4.3. In silico methods

The principle component analyses (PCA) and partial least square (PLS) analyses for IC_{50} values determined for five cancer cell lines and fibroblasts, have been carried out by VolSurf+ in terms of its molecular field descriptors [53]. PCA analysis taking into account also the measured pIC_{50} values has been performed by Multibase 2015 (http://www.numericaldynamics.com/). Classification models were built by using decision tree algorithm See5, release 2.07 [63].

4.4. Biological Assay

4.4.1. Cell culturing

Lung adenocarcinoma (A549), hepatocellular carcinoma (HepG2), ductal pancreatic adenocarcinoma (CFPAC-1), cervical carcinoma (HeLa) and colorectal adenocarcinoma, metastatic (SW620) cell lines, as well as normal human lung fibroblast (WI38) and mouse embryonic fibroblast (3T3) were cultured as monolayers and maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamine, 100 U/ml penicillin and 100 µg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C.

4.4.2. Proliferation assays

The panel cell lines were inoculated onto a series of standard 96-well microtiter plates on day 0, at 5000 cells per well according to the doubling times of specific cell line. Test agents were then added in five, 10-fold dilutions (0.01 to 100 µM) and incubated for further 72 h. Working dilutions were freshly prepared on the day of testing in the growth medium. The solvent (DMSO) was also tested for eventual inhibitory activity by adjusting its concentration to be the same as in the working concentrations (DMSO concentration never exceeded 0.1%). After 72 h of incubation, the cell growth rate was evaluated by performing the MTT assay: experimentally determined absorbance values were transformed into a cell percentage growth (PG) using the formulas proposed by NIH. This method directly relies on control cells behaving normally at the day of assay because it compares the growth of treated cells with the growth of untreated cells in control wells on the same plate – the results are therefore a percentile difference from the calculated expected value. The IC_{50} and LC_{50} values for each compound were calculated from dose-response curves using linear regression analysis by fitting the mean test concentrations that give PG values above and below the reference value. If, however, all of the tested concentrations produce PGs exceeding the respective reference level of effect (*e.g.* PG value of 50) for a given cell line, the highest tested concentration is assigned as the default value (in the screening data report that default value is preceded by a ">" sign). Each test point was performed in quadruplicate in three individual experiments. The results were statistically analyzed (ANOVA, Tukey post-hoc test at $p < 0.05$). Finally, the effects of the tested substances were evaluated by plotting the mean percentage growth for each cell type in comparison to control on dose response graphs.

4.4.3. Apoptosis detection

Detection and quantification of apoptosis and differentiation from necrosis at single cell level was carried out by Annexin-V-FITC Staining kit (Santa Cruz Biotech) according to the manufacturer's instructions. Briefly, cells were seeded into Lab-tek II Chamber Slide with 8 wells and treated with test compounds at their 2xIC50 concentrations for 48 h. The cells were washed with Incubation buffer, and Annexin-V-FITC labelling solution was added. After incubation at room temperature for 15 minutes, chambers and silicon borders of the chamber slides were removed, the cells were fixed with 20% glycerol and analyzed by fluorescence microscopy.

4.4.4. Western blot analysis

Cells were cultured in 6-well plates at seeding density of 200000 cells/well and subjected to treatment with selected compounds at their 2 x IC_{50} concentrations for 48 h. Cells were lysed in RIPA buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na2 EDTA, 1 mM EGTA, 1% NP-40 and 1% sodium deoxycholate supplemented with protease inhibitor cocktail (Roche). Total proteins (50 µg) were resolved on 12% Tris-glycine polyacrylamide gels and transferred to PVDF membranes. Subsequently, membranes were blocked for 1 h at room temperature with 4% BSA in TBST [50 mmol/L Tris base, 150 mmol/L NaCl, 0.1% Tween 20 (pH 7.5)] and probed overnight at 4 °C with primary antibody against either p-SK1 (ECM Biosciences), ASAH1 (Abcam) or 5-lipoxygenase (Santa Cruz Biotech). Membranes were washed with TBST and incubated with either goat anti-mouse (Santa Cruz Biotechnology) or goat anti-rabbit (Santa Cruz Biotechnology) horseradish peroxidase–conjugated secondary antibody at room temperature for 1 h. Individual proteins were visualized by the BM Chemiluminescence Western Blotting Substrate (POD) (Roche) using ImageQuant LAS 500 (GE Healthcare). Densitometry quantitation was determined using the Quantity One 1-D Analysis Software (Bio-Rad, USA).

4.4.5. Antibacterial activity assay

The prepared compounds were tested for their *in vitro* antibacterial activity against Gram-positive bacteria: *S. aureus* (ATCC 25923), *E. faecalis,* vancomycin-resistant *E. faecium* (VRE), and Gram-negative bacteria: *E. coli* (ATCC 25925), *P. aeruginosa* (ATCC 27853), *A. baumannii* (ATCC 19606) and ESBL-producing *K. pneumoniae* (ATCC 27736). This procedure was maintained according to the standard broth microdilution method as recommended in guidelines of Clinical and Laboratory Standards Institute [64,65] 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 Labware, New Jersey, 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 256–0.25 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 x 10^5 CFU/mL, as described (CLSI M7-A7). After inoculation of previously prepared microdilution trays with tested compounds, trays were incubated at 35 ± 2 ºC overnight (16 to 20 h) in an ambient air incubator. *Escherichia coli* ATCC 25922 and *Pseudomonas*

aeruginosa ATCC 27853 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). For the compounds were MIC "trailing" phenomena have been observed [66], such as a very light haze or small buttons (<2 mm) of growth in several successive wells, this faint growth was ignored and the end point was red at the concentration in which there is ≥ 80% reduction in growth as compared to the control. All testings were done in triplicate.

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Supplementary data

Supplementary data associated with this article can be found at http://....

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Research highlights

- 1,2,3-triazole tethered coumarins were synthesized by eco-friendly click chemistry.
- 7-Methylcoumarin–1,2,3-triazole–2-methylbenzimidazole hybrid **33** exhibited IC_{50} in nM range against HepG2 cells.
- **33** induced cell death, mainly due to early apoptosis.
- **33** abrogated 5-lipoxygenase (5-LO) and acid ceramidase (ASAH) expression.
- **17**, **23** and **27** exhibited selective potency against *Enterococcus* species.