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Synthesis and cytotoxic evaluation of benzoxazole/benzothiazole-2-imino-coumarin hybrids and their coumarin analogues as potential anticancer agents

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Two series of 2-imino-coumarin based hybrids: 3-(benzoxazol-2-yl)-2*H*-chromen-2-imines **3-9** (series **A-I**) and 3-(benzothiazol-2-yl)-2*H*-chromen-2-imines **10-16** (series **A-II**), as well as their coumarin analogues: 3-(benzoxazol-2-yl)-2*H*-chromen-2-ones **17-21** (series **B-I**) and 3-(benzothiazol-2-yl)-2*H*-chromen-2-ones **22-28** (series **B-II**) were prepared as potential antitumor agents. The *in vitro* cytotoxic potency of the synthesized compounds was evaluated against five human cancer cell lines: DAN-G, A-427, LCLC-103H, RT-4 and SISO, and relationships between structure and anticancer activity are discussed. Among the compounds tested, 3-(benzo[*d*]oxazol-2-yl)-*N*,*N*-diethyl-2-imino-2*H*-chromen-7-amine (**6**, series **A-I**) and 3-(benzo[*d*]thiazol-2-yl)-6-fluoro-2*H*-chromen-2-one (**26**, series **B-II**) exhibited the most potent cytotoxic activity with IC_{50} values ranging from <0.01 μM to 1.1 μM. In particular, compound **6** demonstrated remarkable cytotoxicity against the A-427 ovarian cancer, the lung cancer LCLC-103H, urinary bladder cancer RT-4 and cervical cancer SISO cell lines with IC_{50} <0.01-0.30 μM, inducing apoptosis in two representative cell lines.

1. Introduction

Since the majority of cancer diseases are multifactorial, a treatment with a single mono functional "targeted" drug may not be sufficient as a therapeutic option. Application of molecular hybridization to the design, discovery and optimization of biological active compounds has emerged as a useful strategy that involves combination of two distinct and independently acting chemical entities in one molecular scaffold to develop hybrid "multitarget-directed compunds". It is commonly accepted that cancer chemotherapy with hybrid compounds might exhibit numerous advantages over the conventional anticancer drugs with a single target (Abbot et al. 2017; Fortin and Bérubé 2013; Gediya and Njar 2009; Kucuksayan and Ozben 2017; Shaveta et al. 2016; Zhao and Huang 2017).

The coumarin moiety (2*H*-chromen-2-one) occupies a central position as a privileged scaffold in the design and discovery of novel therapeutic molecules with high affinity and specificity to different molecular targets (Ibrar et al. 2018; Penta 2015; Sandhu et al. 2014; Stefanachi et al. 2018). Over the last few years, a considerable amount of attention has been paid to coumarin-hybrid compounds with anticancer properties (Elshemy and Zaki 2017; Emami and Dadashpour 2015; Morsy et al. 2017; Thakur et al. 2015). Thus, coumarin hybrids were identified as protein kinase inhibitors (Chand et al. 2013; Nasr et al. 2014; Payne et al. 2010), heat shock protein 90 (Hsp90) inhibitors (Zhao et al. 2011) or telomerase inhibitors (Wu et al. 2014) as well as inhibitors of the tumor-associated carbonic anhydrase isoforms IX and XII (Angapelly et al. 2017; Chandak et al. 2016), inhibitors of aromatase with therapeutic potential in the treatment of hormone-dependent breast cancer (Leonetti et al. 2004; Stefanachi et al. 2011), and histone deacetylase inhibitors (Abdizadeh et al. 2017; Seidel et al. 2014). Furthermore, antitumor effects of the coumarin-based hybrids are also related to apoptosis-inducing capability (Belluti et al. 2010; Elshemy and Zaki 2017; Kamath et al. 2015; Nasr et al. 2014; Sabt et al. 2018; Sashidhara et al. 2013), cell cycle arrest (Chen et al. 2012), inhibition of tubulin polymerization (Batran et al. 2018) and inhibition of angiogenesis (Avin et al. 2014; El-Sawy et al. 2017). Worth noting is the fact that 2-imino-analogues of coumarins, namely 2-imino-coumarins (2*H*-chromen-2-imines), are less known. Nevertheless, some variously substituted 2-imino-coumarins were also reported as potential antitumor agents (Burke et al. 1993; Gill et al. 2017; Huang 1996, Huang et al. 1995) including protein tyrosine kinase inhibitors (Burke et al. 1993; Huang 1996; Huang et al. 1995).

