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Fragment-type 4-azolylcoumarin derivatives with anticancer properties

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Abstract

Several coumarin derivatives with a directly attached azole substituent at C-4 were synthesized and biologically studied for their anticancer properties. The cell lines used for this investigation (HeLa, K-562, MDA-MB-53, and MCF-7) demonstrated different sensitivities. The best response in the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay was shown by K-562 cells, with compounds displaying activity (**3c**, IC_{50} 3.06 μM; **4a**, IC_{50} 5.24 μM; **4c**, IC_{50} 4.7 μM) similar to that of cisplatin (IC_{50} ~6 μM), which was used as the standard. The studied azole-substituted coumarins demonstrated weaker activity toward other cell lines, except for compound **4c**, which was equally potent in the case of MCF-7 cells. Additional biological evaluations supported interference with the cell cycle as a potential mechanism of action and confirmed the absence of toxicity in zebrafish embryos. On the basis of these initial results, 4-azole coumarins should be explored further. Although their activity would need additional optimization, the fact that these compounds are fragment-like structures with MW <300 and clog *P* <3 offers enough flexibility to fine-tune their drug-like properties.

KEYWORDS
anticancer properties, azoles, cell lines, coumarins, embryotoxicity

1 | INTRODUCTION

Among the secondary metabolites produced by plants or other organisms, coumarins are arguably one of the most important classes of heterocyclic compounds.[1,2] The coumarin core can be considered a useful structure for both natural products and medicinally important biologically active compounds.[3] As such, coumarins have been intensively studied in recent decades and numerous classes of these compounds have been synthesized and biologically explored, yielding a range of activities such as anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, and anticancer.[4–10] Apart from being important medicinal leads, coumarins have been widely investigated as fluorescence probes as well.[11–14]

Our recent studies of azole-substituted isocoumarins identified interesting antimicrobial properties of these compounds.[15,16] We also showed that some of these derivatives also possessed potent cytotoxic properties. This prompted our work on easily accessible isosteric 4-azole coumarins with the aim of exploring the effect of azole substituents on their anticancer potential. Surprisingly, 4-azole-substituted coumarins were scarcely studied as medicinal compounds.[17]

Anticancer properties of various coumarin derivatives were comprehensively examined and it is clear that they can

inhibit the growth, proliferation, and metastasis of cancer cells (Figure 1).[18–30]

These effects are exerted via various mechanisms including inhibition of microtubule polymerization, angiogenesis, carbonic anhydrase, the PI3K/AKT/mTOR signaling pathway, acting on apoptosis proteins, or inhibiting tumor multidrug resistance. However, the rich medicinal chemistry of coumarins is yet to create a marketed anticancer drug, and the search for biologically efficient compounds of this type is still a challenging task for medicinal chemists.[31]

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

In recent years, the anticancer properties of coumarin derivatives have been intensively investigated. Numerous compounds with different substitution patterns were synthesized and biologically investigated, and these efforts resulted in the accumulation of a large amount of data in this area. The 4-substituted derivatives seem to be particularly interesting as they act via various mechanisms.[32] Our interest in these derivatives, particularly 4-azole-substituted coumarins, was indirectly generated by results obtained on the isocoumarin series.[15,16] Interestingly, coumarins of this type are rare in the literature, but very few reports suggested that they might provide interesting leads in the search for novel anticancer agents (Figure 2).[33,34]

We synthesized a series of 4-azolyl derivatives and briefly investigated their biological properties.[35] These compounds are easily accessible from coumarin **1** in several straightforward steps as outlined in Scheme 1. Nucleophilic displacement of bromine in derivative **2** was performed in refluxing MeCN and in the presence of a weak base affording product **3** in acceptable yields, while thio-derivative **4** was prepared using Lawesson's reagent. All synthesized derivatives are outlined in Figure 3.

2.2 | Biological studies

2.2.1 | Cytotoxicity

The anticancer properties of these compounds were studied on four cell lines: cervical cancer cells HeLa, myelogenous leukemia cells K-562, and breast cancer cells MDA-MB-453 and MCF-7. The effect of synthesized derivatives on healthy fibroblast MRC-5 cells was also investigated. For this study, the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay was used, which is based on the spectrophotometric determination of formazan produced in the reductive transformation of MTT by a cellular oxidoreductase.[36,37] The result is in direct correlation to the number of metabolically active cells.

In addition to biological profiling, we also determined selected basic molecular properties for the synthesized compounds such as MW and log *P* with the aim of retaining these within the fragment-like properties (Ro3), providing enough flexibility for potential further structural modification.[38] The majority of drugs are weak acids or bases, and some of their properties are related to the ionization state.[39] Although we did not assume that there would be a significant effect of ionization of our compounds on their performance at this stage, pK_a values were calculated and used as a measure of electron-donating ability. Under near-neutral conditions, our compounds were not expected to ionize, but pK_a would be important in any broader structure–activity relationship optimization.

In our experiments, HeLa cells showed low sensitivity toward the azole-derived coumarins (Table 1). The most active compounds **3c** (IC_{50} 20.3 μM) demonstrated significantly lower activity than cisplatin (IC_{50} ~7 μM) used in these experiments as the standard.

The myelogenous leukemia cells K-562 showed different results. Several compounds showed anticancer activity against this cell line, with IC_{50} values slightly below (Table 1, **3c**, IC_{50} 3.06 μM; **4a**, IC_{50} 5.24 μM; **4c**, IC_{50} 4.7 μM) or slightly above (**4b**, IC_{50} 10 μM) that of cisplatin (IC_{50} ~6 μM). The most active compound was the chloropyrazole derivative **3c**, which also showed 12-fold K-562/MRC-5 selectivity. Interestingly, small changes in the structure of the chloropyrazole moiety had a strong impact on the activity against K-562. Replacement of chlorine with bromine (Table 1, **3e**, IC_{50} 167 μM) or iodine (Table 1, **3j**, IC_{50} >200 μM) resulted in almost complete loss of activity, while removal of chlorine (Table 1, **3b**, IC_{50} 112.5 μM) also caused a decrease in potency, but to a lesser extent. In the imidazole series, the most active compound is the ester derivative (Table 1, **3f**, IC_{50} 22 μM). Removal of this substituent creates the parent compound (Table 1, **3d**, IC_{50} 44 μM), which showed lower activity, a trend observed for the pyrazole series, but with a less drastic effect. Introduction of the larger benzimidazole substituent (Table 1, **3h**, IC_{50} 66 μM) decreased potency, while the presence of two chlorines on

