Synthesis of novel 3-substituted coumarin derivatives by transition metal-catalyzed reactions and their antimicrobial and antitumoral evaluations

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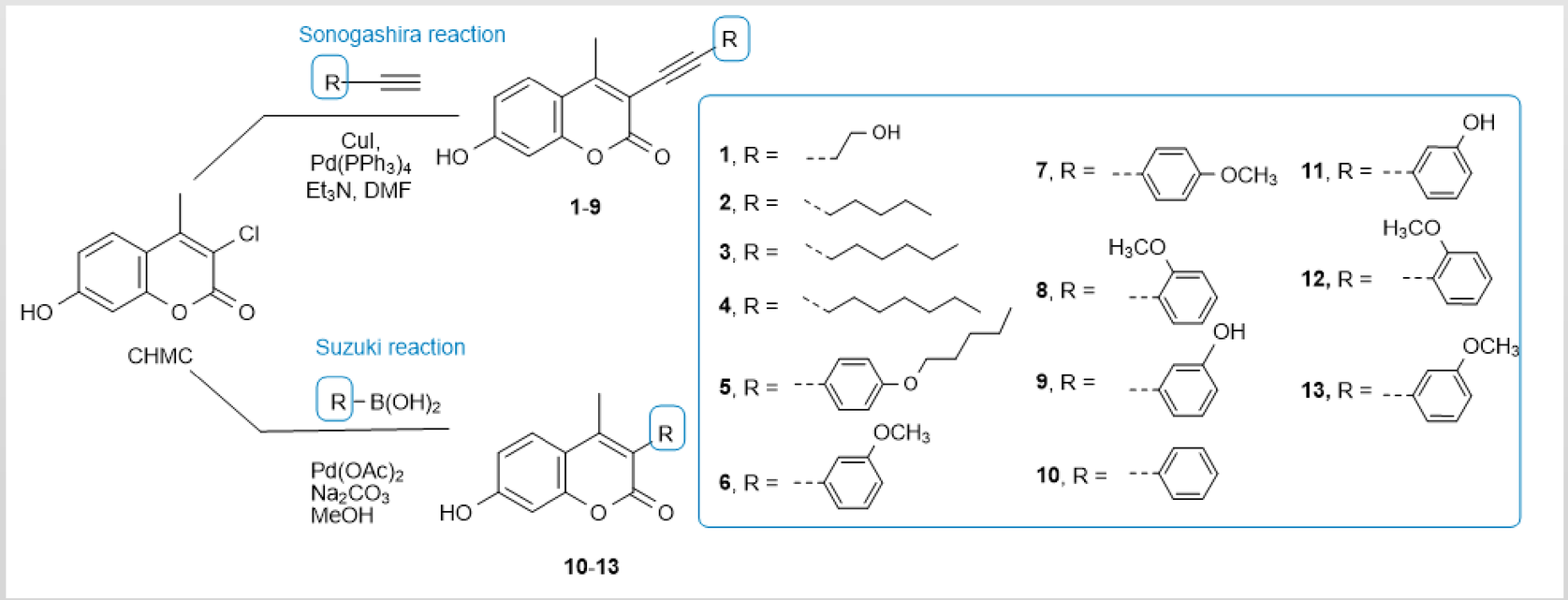
Introduction