Despite the importance of benzoxazole/benzothiazole scaffolds in the design and development of new antineoplastic agents (Abdelgawad et al. 2013, 2017; Altintop et al. 2018; An et al. 2016; Belal et al. 2017; Kamal et al. 2014; Murty et al. 2013; Rajasekhar et al. 2017; Reddy et al. 2016; Sharma et al. 2013; Singh and Singh 2014), only a few reports on the antitumor activity of 3-benzoxazole/3-benzothiazole-coumarin hybrids could be found in the literature (Cournia et al. 2009; Kim et al. 2012, Kim et al. 2009; Lee et al. 2006; Wang et al. 2013; Yaghmaei et al. 2017; Zhang et al. 2015).

Although they have a common chemical motif of 3-heteroaryl-coumarin, there is a variety of mechanisms for their anticancer action including induction of caspase-3 dependent apoptosis (Yaghmaei et al. 2017), inhibition of Skp2 function (Zhang et al. 2015), angiogenesis inhibition (Lee et al. 2006) or disruption of microtubule function (Kim et al. 2012, 2009). A prominent example of this class of compounds is 7-diethylamino-3-(2'-benzoxazolyl)-coumarin (DBC) described as a potential anti-angiogenic agent (Lee et al. 2006) and novel microtubule inhibitor with antimitotic activity in multidrug resistant cancer cells (Kim et al. 2009) (Fig. 1). To the best of our knowledge, there are no publications on the antiproliferative activity of 2-imino-coumarins substituted at position 3 with either the benzoxazole or the benzothiazole moiety.

In this context, and in connection with our research program on 2-imino-2*H*-chromen-3-yl-1,3,5-triazine chemistry and biological activities (Makowska et al. 2018), we extended our studies on the synthesis of 2-imino-coumarin hybrids, characterized by the presence of benzoxazole (series **A-I**) or benzothiazole ring system (series **A-II**) at position 3 as well as their corresponding coumarin analogues (series **B-I** and **B-II**). We have also explored structure-activity relationships for *in vitro* antitumor properties of these classes of compounds (Fig. 1), and performed preliminary experiments on the mechanism of cell death.

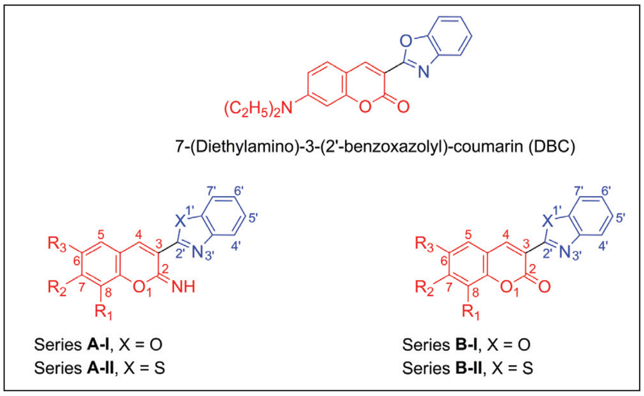
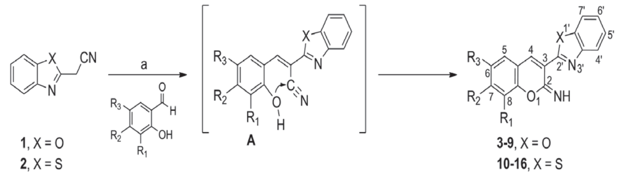


Fig. 1: Structures of reported and studied in this work hybrid molecules containing benzoxazole/benzothiazole and 2-imino-coumarin/coumarin scaffolds.