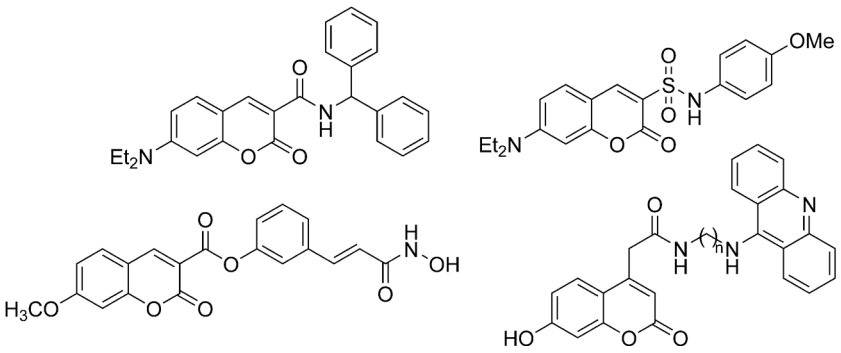
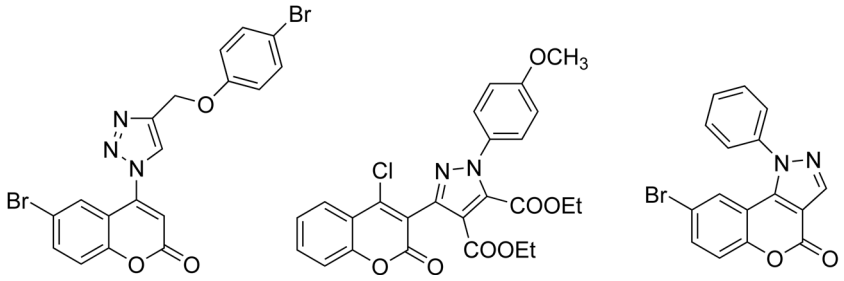
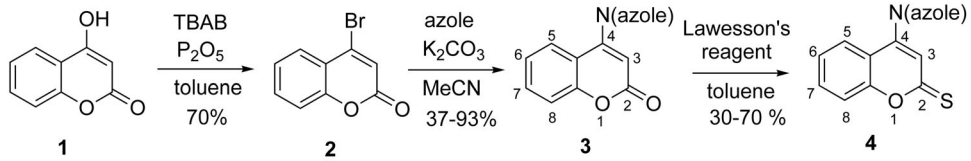


FIGURE 1 Examples of anticancer coumarins

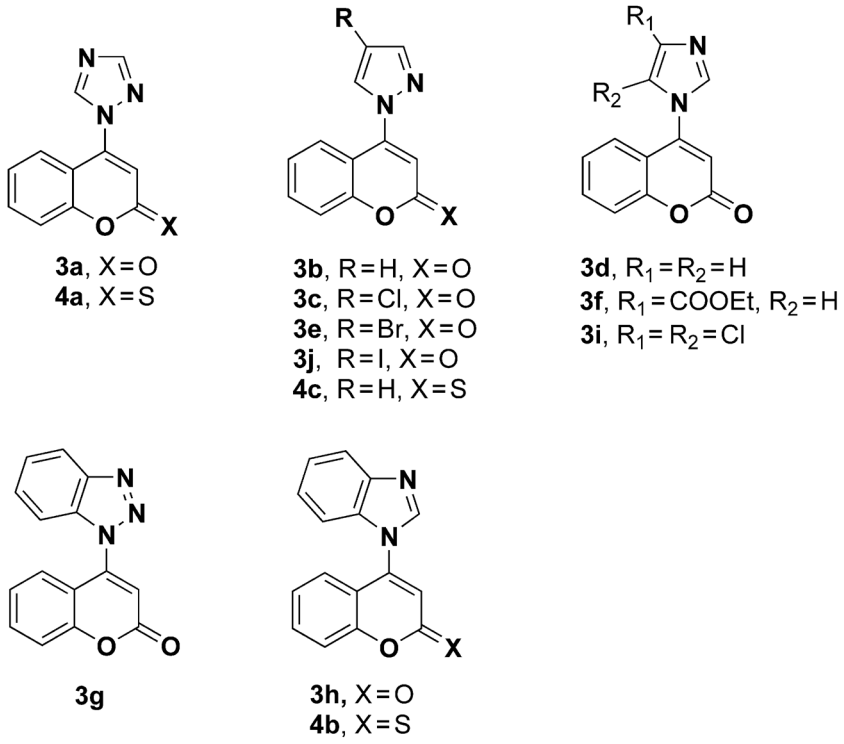
FIGURE 2 Some azole-derived coumarins





SCHEME 1 Synthesis of 4-azolylcoumarins

FIGURE 3 Structures of the synthesized 4-azolylcoumarins



the imidazole ring (Table 1, **3i**, IC_{50} >200 μM) proved to be detrimental to the biological properties. Based on compounds from the pyrazole and the imidazole series, it is evident that the substituent at the heterocyclic ring is very important, but further study is necessary to establish its precise role. The comparison of compounds with the parent azoles as coumarin substituents at the C(4), exemplified by **3a**, **3b**, and **3d**, is very interesting. All these compounds in K562 cell line experiments were less active than the most potent derivative **3c.** However, within the parent compounds, the activity trend was somehow unexpected. Namely, the pyrazole derivative was the least active (Table 1, **3b**, IC_{50} 112.5 μM), while triazole (Table 1, **3a**, IC_{50} 73 μM) and, in particular, imidazole (Table 1, **3d**, IC_{50} 44 μM) showed better potency. The observed results do not seem to correlate to the calculated log *P* or pK_a and may suggest the involvement of the ring nitrogens in binding as an electron donor (e.g., H-bond acceptor). At least in the parent series, position 3 for nitrogen in the heterocyclic substituent and its stronger electron-donor ability seemed to promote better potency and this is perhaps further supported by the activity observed for **3g** (Table 1, IC_{50} 59 μM) and **3h** (Table 1, IC_{50} 66 μM) in comparison with **3b** (Table 1, IC_{50} 112.5 μM). Replacement of the carbonyl with the thiocarbonyl moiety was found to have a strong impact on the activity. Thus, thio-derivative **4a** (Table 1, IC_{50} 5.24 μM) showed better potency than the corresponding O-analogue **3a** (Table 1, IC_{50} 73 μM). The same trend was observed for

TABLE 1 IC_{50} values of 4-azolylcoumarins against cancer cell lines and healthy cells

Compound	IC_{50} (μM)					log *P*	pK_a	MW
	HeLa	K562	MDAMB453	MCF-7	MRC5			
3a	82.8	73	100	129.2	119.7	0.73	0.41	213
3b	154.1	112.5	156.7	197.2	95.6	1.42	1.6	212
3c	20.3	3.06	30.2	22.1	36.6	2.02	0.64	247
3d	95	44	>200	>200	192	1.1	4.04	212
3e	>200	167	>200	194	146	2.19	0.84	290
3f	50	22	126	121	157	1.64	1.23	284
3g	103	59	170	111	84	2.44	0.21	263
3h	68	66	143	198	156	2.5	2.89	262
3i	60	>200	32	>200	>200	2.24	0.41	280
3j	>200	>200	>200	>200	>200	2.35	0.88	338
4a	30.4	5.24	49.4	39.2	43.2	1.62	0.4	229
4b	23	10	99	20	18	3.39	2.9	278
4c	35	4.7	80	6.2	5	2.31	1.61	228
Cisplatin	7	6	6.7	5.7	–	–	–	–