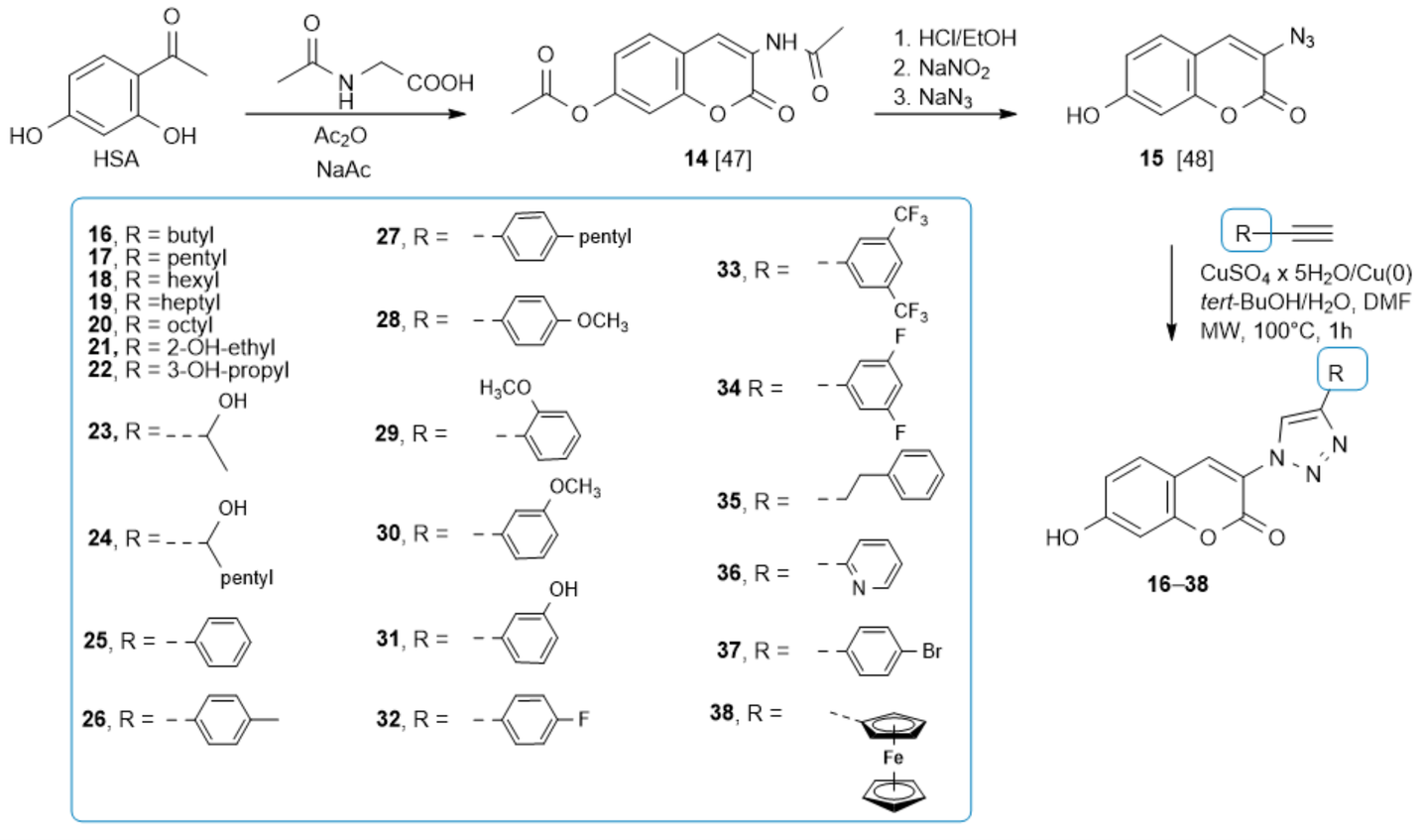
Resistance to currently available antitumour [1] and antimicrobial drugs presents a serious healthcare issue and calls for an intensive search for new, more efficient, and selective therapeutic agents. The development of new antibiotics is not keeping pace with the rapid evolution of resistance to almost all clinically available drugs. [2] Coumarin has an important place in medicinal chemistry, as molecules incorporating the coumarin scaffold show a broad spectrum of pharmacological activities, including anticancer, antimicrobial, antiviral, antioxidant, anticoagulant, anti-inflammatory, and anti-enzymatic. [3] In our previous study, we prepared a number of conformationally unrestricted 4-(1,2,3-triazolyl)coumarin derivatives with alkyl, phenyl, and heterocyclic moieties attached at the C-4 position of 1,2,3-triazole, which exhibited anticancer and antibacterial activity *in vitro*. [4,5] In this work, we present synthesis of new coumarin-based compounds by using transition metal-catalysed reactions and evaluation of their antibacterial and antitumor potential.