2. Investigations, results and discussion

2.1. Chemistry

Following the convenient synthetic routes towards 3-heterocyclic substituted 2-imino-coumarins (Chemate and Sekar 2015; Dryanska 1987, 1991; Gorobets et al. 2002; Komatsu et al. 2007; Kovalenko et al. 1998), we prepared the target 3-(benzoxazol-2-yl)-2-imino-coumarins **3-9** (series **A-I**) and 3-(benzothiazol-2-yl)-2-imino-coumarins **10-16** (series **A-II**) by reacting the readily available (1,3-benzoxazol-2-yl)acetonitrile (**1**) (Sakamoto et al. 1995) or (1,3-benzothiazol-2-yl)acetonitrile (**2**) (Saito et al. 1983), respectively, with the corresponding 2-hydroxybenzaldehydes. As shown in Scheme 1, the intermediate of type **A** formed in the first stage of the reaction sequence undergoes a spontaneous intramolecular addition of the hydroxyl OH group to a carbon-triple-bond-nitrogen CN function to give the final 2-imino-coumarins. This typical Knoevenagel condensation reaction is usually carried out in the presence of a base catalyst such as piperidine or aqueous sodium hydroxide in ethanol (Chemate and Sekar 2015; Dryanska 1987; Gorobets et al. 2002; Komatsu et al. 2007; Kovalenko 1998). It is pertinent to note that under similar conditions 2-imino-coumarins may undergo transformation into coumarins resulting from the hydrolysis of the imino group (Chao et al. 2010; Hassan 2009; Volmajer et al. 2005).



3, X = O, R_1 = Cl, R_2 = H, R_3 = H
4, X = O, R_1 = OCH_3, R_2 = H, R_3 = H
5, X = O, R_1 = Br, R_2 = H, R_3 = H
6, X = O, R_1 = H, R_2 = $(C_2H_5)_2N$, R_3 = H
7, X = O, R_1 = H, R_2 = H, R_3 = F
8, X = O, R_1 = H, R_2 = H, R_3 = CH_3
9, X = O, R_1 = H, R_2 = H, R_3 = Cl

10, X = S, R_1 = Cl, R_2 = H, R_3 = H
11, X = S, R_1 = OCH_3, R_2 = H, R_3 = H
12, X = S, R_1 = Br, R_2 = H, R_3 = H
13, X = S, R_1 = H, R_2 = $(C_2H_5)_2N$, R_3 = H
14, X = S, R_1 = H, R_2 = H, R_3 = F
15, X = S, R_1 = H, R_2 = H, R_3 = CH_3
16, X = S, R_1 = H, R_2 = H, R_3 = Cl

Scheme 1: Synthetic route to compounds **3-9** (series **A-I**) and **10-16** (series **A-II**). Reagents and condition: (a) EtOH, Et_3N, 3 min, 40-50 °C, 71-96% yield for **3-4**, **8** and **10-11**, **14-15** or EtOH, Et_3N, 1 min, 40 °C then ambient temperature (20-22 °C), 15 min, 71-93% yield for **5**, **7**, **9** and **12**, **16** or EtOH, Et_3N, 15 min, 50 °C then ambient temperature (20-22 °C), 18 h, 38-68% yield for **6** and **13**.

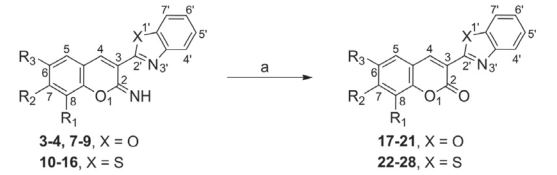
We have found in our study that for 2-imino-coumarin products **3-9** and **10-16** the best results in terms of yields and purity were obtained when the condensation process was catalyzed by triethylamine in 98 % ethanol at 20-50 °C. The above reactions proceed smoothly by short heating of the substrates in ethanol at 40-50 °C (**3-4**, **8** and **10-11**, **14-15**, 71-96% yield) or at room temperature within 15 min (**5**, **7**, **9** and **12**, **16**, 71-93% yield). However, in the case of the less active 2-hydroxybenzaldehyde containing the electron-donating diethylamino substituent the formation of the 2-imino-coumarin hybrids **6** and **13** could be accomplished upon heating of the substrates at 50 °C for 15 min and extending reaction time at room temperature to 18 h.

Structures of the 2-imino-coumarin derivatives **3-9** and **10-16** were confirmed by elemental analyses as well as IR and ^1H NMR spectroscopic data. The IR spectra of these compounds contain a strong absorption of the imino C2=N-H group in the range of 3302 to 3213 cm^{-1}. In turn, ^1H NMR spectra present a characteristic singlet attributable to the proton of C4-H of the coumarin ring in the range of 8.50 to 8.78 ppm, while the proton signal of the imino C2=N-H group appears in the range of 9.04 to 10.54 ppm.