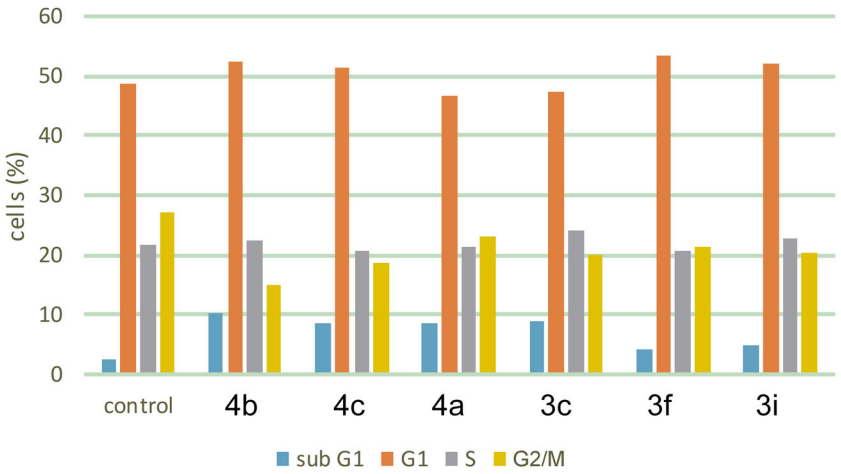


FIGURE 4 Cell cycle distribution of HeLa cells after 24 h of continuous action of the investigated agents

compounds **4b** (Table 1, IC_{50} 10 μM) and **4c** (Table 1, IC_{50} 4.7 μM) compared to compounds **3h** (Table 1, IC_{50} 66 μM) and **3b** (Table 1, IC_{50} 112.5 μM). Unfortunately, potency toward healthy MRC-5 cell lines also increased, hence downgrading the K-562/MRC-5 selectivity.

Regarding the MDA-MB-453 cell line, compared to cisplatin as the standard, a weak activity is generally observed, with most active compounds being chloro derivative **3c** (IC_{50} 30.2 μM) and dichloro **3i** (IC_{50} 32 μM). The dichloro derivative showed better MDA-MB-453/MRC-5 selectivity.

Finally, profiling all compounds against the MCF-7 cell line demonstrated some of the above-mentioned trends but also some specificity. Among the derivatives with a carbonyl group, once again, chloro compound **3c** (IC_{50} 22.1 μM) was the most active. Replacement of carbonyl with thiocarbonyl produced the most active compound for this cell line **4c** (IC_{50} 6.2 μM) but, unfortunately, with complete loss of MCF-7/MRC-5 selectivity.

2.2.2 | Cell cycle analysis

To further examine the mechanisms of action of our compounds, determination of the cell cycle distribution was performed in HeLa cells using compounds **3c**, **3f**, **3i**, and **4a**–**c**. After exposure to the investigated compounds at concentrations corresponding to IC_{50} values, cells were harvested, stained with propidium iodide (PI), and subjected to flow cytometry. As shown in Figure 4 and Table S1, after 24-h exposure to the investigated compounds, the numbers of HeLa

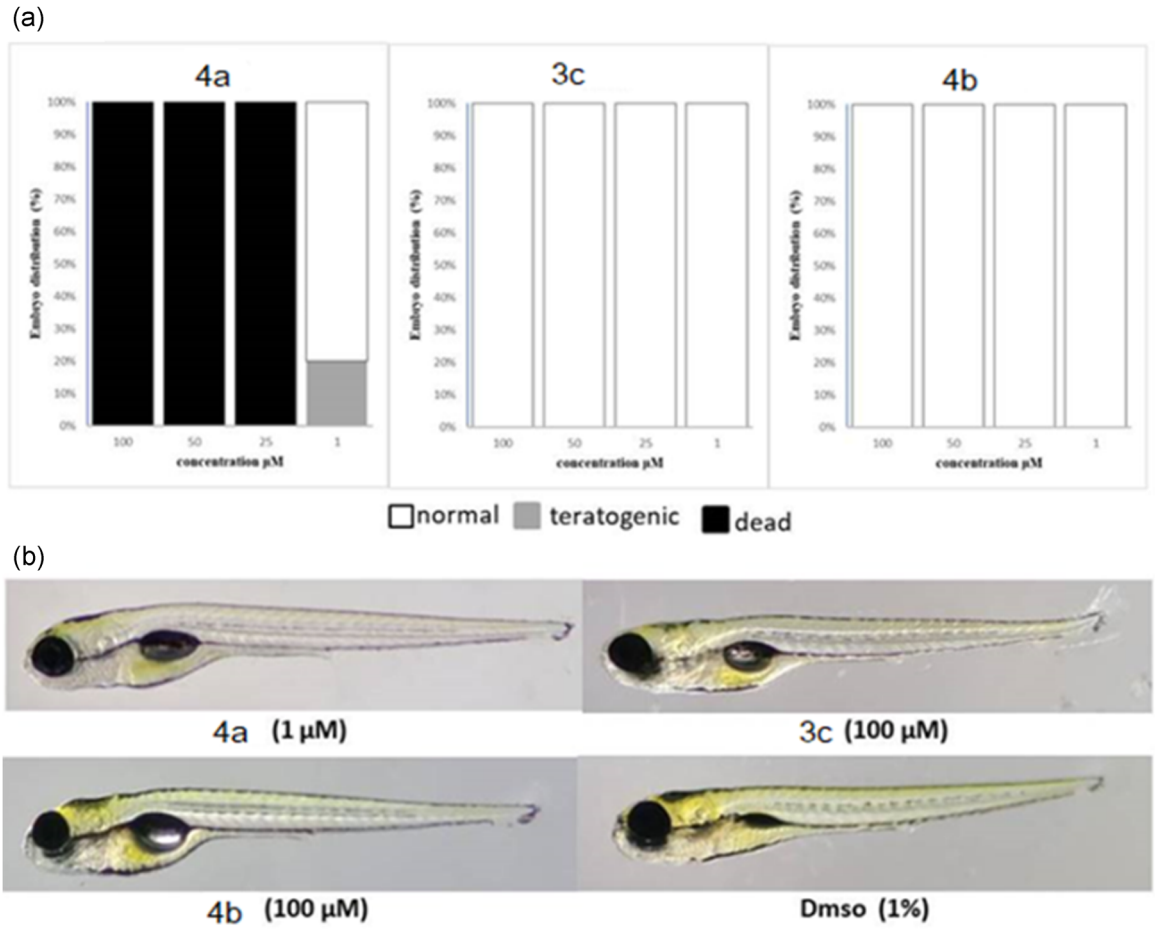


FIGURE 5 Effects of **4a**, **3c**, and **4b** on the development of zebrafish embryos: (a) Percent of normal, teratogenic, and normal embryos for each tested compound at 100, 50, 25, and 1 µM. (b) Images of zebrafish embryos treated with **4a**, **3c**, and **4b** at 1, 100, and 100 µM concentrations, respectively, and 1% (v/v) dimethyl sulfoxide as a control

cells in the subG1 phase increased in all the experiments. This accumulation indicated induction of cell death and this was further confirmed by the decrease in viable cells in the G2/M phase, particularly in the case of compounds **4b**, **4c**, and **3c**, implying that the active compounds interfere with the highly regulated cell cycle process. At this stage, it is difficult to predict the origin of the activity of the studied compounds as the cell cycle is a very complex and finely regulated process with many potential targets from initially important CDK4/6 to proteins involved in spindle assembly.[40] The increase in cell number in the subG1 phase may suggest that the cells are incapable of crossing the G1 checkpoint, which could be a result of DNA damage.[41] This effect might be correlated to the functions of p53/p21, CDK2/4/6, and related cyclines, but for unambiguous rationalization, this needs to be further investigated. The studied compounds possess structural properties of kinase inhibitors as they can adopt a near-planar conformation (mimicking the adenine part of ATP) with heteroatoms that can create at least one H bond with the hinge region (the kinase binding modes I and II).[42] Interactions with several targets are also conceivable as a small size of the compounds implies more flexibility toward various biomolecules.