Chemistry

Novel compounds (**1**-**13** and **16**-**38**) incorporating the coumarin nucleus were prepared using transition metal-catalysed reactions and following procedures illustrated in Schemes 1 and 2. To obtain compounds **1–9**, alkynyl substituents were introduced at the position 3 of the 7-hydroxy-4-methyl coumarin core *via* Sonogashira cross-coupling reaction between 3-chloro-7-hydroxy-4-methylcoumarin (CHMC) and the corresponding alkyl- or aryl-terminal alkynes in dimethylformamide (DMF) using $Pd(PPh_3)_4$), Et_3N and CuI (Scheme 1). Coumarin derivatives **10-13**, having a phenyl moiety with various substitution patterns at the position 3 of the coumarin, were prepared using the Suzuki-Miyaura cross-coupling reaction of CHMC with appropriate arylboronic acids in the presence of $Pd(OAc)_2$. Coumarin derivatives **16-38** incorporating 4-alkyl-, 4-aryl-, and 4-ferrocenyl-1,2,3-triazole ring at the position 3 of the coumarin moiety were synthesised by the microwave-assisted "click" copper-catalysed azide-alkyne cycloaddition (CuAAC) reaction of the 3-azido derivative **15** with corresponding terminal alkynes (Scheme 2) in a mixture of organic solvent and water. As a source of catalytic copper(I), elemental copper in the presence of 1 M copper sulphate ($CuSO_4$ x 5 H_2O) was used.



Scheme 1. Synthesis of alkynyl (**1**-**4**) and arylethynyl (**5**-**9**) coumarin derivatives through the Sonogashira coupling reaction and of 3-phenylcoumarin derivatives **10**-**13** through the Suzuki-Miyaura cross-coupling reaction



Scheme 2. Synthesis of 7-hydroxy-3-(1,2,3-triazolyl)coumarin derivatives **16**-**38** using CuAAC

Conclusions

The obtained results of antiproliferative activity of newly synthesized hybrids of 1,2,3-triazolyl-coumarin indicate that the activity depends on the type of substituent at position C-4 of the 1,2,3-triazole ring. In general, *m*-substituted electron-donating groups at phenyl ring in 30 and 31 improved growth inhibition, while unsubstituted, *p*- and *o*-substituted aryl caused the lack of activity. Compound 33 with electron-withdrawing trifluoromethyl substituent at positions 3 and 5 of the phenyl ring was the most promising, with strong and selective activity against HeLa cells (IC_{50}=9.9 μM), which was superior to the activity of the reference drug 5-fluorouracil (IC_{50}=18 μM).

Results of antibacterial evaluation showed that 7-hydroxycoumarin **10** with unsubstituted phenyl ring directly attached at C-3 position of coumarin was active against Gram-positive bacteria, *Staphylococcus* and *Enterococcus* species in particular, and more importantly, this compound was active against clinically isolated methicillin-resistant *S. aureus* and vancomycin-resistant *E. faecium.* Although promising, it was not active as reference drugs gentamicin and ampicillin. Compound **10** owes this activity to the phenyl group at C-4 position of the 7-hydroxy-4-methylcoumarin, since other tested compounds had MICs higher than 128 μg/mL against all tested bacterial strains.