It should be noted that 3-(benzothiazol-2-yl)-2-imino-coumarins **11** (Dryanska 1991) and **13** (Ahamed and Ghosh 2013; Gorobets et al. 2002; Jiang et al. 2018; Khan et al. 2018; Kim et al. 2011; Komatsu et al. 2007; Park and Kim 2013; Wang et al. 2014) have been previously reported. However, no biological properties were reported.

It is well known that imino-coumarins are useful intermediates in the synthesis of coumarin derivatives (Dryanska 1987; Khilya et al. 2001; Kovalenko et al. 1998; Wang et al. 2013). Thus, as shown in Scheme 2, the resulting benzoxazole-iminocoumarins **3-4**, **7-9** and benzothiazole-iminocoumarins **10-16** were further converted into the both known and novel benzoxazole-coumarins **17**, **18** (Lack et al. 2011), **19**, **20** (Ciba Ltd. 1963), **21** (Ciba Ltd. 1963; Han et al. 2009; Jiang et al. 2016; Min et al. 2012) (series **B-I**) or benzothiazole-coumarins **22**, **23** (Chao et al. 2010; Dryanska 1991; Khilya et al. 2001), **24**, **25** (Lee et al. 2006; Min et al. 2012; Soto-Ortega et al. 2011; Zhou et al. 2010), **26**, **27** (Chao et al. 2010; Min et al. 20121), **28** (Hassan 2009; Min et al. 2010; Youssef et al. 2006; Zhou et al. 2010) (series **B-II**), respectively, by means of hydrolysis of the imino group upon heating in dimethylformamide (DMF) containing 10% water.

This method is based on our previous findings that the imino-coumarins upon treatment with refluxing aqueous DMF furnished coumarins and no mineral acid was required for the hydrolysis of the imino group (Makowska et al. 2018).

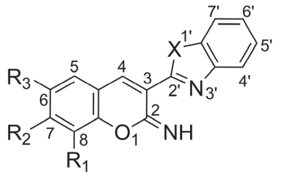


17, X = O, R_1 = Cl, R_2 = H, R_3 = H
18, X = O, R_1 = OCH_3, R_2 = H, R_3 = H
19, X = O, R_1 = H, R_2 = H, R_3 = F
20, X = O, R_1 = H, R_2 = H, R_3 = CH_3
21, X = O, R_1 = H, R_2 = H, R_3 = Cl

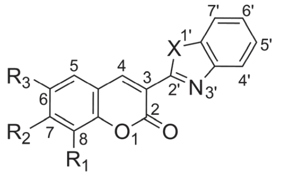
22, X = S, R_1 = Cl, R_2 = H, R_3 = H
23, X = S, R_1 = OCH_3, R_2 = H, R_3 = H
24, X = S, R_1 = Br, R_2 = H, R_3 = H
25, X = S, R_1 = H, R_2 = $(C_2H_5)_2N$, R_3 = H
26, X = S, R_1 = H, R_2 = H, R_3 = F
27, X = S, R_1 = H, R_2 = H, R_3 = CH_3
28, X = S, R_1 = H, R_2 = H, R_3 = Cl

Scheme 2: Synthetic route to compounds **17-21** (series **B-I**) and **22-28** (series **B-II**). Reagents and conditions: (a) DMF/H_2O, Δ, 10 min, 80-85% yield for **17-21** or DMF/H_2O, Δ, 30 min, 70-93% yield for **22-28**.

Table 1: Results of primary screening in three cancer cell lines (5637, A-427, LCLC-103H)[a]



Series **A-I**, X = O
Series **A-II**, X = S



Series **B-I**, X = O
Series **B-II**, X = S

Substructure	Series A		Substructure	Series B	
	Compd.	**Activity[b]**		**Compd.**	**Activity[b]**
I	**3**	A	**I**	**17**	NA
I	**4**	NA	**I**	**18**	NA
I	**5**	A	**I**	**19**	A
I	**6**	A	**I**	**20**	NA
I	**7**	A	**I**	**21**	NA
I	**8**	A	**II**	**22**	A
I	**9**	NA	**II**	**23**	NA
II	**10**	A	**II**	**24**	NA
II	**11**	NA	**II**	**25**	A
II	**12**	A	**II**	**26**	A
II	**13**	A	**II**	**27**	NA
II	**14**	A	**II**	**28**	A
II	**15**	A			
II	**16**	NA			

Compounds were considered active if they inhibited cell growth at 20 µM by greater than 50% in at least one cell line after 96 h

[a] See Bracht et al. (2006).