2.2.3 | Embryotoxicity of selected compounds

Biological exploration of the azolylcoumarins revealed a certain level of toxicity in in vitro experiments on the MRC-5 cell line. Therefore, we also explored in vivo toxicity of selected compounds in the zebrafish model. Zebrafish is a vertebrate model system often used to assess the toxicology of biologically active compounds. It has genetic similarity to and good correlation with humans, while zebrafish body transparency allows easy and practical visual inspection for any malformation during embryonic development.[43,44] The effects of selected coumarins were examined at four concentrations (100, 50, 25, and 1 µM), close to the IC_{50} values determined in the cytotoxicity assessment (Table 1). Embryos treated with **4a** died after the first 24 h at the three highest concentrations. When treated with 1 µM of **4a**, 80% of the embryos developed normally and 20% showed cardiovascular abnormalities, which was marked as a teratogenic effect (Figure 5a). None of the remaining tested substances, at four concentrations, showed lethal or teratogenic effects on embryos within 5 days (Figure 5b). The developed embryos did not show any malformations on the skeleton or internal organs, suggesting the suitability of **3c** and **4b** for further development as anticancer drug leads.

3 | CONCLUSION

Several azolylcoumarin derivatives with fragment-type properties were synthesized and their anticancer potential was studied. Biological profiling was carried out on selected cell lines, among which the myelogenous leukemia cells K-562 proved to be the most sensitive. The most active derivative **3c** showed slightly better potency than cisplatin, which is used as a standard. In addition, when tested on the healthy fibroblast MRC-5 cells, this compound demonstrated lower activity with 12-fold K-562/MRC-5 selectivity. Further biological experiments suggested interference of **3c** with the cell cycle and they also demonstrated the absence of toxicity in the zebrafish model. The biological profile of **3c**, together with the fact that this is a fragment-like compound with low molecular weight and low clog *P*, makes it a good candidate for further development as an anticancer agent in this class of compounds.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Infrared (IR) spectra were recorded on an IR Thermo Scientific NICOLET iS10 (4950) spectrometer. Melting points were determined using a Boetius PHMK 05 apparatus without correction. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Ascend 400 (400 MHz) spectrometer (see the Supporting Information). Deuterochloroform was used as a solvent, and chemical shifts are given in parts per million (δ) downfield from tetramethylsilane as the internal standard. Mass spectral data were recorded using an Agilent Technologies 6520 Q-TOF spectrometer coupled with an Agilent 1200 HPLC, LTQ Orbitrap XL and an Agilent Technologies 5975C MS coupled with an Agilent Technologies 6890N GC. Flash chromatography used a silica gel 60 (230–400 mesh), while thin-layer chromatography (TLC) was carried out using alumina plates with a 0.25 mm silica layer (Kieselgel 60 F_{254}; Merck). Compounds were visualized by staining with potassium permanganate solution and Dragendorff reagent. The starting compound, 4-bromocoumarin (4-bromo-chromen-2-one), was synthesized from 4-hydroxycoumarin, TBAB, and P_2O_5 following the literature procedure.[45] The physicochemical properties of synthesized compounds were determined using MarvinSketch version 21.3.0-12862, 2021, ChemAxon (http://www.chemaxon.com).

The InChI codes of the investigated compound, together with some biological activity data, are provided as the Supporting Information.

4.1.2 | General procedure for the synthesis of 4-azolylcoumarins 3[46]

The mixture of 4-bromocoumarin (23 mg, 0.10 mmol), azole (0.12 mmol), and K_2CO_3 (21 mg, 0.15 mmol) in acetonitrile (2 ml) was heated in a nitrogen atmosphere at 82°C for 16 h. After completion of the reaction, as indicated by TLC, the mixture was cooled to room temperature, and the solvent was removed under reduced pressure. The crude mixture was purified by flash chromatography to afford the product.

*4-[1,2,4]Triazol-1-yl-chromen-2-one (**3a**)*

Compound **3a** was synthesized from 4-bromocoumarin and 1,2,4-triazole following the general procedure. Flash chromatography (SiO_2, 8:2 v/v diethyl ether/petroleum ether) afforded the product **3a** (46%) as white needles, mp: 234–236°C. IR (attenuated total reflection [ATR]) cm^{-1}: 1771, 1721, 1438, 1282, 1217, 1003, 947, 767; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 8.29 (s, 1H), 7.95 (d, 1H, J = 8.0 Hz), 7.68 (t, 1H, J = 8.0 Hz), 7.47 (d, 1H, J = 8.4 Hz), 7.37 (t, 1H, J = 7.6 Hz), 6.56 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.7, 154.4, 153.9, 144.2, 133.6, 125.4, 124.9, 114.4, 109.4; mass spectrometry (electrospray ionization) [MS (EI)]: m/z 213.0 $[M]^+$, 185.0, 143.9, 130.9, 115.9, 103.0, 88.0, 76.0, 63.0; high-resolution mass spectrometry (HRMS) (ESI/quadrupole time-of-flight [Q-TOF]) m/z calcd. for $[C_{11}H_7N_3O_2 + H^+]$: 214.0616; found, 214.0616.

*4-Pyrazol-1-yl-chromen-2-one (**3b**)*

Compound **3b** was synthesized from 4-bromocoumarin and pyrazole following the general procedure. Flash chromatography (SiO_2, 1:1 v/v diethyl ether/petroleum ether) afforded the product **3b** (73%) as a colorless solid, mp: 125–127°C. IR (ATR) cm^{-1}: 1722, 1619, 1437, 1395, 1188, 969, 942, 752, 653; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, 1H, J = 8.0 Hz), 7.93 (s, 1H), 7.92 (s, 1H), 7.62 (t, 1H, J = 7.6 Hz), 7.43 (d, 1H, J = 8.4 Hz), 7.34 (t, 1H, J = 7.6 Hz), 8.81 (s, 1H), 6.45 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.7, 154.5, 149.5, 143.5, 132.8, 130.6, 126.7, 124.5, 117.4, 115.0, 108.9, 107.1; MS (EI): m/z 212.0 $[M]^+$, 184.0, 171.9, 155.0, 144.0, 129.0, 116.0, 103.0, 88.0, 78.0, 63.0, 51.0, 39.0; HRMS (ESI/Q-TOF) m/z calcd. for $[C_{12}H_9N_2O_2 + H^+]$: 213.0664; found, 213.0661.