References

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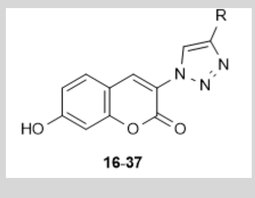
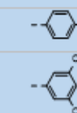
Antiproliferative activity

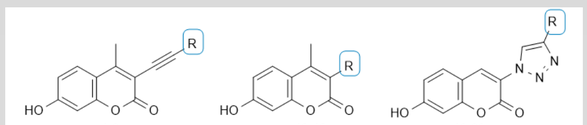
Table 1. Antiproliferative activity of compounds against two human tumour cell lines T-cell leukemia cells (CEM) and cervical carcinoma cells (HeLa) and human dermal microvascular endothelial cell line (HMEC 1)and their clogP[a] values



Compound	R	IC_{50}[b] (μM) CEM	HeLa	HMEC-1	clogP[a]
16	butyl	31±7	49±2	57±6	3.13
17	pentyl	71±41	26±6	58±6	3.65
21	2-OH-ethyl	>100	>100	>100	0.23
22	3-OH-propyl	>100	>100	>100	0.61
23		>100	>100	>100	0.54
25		>100	>100	>100	3.37
26		>100	>100	>100	3.87
28		>100	>100	>100	3.30
29		>100	94±10	97±4	2.74
30		72±40	26±1	64±1	3.30
31		>100	26±1	>100	2.73
32		≥100	85±25	80±12	3.51
33		59±21	9.9±1.7	67±8	5.14
34		>100	>100	>100	3.66
35		>100	>100	>100	3.49
36		>100	>100	81±4	2.09
37		30±17	40±31	80±2	4.23
5-fluorouracil		0.18±0.02	18±5	-	

Antimicrobial activity

Table 2. Antibacterial activity of compounds against gram-positive (*Staphylococcus aureus,* MRSA (methicillin-resistant *Staphylococcus aureus*), *Enterococcus faecalis* and VRE (vancomycin-resistant) *Enterococcus faecium*) and gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli* ESBL (extended-spectrum *b* -lactamases), *Klebsiella pneumoniae* ESBL and *Acinetobacter baumanii*.



Compd.	MIC (μg/mL)[e] Gram-positive bacteria: S. aureus[b]	MRSA[c]	E.faecalis[d]	E.faecium VRE[f]	Gram-negative bacteria: E. coli[g]	K. pneumoniae[h]	P. aeruginosa[i]	A. baumannii[j]	E. coli ESBL[k]	K. pneumoniae ESB[l]	cLogP[m]
1	>128	>128	>128	128	>128	>128	>128	>128	>128	>128	1.46
2	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	5.03
3	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	5.57
4	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	6.10
5	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	6.80
10	16	16	32	32	>128	>128	>128	>128	>128	>128	3.92
11	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	3.27
16	>128	128	>128	>128	>128	>128	>128	>128	>128	>128	3.13
17	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	3.65
21	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	0.23
22	>128	128	>128	>128	>128	>128	>128	>128	>128	>128	0.61
23	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	0.54
25	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	3.37
26	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	3.87
28	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	3.30
29	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	2.74
30	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	3.30
31	>128	128	>128	128	>128	>128	>128	>128	>128	>128	2.73
32	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	3.51
33	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	5.14
34	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	3.66
35	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	3.49
36	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	2.09
37	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	4.23
38	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	3.01
Gentamicin	0.125	0.25	8	0.25							
Ampicillin	1	4	2	1							

[a] MIC (minimum inhibitory concentration); [b] *Staphylococcus aureus* ATCC 25923; [c] MRSA (methicillin-resistant *Staphylococcus aureus*) 11710; [d] *Enterococcus faecalis* ATCC; [e] MRSA 13276; [f] VRE (vancomycin-resistant) *Enterococcus faecium* MKB 3019; [g] Escherichia coli ATCC 2592; [h] *Klebsiella pneumoniae* ATCC 700603; [i] *Pseudomonas aeruginosa* ATCC 27853; [j] *Acinetobacter. baumannii* ATCC 1960; [k] ESBL (extended-spectrum *b* -lactamases (resistant to most *b*-lactam antibiotics)) *Escherichia coli* 26001; [l] ESBL *Klebsiella pneumoniae* 9350; [m] *Acinetobacter baumanii* 9768; [n] clogP (calculated logP values) were obtained by using ChemDraw Professional 15.