[b] Activity denoted as: A = active, NA = not active.

All of the final compounds **17-28** were characterized by elemental analyses and spectroscopic data (IR and ^{1}H NMR) presented in the Experimental part. Their IR spectra revealed absence of absorption bands in the range of 3302 to 3213 cm^{-1} present in the 2-imino-coumarin analogues, while a strong absorption in the range of 1759 to 1711 cm^{-1} corresponds to the carbonyl C=O group. In the ^{1}H NMR spectra recorded in DMSO-d_6 solution a characteristic singlet of C4-H proton of the coumarin ring is found at 9.04-10.40 ppm. There are no signals attributable to C2=NH imino group.

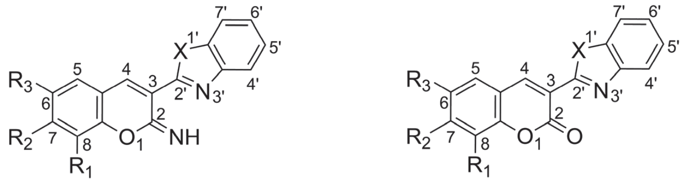
2.2. *In vitro cytotoxic activity*

To evaluate the potential usefulness of the prepared benzoxazole-iminocoumarin **3-9** (series **A-I**) and benzothiazole-iminocoumarin **10-16** (series **A-II**) hybrid compounds and their coumarin analogues **17-28** (series **B-I** and **B-II**, respectively) as antitumor agents, we first performed primary screening of the compounds by treating three cell lines of different origin: human urinary bladder cancer 5637, human lung cancer A-427 and LCLC-103H with the compounds all at a concentration of 20 µM. Compounds that inhibited cell proliferation by 50 % or more in at least one cell line, as established by a crystal violet-based microtiter plate assay (Bracht et al. 2006), were considered active (Table 1). Thus, for the active benzoxazoles **3**, **5-8** (series **A-I**) and **19** (series **B-I**) as well as benzothiazoles **10**, **12-15** (series **A-II**) and **22**, **25-26**, **28** (series **B-II**), a secondary screening was done to determine their antiproliferative potency in a panel of five human cancer cell lines, including human pancreatic cancer cell line DAN-G, human lung cancer cell lines A-427 and LCLC-103H, human urinary bladder cancer cell line RT-4 and human cervix cancer cell line SISO. The results of the secondary screening are presented in Table 2 and Figs. 2-3 as the average IC$_{50}$ values calculated from dose-response data obtained from 3 independent experiments after 96 h exposure. In general, the compounds tested displayed modest to high cell growth inhibitory potency (IC$_{50}$ values between <0.01 µM and 42.56 µM) compared to the widely used anticancer agent cisplatin (IC$_{50}$ in the range of 0.24-1.96 µM, Table 2). Other reference compound was coumarin, which was inactive in all cell lines up to 20 µM, and 7-diethylaminocoumarin, which was inactive at concentrations up to 50 µM except in the A-427 line, where it was weakly active.

Among the benzoxazole-iminocoumarins of series **A-I** (compounds **3**, **5**-**8**), the most potent was derivative **6** containing a diethylamino substituent at position 7 of the imino-coumarin moiety, for which the calculated IC$_{50}$ values were in the range of 0.06-0.29 µM (Table 2, Fig. 2). Compared to cisplatin, compound **6** proved to be more active in the DAN-G, A-427, LCLC-103H and RT-4 cell lines (IC$_{50}$ = 0.06-0.29 µM vs. 0.73-1.96 µM) (Table 2, Fig. 2). Replacement of the 7-diethylamino group for 6-F-substituent in the imino-coumarin scaffold resulted in slight decrease in antiproliferative potency (compare **6** with **7**: IC$_{50}$ = 0.06-0.29 µM vs. 0.99-3.22 µM), while the introduction of 8-Cl (**3**), 8-Br (**5**) or 6-CH$_3$ (**8**) substituents into parent structure led to compounds with moderate activity (IC$_{50}$ values in the range between 11.89 µM and 42.56 µM). The least active in this series was compound **3** bearing a chlorine atom at position 8 of the imino-coumarin ring (Table 2, Fig. 2).