*4-(4-Chloro-pyrazol-1-yl)-chromen-2-one (**3c**)*

Compound **3c** was synthesized from 4-bromocoumarin and 4-chloropyrazole following the general procedure. Flash chromatography (SiO_2, 7:3 v/v petroleum ether/diethyl ether) afforded the product **3c** (50%) as a colorless solid, mp: 187–190°C. IR (ATR) cm^{-1}: 1722, 1621, 1436, 1246, 986, 947, 871, 758; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (dd, 1H, J = 8.0 and 1.2 Hz), 7.92 (s, 1H), 7.84 (s, 1H), 7.64 (t, 1H, J = 7.7 Hz), 7.44 (d,1H, J = 8.0 Hz), 7.36 (t, 1H, J = 8.0 Hz), 6.44 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.3, 154.5, 148.8, 142.1, 133.1, 128.2, 126.2, 124.7, 117.6, 114.57, 114.4, 107.6; MS (EI): m/z 246.0 $[M]^+$, 218.0, 189.0, 163.0, 144.0, 116.0, 103.0, 89.0, 75.0, 63.0, 39.0; HRMS (ESI/Q-TOF) m/z calcd. for $[C_{12}H_7ClN_2O_2 + H^+]$: 247.0274; found, 247.0271.

*4-Imidazol-1-yl-chromen-2-one (**3d**)*

Compound **3d** was synthesized from 4-bromocoumarin and imidazole following the general procedure. Flash chromatography (SiO_2, 8:2 v/v diethyl ether/ethyl acetate) afforded the product **3d** (88%) as

colorless needles, mp: 181–183.5°C. IR (ATR) cm^{-1}: 1732, 1620, 1608, 1482, 1405, 1243, 1086, 948, 872, 759, 658; ^1H NMR (400 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.70 (t, 1H, J = 8.0 Hz), 7.58 (d, 1H, J = 8.0 Hz), 7.47 (d, 1H, J = 8.0 Hz), 7.38–7.35 (m, 2H) 7.31 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.9, 154.3, 147.9, 136.8, 133.5, 131.3, 125.0, 124.2, 119.7, 117.9, 115.6, 110.3; MS (EI): m/z 212.0 $[M]^+$, 185.0, 171.0, 156.0, 145.0, 130.0, 117.0, 101.0, 89.0, 76.0, 63.0, 51.0, 39.1; HRMS (HESI/orbitrap) m/z calcd. for $[C_{12}H_8N_2O_2 + H^+]$: 213.06640; found, 213.06586.

*4-(4-Bromo-pyrazol-1-yl)-chromen-2-one (**3e**)*

Compound **3e** was synthesized from 4-bromocoumarin and 4-bromopyrazole following the general procedure. Flash chromatography (SiO_2, 6:4 v/v petroleum ether/diethyl ether) afforded the product, which was then washed with petroleum ether. Pure compound **3e** was isolated as white needles (47%), mp: 170–173°C. IR (ATR) cm^{-1}: 1723, 1452, 1185, 984, 945, 757; ^1H NMR (400 MHz, $CDCl_3$) δ 8.15 (dd, 1H, J = 8.0 and 0.8 Hz), 7.95 (s, 1H), 7.87 (s, 1H), 7.65 (dd, 1H, J = 11.4, 4.2 Hz). 7.44 (d, 1H, J = 8.4 Hz), 7.35 (t, 1H, J = 8.4 Hz), 6.44 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.3, 154.5, 148.7, 144.0, 133.1, 130.5, 126.1, 124.7, 117.6, 114.5, 107.7, 97.7; MS (EI): m/z 290.0 $[M]^+$, 262.0, 235.0, 206.9, 183.1, 172.0, 155.0, 144.0, 128.0, 103.0, 89.0, 77.1, 63.1, 51.0, 39.0; HRMS (HESI/orbitrap) m/z calcd. for $[C_{12}H_7BrN_2O_2 + H^+]$: 290.97692; found, 290.97621.

*1-(2-Oxo-2H-chromen-4-yl)-1H-imidazole-4-carboxylic acid ethyl ester (**3f**)*

Compound **3f** was synthesized from 4-bromocoumarin and ethyl imidazole-4-carboxylate following the general procedure. Flash chromatography (SiO_2, 9:1 v/v diethyl ether/ethyl acetate) afforded the product **3f** (37%) as a colorless solid, mp: 153–155°C. IR (ATR) cm^{-1}: 1745, 1720, 1622, 1484, 1217, 945, 763; ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, 1H, J = 1.6 Hz), 7.86 (d, 1H, J = 0.8 Hz), 7.77–7.65 (m, 1H), 7.48 (dd, 2H, J = 14.1, 5.1 Hz), 7.39 (t, 1H, J = 7.6 Hz). 6.48 (s, 1H), 4.44 (q, 2H, J = 7.2 Hz), 1.43 (t, 3H, J = 7.2 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.03, 159.36, 154.23, 146.96, 137.2, 136.1, 133.9, 125.3, 124.9, 123.8, 118.0, 115.1, 111.4, 61.2, 14.4; MS (EI): m/z 284.1 $[M]^+$, 269.0, 256.0, 239.0, 212.0, 199.0, 185.0, 172.0, 155.1, 145.0, 129.0, 118.0, 89.0, 63.0, 51.0, 39.0; HRMS (HESI/orbitrap) m/z calcd. for $[C_{15}H_{12}N_2O_4 + H^+]$: 285.08753; found, 285.08685.

*4-Benzotriazol-1-yl-chromen-2-one (**3g**)*

Compound **3g** was synthesized from 4-bromocoumarin and benzotriazole following the general procedure. Flash chromatography (SiO_2, 6:3:1 v/v/v petroleum ether/diethyl ether/dichloromethane) afforded the product **3g** (80%) as a colorless solid, mp: 190–193°C. IR (ATR) cm^{-1}: 1728, 1623, 1606, 1494, 1426, 1185, 1027, 937, 744; ^1H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, 1H, J = 8.4 Hz), 7.85 (d, 1H, J = 8.0 Hz), 7.72–7.68 (m, 3H), 7.57–7.51 (m, 2H), 7.36 (t, 1H, J = 7.2 Hz), 6.70 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.1, 154.6, 146.8, 146.2, 133.5, 132.8, 129.6, 126.1, 125.6, 124.9, 120.9, 117.6, 115.0, 110.5, 109.9; MS (EI): m/z 263.0 $[M]^+$, 235.0, 207.0, 190.0, 179.0, 145.0, 117.0, 101.0, 89.0, 76.0, 63.0, 50.0, 39.1; HRMS (HESI/orbitrap) m/z calcd. for $[C_{15}H_9N_3O_2 + H^+]$: 264.07730; found, 264.07658.

*4-Benzoimidazol-1-yl-chromen-2-one (**3h**)*

Compound **3h** was synthesized from 4-bromocoumarin and benzimidazole following the general procedure. Flash chromatography (SiO_2, diethyl ether) afforded the product **3h** (85%) as a colorless solid, mp: 184–187°C. IR (ATR) cm^{-1}: 1723, 1623, 1605, 1144, 763, 740; ^1H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.94 (d, 1H, J = 7.6 Hz), 7.75–7.63 (m, 1H), 7.52 (d, 1H, J = 8.0 Hz), 7.46–7.39 (m, 4H), 7.32 (dd, 1H, J = 11.3, 4.0 Hz), 6.59 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 196.1, 157.7, 144.0, 141.7, 139.4, 133.7, 133.4, 125.8, 124.8, 124.6, 124.4, 124.1, 121.2, 117.7, 117.4, 111.2; MS (EI): m/z $[M]^+$ 262.1, 245.0, 233.1, 221.1, 206.1, 179.1, 145.0, 116.0, 89.0, 76.0, 63.0, 39.1; HRMS (HESI/orbitrap) m/z calcd. for $[C_{16}H_{10}N_2O_2 + H^+]$: 263.08205; found, 263.08234.

*4-(4,5-Dichloro-imidazol-1-yl)-chromen-2-one (**3i**)*

Compound **3i** was synthesized from 4-bromocoumarin and 4,5-dichloroimidazole following the general procedure. Flash chromatography (SiO_2, 6:4 v/v petroleum ether/ethyl acetate) afforded the product **3i** (62%) as a colorless solid, mp: 190–192°C. IR (ATR) cm^{-1}: 1727, 1622, 1606, 1397, 1278, 1249, 945, 882, 765; ^1H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, 1H, J = 7.6 Hz), 7.62 (s, 1H), 7.48 (d, 1H, J = 8.4 Hz), 7.36 (t, 1H, J = 7.6 Hz), 7.20 (d, 1H, J = 8.0 Hz), 6.49 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.1, 154.0, 145.4, 134.6, 133.9, 129.0, 125.3, 124.1, 117.7, 115.9, 115.2, 114.3; MS (EI): m/z 280.0 $[M]^+$, 252.9, 245.0, 217.0, 190.0, 178.0, 156.0, 101.0, 89.0, 75.0, 63.0, 51.0, 39.1; HRMS (HESI/orbitrap) m/z calcd. for $[C_{12}H_6Cl_2N_2O_2 + H^+]$: 280.98846; found, 280.98806.

*4-(4-Iodo-pyrazol-1-yl)-chromen-2-one (**3j**)*

Compound **3j** was synthesized from 4-bromocoumarin and 4-iodopyrazole following the general procedure. Flash chromatography (SiO_2, 7:3 v/v petroleum ether/diethyl ether) afforded the product **3j** (93%) as a colorless solid, mp: 173–176°C. IR (ATR) cm^{-1}: 1720, 1621, 1429, 1185, 984, 937, 765, 742; ^1H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, 1H, J = 8.4 Hz), 7.98 (s, 1H), 7.89 (s, 1H), 7.63 (t, 1H, J = 8.0 Hz), 7.42 (d, 1H, J = 8.0 Hz), 7.34 (t, 1H, J = 8.0 Hz), 6.44 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.4, 154.4, 148.6, 148.3, 134.9, 133.1, 126.1, 124.6, 117.5, 114.5, 107.6; MS (EI): m/z 338.0 $[M]^+$, 310.0, 255.0, 156.0, 144.0, 127.0, 116.0, 101.0, 89.0, 63.0, 51.1, 39.1; HRMS (HESI/orbitrap) m/z calcd. for $[C_{12}H_7IN_2O_2 + H^+]$: 338.96305, found 338.96321.

4.1.3 | General procedure for the synthesis of 4-azolylthiocoumarins **4**[47]

A mixture of 4-azolylcoumarin (0.10 mmol) and Lawesson's reagent (40.4 mg, 0.1 mmol) in anhydrous toluene (10 ml) was stirred at 110°C for several hours. After completion of the reaction, as

indicated by TLC, the mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude mixture was purified by flash chromatography to afford the product.

*4-[1,2,4]Triazol-1-yl-chromene-2-thione (**4a**)*

Compound **4a** was synthesized from 4-[1,2,4]triazol-1-yl-chromen-2-one and Lawesson's reagent following the general procedure. Flash chromatography (SiO_2, 8:2 v/v diethyl ether/petroleum ether) afforded the product **4a** (52%) as a yellow solid, mp: 166–169°C. IR (ATR) cm^{-1}: 1606, 1554, 1511, 1438, 1366, 1179, 1004, 758, 667; ^1H NMR (400 MHz, $CDCl_3$) δ 8.62 (s, 1H), 8.29 (s, 1H), 8.06 (d, 1H, J = 7.2 Hz), 7.71 (t, 1H, J = 7.2 Hz), 7.58 (d, 1H, J = 8.4 Hz), 7.42 (t, 1H, J = 8.0 Hz), 7.33 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 195.6, 157.7, 154.1, 144.1, 138.8, 133.6, 125.9, 125.4, 121.9, 117.4, 115.8; MS (EI): m/z 229.0 $[M]^+$, 212.9, 202.0, 185.0, 175.0, 159.9, 145.9, 131.9, 119.0, 103.0, 89.0, 63.1, 39.1; HRMS (ESI/Q-TOF) m/z calcd. for $[C_{11}H_7N_3OS + H^+]$: 230.0388; found, 230.0385.

*4-Benzoimidazol-1-yl-chromene-2-thione (**4b**)*

Compound **4b** was synthesized from 4-benzoimidazol-1-yl-chromen-2-one and Lawesson's reagent following the general procedure. Flash chromatography (SiO_2, 8:2 v/v diethyl ether/petroleum ether) afforded the product **4b** (70%) as a yellow solid, mp: 197–200°C. IR (ATR) cm^{-1}: 1602, 1533, 1506, 1399, 1179, 1113, 727; ^1H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.93 (d, 1H, J = 7.6 Hz), 7.72 (t, 1H, J = 7.8 Hz). 7.63 (d, 1H, J = 8.4 Hz), 7.48 (d, 1H, J = 8.0 Hz), 7.45–7.40 (m, 3H), 7.36–7.35 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 196.1, 157.7, 144.0, 141.7, 139.4, 133.7, 133.4, 125.8, 124.8, 124.6, 124.4, 124.1, 121.2, 117.7, 117.4, 111.2; MS (EI): m/z 278.0 $[M]^+$, 262.0, 245.0, 234.0, 221.0, 206.0, 160.9, 101.0, 89.0, 63.0; HRMS (HESI/orbitrap) m/z calcd. for $[C_{16}H_{10}N_2OS + H^+]$: 279.05921; found, 279.05880.

*4-Pyrazol-1-yl-chromene-2-thione (**4c**)*

Compound **4c** was synthesized from 4-pyrazol-1-yl-chromen-2-one and Lawesson's reagent following the general procedure. Flash chromatography (SiO_2, 8:2 v/v petroleum ether/diethyl ether) afforded the product **4c** (30%) as yellow needles, mp: 163–165.5°C. IR (ATR) cm^{-1}: 1600, 1548, 1441, 1393, 1354, 1139, 975, 773, 751; ^1H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, 1H, J = 8.0 Hz), 7.96–7.93 (m, 2H), 7.66 (t, 1H, J = 8.0 Hz), 7.55 (d, 1H, J = 8.4 Hz), 7.39 (t, 1H, J = 8.0 Hz), 7.29 (s, 1H), 6.62 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 196.5, 157.9, 144.0, 141.8, 133.0, 130.5, 126.9, 125.5, 120.1, 117.3, 116.3, 109.3; MS (EI): m/z 228.0 $[M]^+$, 212.0, 201.0, 184.0, 169.0, 132.0, 114.0, 89.0, 63.0, 51.1, 39.1; HRMS (HESI/orbitrap) m/z calcd. for $[C_{12}H_8N_2OS + H^+]$: 229.04356; found, 229.04307.