Next, it was found that replacement of the benzoxazole for benzothiazole ring in the imino-coumarin hybrids generally increased the antiproliferative potency as evidenced by comparison of IC$_{50}$ values of benzoxazole-iminocoumarins **3**, **5**, **7** and **8** (series **A-I**, Table 2) with activities of the related benzothiazole analogues **10**, **12**, **14** and **15** (series **A-II**, Table 2). The exception to this was the

Table 2: Results of secondary screening (IC_{50} ± SD, µM) of benzazole-iminocoumarin derivatives of series A (A-I, X = O, A-II, X = S), benzazole-coumarin derivatives of series B (B-I, X = O, B-II, X = S) and reference compounds for growth inhibition of five human cancer cell lines after 96 h



Series **A-I**, X = O
Series **A-II**, X = S

Series **B-I**, X = O
Series **B-II**, X = S

						Cell line				
Compd.	**Series**	**R_1**	**R_2**	**R_3**	**X**	**DAN-G**	**A-427**	**LCLC-103H**	**RT-4**	**SISO**
3	A-I	Cl	H	H	O	19.44 ± 5.50	28.76 ± 8.29	42.56 ± 10.52	28.64 ± 13.26	16.61 ± 13.79
5	A-I	Br	H	H	O	17.71 ± 1.95	11.89 ± 0.86	16.65 ± 2.21	27.32 ± 12.83	22.02 ± 1.60
6	A-I	H	$(C_2H_5)_2N$	H	O	0.10 ± 0.04	0.12± 0.05	0.06± 0.05	0.27 ± 0.11	0.29 ± 0.06
7	A-I	H	H	F	O	1.96 ± 0.37	2.38 ± 0.63	0.99 ± 0.21	3.22 ± 1.2	2.44 ± 0.89
8	A-I	H	H	CH_3	O	20.76 ± 1.91	17.74 ± 1.02	31.32 ± 5.76	14.72 ± 2.73	34.69 ± 7.95
10	A-II	Cl	H	H	S	14.09 ± 0.88	14.56 ± 0.55	8.69 ± 0.63	1.44 ± 0.13	13.8 ± 0.55
12	A-II	Br	H	H	S	15.93 ± 1.11	12.26 ± 0.92	10.29 ± 2.13	13.89 ± 3.89	18.89 ± 4.95
13	A-II	H	$(C_2H_5)_2N$	H	S	1.05 ± 0.13	1.22 ± 0.23	0.82 ± 0.09	0.54 ± 0.39	1.13 ± 0.17
14	A-II	H	H	F	S	1.45 ± 0.61	1.36 ± 0.97	0.98 ± 0.71	1.11 ± 0.81	1.12 ± 0.49
15	A-II	H	H	CH_3	S	12.74 ± 0.91	13.05 ± 1.01	13.67 ± 1.34	14.03 ± 1.1	13.14 ± 0.72
19	B-I	H	H	F	O	17.61 1.4	18.94 ± 3.22	21.65 ± 2.33	14.91 ± 0.13	17.37 ± 0.47
22	B-II	Cl	H	H	S	8.91 ± 2.4	16.89 ± 1.11	8.01 ± 0.34	0.52 ± 0.19	12.76 ± 0.54
25	B-II	H	$(C_2H_5)_2N$	H	S	1.69 ± 0.1	1.37 ± 0.11	0.86 ± 0.09	2.24 ± 0.38	1.81 ± 0.12
26	B-II	H	H	F	S	0.2 ± 0.2	0.15 ± 0.09	0.33 ± 0.39	1.1 ± 0.24	0.25 ± 0.12
28	B-II	H	H	Cl	S	9.11 ± 1.3	6.1 ± 0.91	10.5 ± 4.09	2.52 ± 3.58	7.39 ± 0.34
Coumarin						>20	>20	>20	>20	>20
7-Diethylaminocoumarin						>50	17.55 ± 5.73	>50	>50	>50
Cisplatin (CDDP)[a]						0.73 ± 0.34	1.96 ± 0.54	0.90 ± 0.19	1.61 ± 0.16	0.24 ± 0.06

[a] See Bracht et al. (2006).