4.2 | Biology

4.2.1 | Cell lines

Human cervix adenocarcinoma cell line (HeLa), human chronic myelogenous leukemia cells (K562), human breast cancer cell lines (MDA-MB-453 and MCF7), and the noncancerous cell line MRC-5 (human embryonic lung fibroblasts) were grown in complete RPMI-1640 medium.

4.2.2 | Determination of cell survival

Stock solutions of the investigated compounds (10 mM) were prepared in dimethyl sulfoxide (DMSO) and then dissolved in complete medium to achieve adequate working concentrations. The final concentration of the DMSO solvent never exceeded 0.5%, a concentration considered nontoxic to the cells. Target adherent cells HeLa (2500 cells/well), MDA-MB-453 (3000 cells/well), MCF7 (7000 cells/well), and MRC-5 (5000 cells/well) were seeded into the wells of a 96-well flat-bottom microtiter plate. Twenty-four hours later, after the cell adherence, different concentrations of investigated compounds were added to the wells, except for the controls, where only the complete medium was added. For nonadherent K562 cells (6000 cells/well), the compounds were applied 2 h after cell seeding. Culture medium with the corresponding concentrations of the investigated compounds, but without cells, was used as a blank. The cultures were incubated for 72 h, and the effects of the investigated compounds on cancer and normal cell survival were determined using the MTT variant of the microculture tetrazolium assay (MTA), according to Mosmann,[36] with modification by Ohno and Abe,[37] 72 h after the addition of the investigated compounds. Briefly, 20 µl of MTT dye solution (5 mg/ml of MTT in phosphate-buffered saline) was added to each well. Samples were incubated for an additional 4 h at 37°C in a humidified atmosphere of 5% CO_2 (v/v). Afterward, 100 µl of sodium dodecyl sulfate was added to extract the insoluble formazan, which represents the product of the conversion of the MTT dye by viable cells. The number of viable cells in each well is proportional to the intensity of the absorbance (A) of light, which was measured in a microtiter plate reader at 570 nm after 24 h. To determine cell survival (S%), the A of a sample with cells grown in the presence of various concentrations of the investigated compounds was divided by the control optical density (the A of control cells grown only in nutrient medium) and multiplied by 100. The A of the blank was always subtracted from the A of the corresponding sample incubated with the target cells. All experiments were performed in triplicate.

4.2.3 | Cell cycle determination

Aliquots of 5×10^5 control cells or cells treated with investigated compounds for 24 h (concentrations corresponded to IC_{50} values for 72 h determined in the MTT test) were fixed in 70% ethanol on ice for 1 week and centrifuged. The pellet was treated with RNase A (100 µg/ml) at 37°C for 30 min and then incubated with 40 µg/ml PI for at least 30 min. Cells were analyzed using a FACSCalibur flow cytometer (BD Biosciences Franklin Lakes) equipped with a 15 mW, air-cooled 488 nm argon-ion laser for excitation of PI. PI fluorescence

was collected with FL2 detector (yellow orange channel) after passing a 585/42-nm band pass filter. A FACSCalibur flow cytometer is equipped with an FL2 upgraded doublet discrimination module, which enables screening and then exclusion of the possible occurrence of cell doublets, clumps, and debris by plotting FL2-area versus FL2-width signals.[48] PI fluorescence data were collected using linear amplification. A minimum of 10,000 events was collected on each sample. Finally, data were analyzed using CELLQuest 3.2.1.f1 software (BD Biosciences).

4.2.4 | In vivo zebrafish toxicity

General rules of the OECD guidelines for the testing of chemicals (No, O.T., 236: fish embryo acute toxicity [FET] test; OECD guidelines for the testing of chemicals, section, 2013. 2: p. 1–22) were followed while the zebrafish embryotoxicity assay was performed. The wild-type zebrafish (*Danio rerio*) strain was obtained from a commercial supplier (Pet Centre) and held under controlled environmental conditions (water temperature 28°C, 14 h under light and 10 h in the dark) and fed regularly three times daily with commercially dry flake food supplemented with *Artemia nauplii* (TetraMin™ flakes; Tetra Melle). After mating adult females and males (ratio 1: 2), the obtained embryos were collected and washed from detritus. Only fertilized embryos were selected and distributed into 24-well plates containing 10 embryos per well (Sarstedt). Each well contained exactly 1 ml of water for embryos (0.2 g/l of Instant Ocean® Salt in distilled water). Embryos at the 6-h post-fertilization stage were treated with selected compounds at appropriate concentrations (maximum DMSO concentration in control 1%, v/v) and incubated at 28°C.[49] Experiments were performed in triplicate using 30 embryos per concentration. Over 5 days, the appearance of different morpho-physiological parameters in embryo development was monitored and dead embryos were counted and discarded every 24 h.[50] On the fifth day, the embryos were anesthetized by the addition of a 0.1% (w/v) tricaine solution (Sigma-Aldrich), observed under a stereomicroscope (SMZ143-N2GG; Motic), photographed, and killed by freezing at −20°C for ≥24 h. All experiments involving zebrafish were performed in compliance with the European directive 2010/63/EU and the ethical guidelines of the Guide for Care and Use of Laboratory Animals of the Institute of Molecular Genetics and Genetic Engineering, University of Belgrade.

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CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interests.

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REFERENCES

[1] K. Robe, E. Izquierdo, F. Vignols, H. Rouached, C. Dubos, *Trends Plant Sci.* **2021**, *26*, 248.
[2] M. J. J. Stassen, S.-H. Hsu, C. M. J. Pieterse, I. A. Stringlis, *Trends Plant Sci.* **2021**, *26*, 169.
[3] R. W. DeSimone, K. S. Currie, S. A. Mitchell, J. W. Darrow, D. A. Pippin, *Comb. Chem. High Throughput Screening* **2004**, *7*, 473.
[4] H.-L. Qin, Z.-W. Zhang, L. Ravindar, K. P. Rakesh, *Eur. J. Med. Chem.* **2020**, *207*, 112832.
[5] Z. Xu, Q. Chen, Y. Zhang, C. Liang, *Fitoterapia* **2021**, *150*, 104863.
[6] J. S. Prusty, A. Kumar, *Mol. Divers.* **2020**, *24*, 1367.
[7] T. Al-Warhi, A. Sabt, E. B. Elkaeed, W. M. Eldehna, *Bioorg. Chem.* **2020**, *103*, 104163.
[8] S. Balcıoğlu, M. Olgun Karataş, B. Ateş, B. Alıcı, İ. Özdemir, *Bioorg. Med. Chem. Lett.* **2020**, *30*, 126805.
[9] M. E. Riveiro, N. De Kimpe, A. Moglioni, R. Vázquez, F. Monczor, C. Shayo, C. Davio, *Curr. Med. Chem.* **2010**, *17*, 1325.
[10] X.-F. Song, J. Fan, L. Liu, X.-F. Liu, F. Gao, *Arch. Pharm.* **2020**, *353*, 2000025.
[11] J. Galeta, R. Dzijak, J. Obořil, M. Dračínský, M. Vrabel, *Chem.–Eur. J.* **2020**, *26*, 9945.
[12] N. M. Sarih, A. Ciupa, S. Moss, P. Myers, A. G. Slater, Z. Abdullah, H. A. Tajuddin, S. Maher, *Sci. Rep.* **2020**, *10*, 7421.
[13] X. Sun, T. Liu, J. Sun, X. Wang, *RSC Adv.* **2020**, *10*, 10826.
[14] M. Tasior, D. Kim, S. Singha, M. Krzeszewski, K. H. Ahn, D. T. Gryko, *J. Mater. Chem. C* **2015**, *3*, 1421.
[15] M. Simic, N. Paunovic, I. Boric, J. Randjelovic, S. Vojnovic, J. Nikodinovic-Runic, M. Pekmezovic, V. Savic, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 235.
[16] M. R. Simić, S. Erić, I. Borić, A. Lubelska, G. Latacz, K. Kieć-Kononowicz, S. Vojnović, J. Nikodinović-Runić, V. Savic, *J. Serb. Chem. Soc.* **2021**.*86*, 639
[17] W. Zhang, Z. Li, M. Zhou, F. Wu, X. Hou, H. Luo, H. Liu, X. Han, G. Yan, Z. Ding, R. Li, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 799.
[18] S. Umar, R. Soni, S. D. Durgapal, S. Soman, S. Balakrishnan, *J. Biochem. Mol. Toxicol*, **2020**, *34*, e22553.
[19] J. Zhang, Y. Tan, G. Li, L. Chen, M. Nie, Z. Wang, H. Ji, *Molecules* **2021**, *26*, 786.
[20] E. Küpeli Akkol, Y. Genç, B. Karpuz, E. Sobarzo-Sánchez, R. Capasso, *Cancers* **2020**, *12*, 1959.
[21] J. Ding, J. Liu, Z. Zhang, J. Guo, M. Cheng, Y. Wan, R. Wang, Y. Fang, Z. Guan, Y. Jin, S.-S. Xie, *Bioorg. Chem.* **2020**, *101*, 104023.
[22] Y. Yamaguchi, N. Nishizono, K. Oda, *Biol. Pharm. Bull.* **2020**, *43*, 1179.
[23] Z. M. Faten, W. R. Youstina, S. A. Moustafa, M. E.-D. Ibrahim, *Anticancer Agents Med. Chem.* **2020**, *20*, 2246.
[24] S. G. Nerella, K. Pardeep, D. B. Rose, *Mini Rev. Med. Chem.* **2020**, *20*, 1754.
[25] H. Ji, Y. Tan, N. Gan, J. Zhang, S. Li, X. Zheng, Z. Wang, W. Yi, *Bioorg. Med. Chem.* **2021**, *29*, 115870.
[26] K. M. Chandra, N. S. Goud, M. Arifuddin, M. Alvala, R. Alvala, A. Angeli, C. T. Supuran, *Bioorg. Med. Chem. Lett.* **2021**, *39*, 127877.
[27] Y. Li, Q. Tang, Y. Xie, D. He, K. Yang, L. Zheng, *Bioorg. Med. Chem. Lett.* **2021**, *33*, 127750.
[28] J. Shi, W. Lu, J. Chen, L. Sun, S. Yang, M. Zhou, L. Xu, Y. Ma, L. Yu, *Arch. Pharm.* **2021**, *354*, 2000236.
[29] E. Konkoľová, M. Hudáčová, S. Hamuľaková, R. Jendželovský, J. Vargová, J. Ševc, P. Fedoročko, M. Kožurková, *Molecules* **2021**, *26*, 1133.
[30] F. A. Ragab, A. A. M. Eissa, S. H. Fahim, M. A. Salem, M. A. Gamal, Y. M. Nissan, *Arch. Pharm.* **2021**. In press. *354*(*8*), e210029. http://doi.org/10.1002/(ISSN)1521-4184

[31] S. J. Stanway, A. Purohit, L. W. L. Woo, S. Sufi, D. Vigushin, R. Ward, R. H. Wilson, F. Z. Stanczyk, N. Dobbs, E. Kulinskaya, M. Elliott, B. V. L. Potter, M. J. Reed, R. C. Coombes, *Clin. Cancer Res.* **2006**, *12*, 1585.
[32] J. Dandriyal, R. Singla, M. Kumar, V. Jaitak, *Eur. J. Med. Chem.* **2016**, *119*, 141.
[33] J. A. Kumar, G. Saidachary, G. Mallesham, B. Sridhar, N. Jain, S. V. Kalivendi, V. J. Rao d, B. C. Raju, *Eur. J. Med. Chem.* **2013**, *65*, 389.
[34] A. Bonardi, M. Falsini, D. Catarzi, F. Varano, L. Di Cesare Mannelli, B. Tenci, C. Ghelardini, A. Angeli, C. T. Supuran, V. Colotta, *Eur. J. Med. Chem.* **2018**, *146*, 47.
[35] O. Ganina, A. Fedorov, I. Beletskaya, *Synthesis* **2009**, *2009*, 3689.
[36] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55.
[37] M. Ohno, T. Abe, *J. Immunol. Methods* **1991**, *145*, 199.
[38] P. Kirsch, A. M. Hartman, A. K. H. Hirsch, M. Empting, *Molecules* **2019**, *24*, 4309.
[39] P. S. Charifson, W. P. Walters, *J. Med. Chem.* **2014**, *57*, 9701.
[40] C. J. Sherr, J. Bartek, *Annu. Rev. Cancer Biol.* **2017**, *1*, 41.
[41] M. L. Agarwal, W. R. Taylor, M. V. Chernov, O. B. Chernova, G. R. Stark, *J. Biol. Chem.* **1998**, *273*, 1.
[42] Z. Zhao, H. Wu, L. Wang, Y. Liu, S. Knapp, Q. Liu, N. S. Gray, *ACS Chem. Biol.* **2014**, *9*, 1230.
[43] C. Chakraborty, C. H. Hsu, Z. H. Wen, C. S. Lin, G. Agoramoorthy, *Curr. Drug Metab.* **2009**, *10*, 116.
[44] C. A. MacRae, R. T. Peterson, *Nat. Rev. Drug Discov.* **2015**, *14*, 721.
[45] M. E. Jung, D. A. Allen, *Org. Lett.* **2009**, *11*, 757.
[46] Q. Ji, Z. Ge, Z. Ge, K. Chen, H. Wu, X. Liu, Y. Huang, L. Yuan, X. Yang, F. Liao, *Eur. J. Med. Chem.* **2016**, *108*, 166.
[47] P. Manivel, S. M. Roopan, D. P. Kumar, F. N. Khan, *Phosphorus, Sulfur Silicon Relat. Elem.* **2009**, *184*, 2576.
[48] M. Carbonari, T. Tedesco, M. Fiorilli, *Cytometry* **2001**, *44*, 120.
[49] C. B. Kimmel, W. W. Ballard, S. R. Kimmel, B. Ullmann, T. F. Schilling, *Dev. Dyn.* **1995**, *203*, 253.
[50] R. Nagel, *ALTEX* **2002**, *19*(*suppl 1*), 38.

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