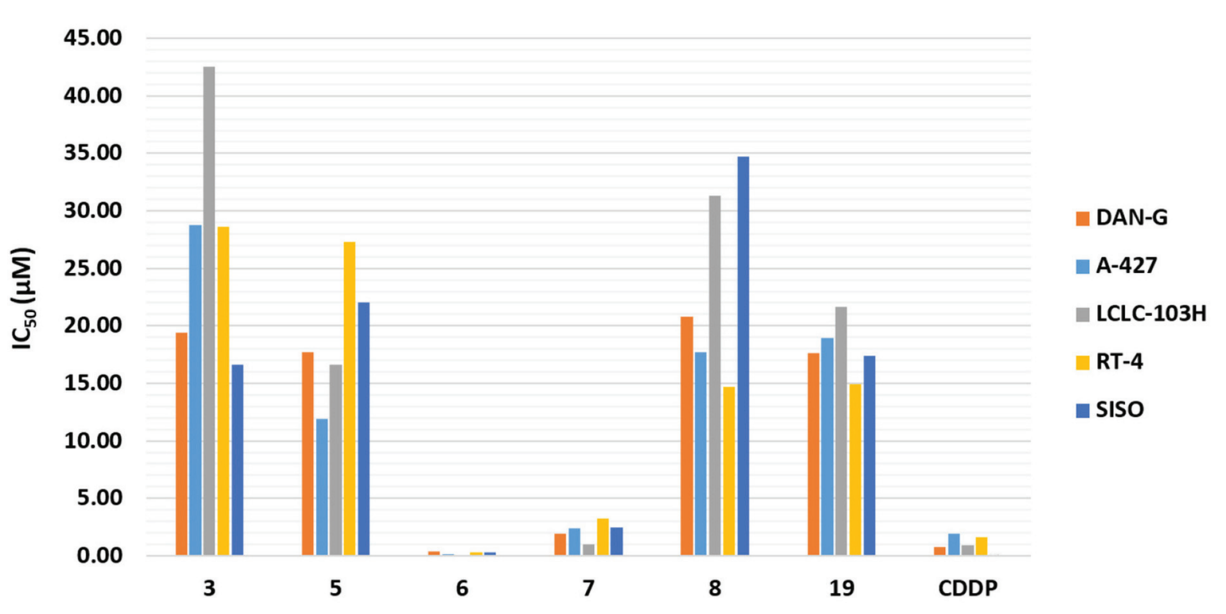


Fig. 2: Average IC_{50} (µM) values of 3-(benzoxazol-2-yl)-2*H*-chromen-2-imines **3**, **5-8** (series **A-I**) and 3-(benzoxazol-2-yl)-2*H*-chromen-2-one **19** (series **B-I**) on five cancer cell lines (DAN-G, A-427, LCLC-103H, RT-4 and SISO) compared to cisplatin (CDDP).

2-imino-coumarin pair **6** and **13**, where the benzoxazole analogue **6** was actually more active than the benzothiazole counterpart **13** (IC_{50} = 0.06-0.29 µM vs. 0.54-1.22 µM). Similarly to benzoxazole-iminocoumarins the best activity was found for 7-diethylamino- and 6-F-substituted benzothiazole-iminocoumarins **13** and **14** (IC_{50} = 0.54-1.22 µM and 0.98-1.45 µM, respectively) (Table 2, Fig. 3).

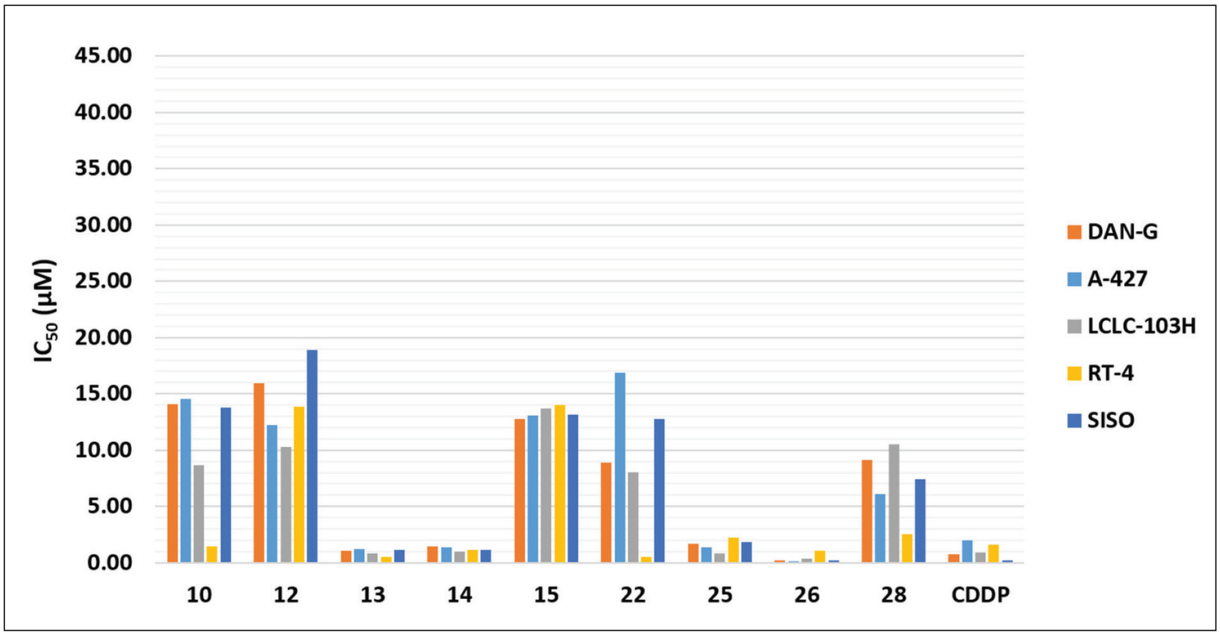


Fig. 3: Average IC_{50} (µM) values of 3-(benzothiazol-2-yl)-2*H*-chromen-2-imines **10**, **12-15** (series **A-II**) and 3-(benzothiazol-2-yl)-2*H*-chromen-2-ones **22**, **25-26**, **28** (series **B-II**) on five human cancer cell lines (DAN-G, A-427, LCLC-103H, RT-4 and SISO) compared to cisplatin (CDDP).

Furthermore, in the group of the benzoxazole-coumarin analogues of series **B-I** (compounds **17-21**), only compound **19** with 6-F substituent displayed significant growth inhibitory properties toward tested tumor cell lines, although showed lower potency than its 2-imino-coumarin counterpart **7** (IC_{50} = 14.91-21.65 µM vs. 0.99-3.22 µM), (Table 2, Fig. 2). Other derivatives with variously substituted coumarin ring systems did not pass the preliminary test (Table 1). It was assumed that the lack of activity could in part be due to the very low solubility of compounds **17-18**, **20-21** under the conditions of the microtiter plate assay. On the contrary, transformation of benzothiazole-iminocumarins **10-16** into the corresponding coumarins **22-28** resulted in compounds **22**, **25**-**26** and **28** (series **B-II**) that retained a pronounced antiproliferative activity (IC_{50} values in the range of 0.15-16.89 µM, Table 2, Fig. 3).

It is pertinent to know that the benzothiazole-coumarins **25** and **28** were evaluated previously against cancer cell lines different from those used in our studies (Hassan 2009; Lee et al. 2006). Nevertheless, in contrast to our observation, the compounds **25** (Lee et al. 2006) and **28** (Hassan 2009) were reported to be devoid of cytotoxic activity against the tested cell lines. The most active compound in this category was the 6-F-substituted derivative **26** which inhibited cancer proliferation with IC_{50} values ranging from 0.15 µM to 1.1 µM and showed greater potency than cisplatin in the DAN-G, A-427 and LCLC-103H cell lines (Table 2, Fig. 3).

Concerning selectivity of the compounds evaluated it should be noted that although the LCLC-103H and RT-4 cell lines were the most sensitive to the compounds tested, the results generally show no great selectivity toward any specific cancer cell line. Moreover, from the pattern of IC_{50} values it can be concluded that a significant antiproliferative activity was associated with the electron-donating

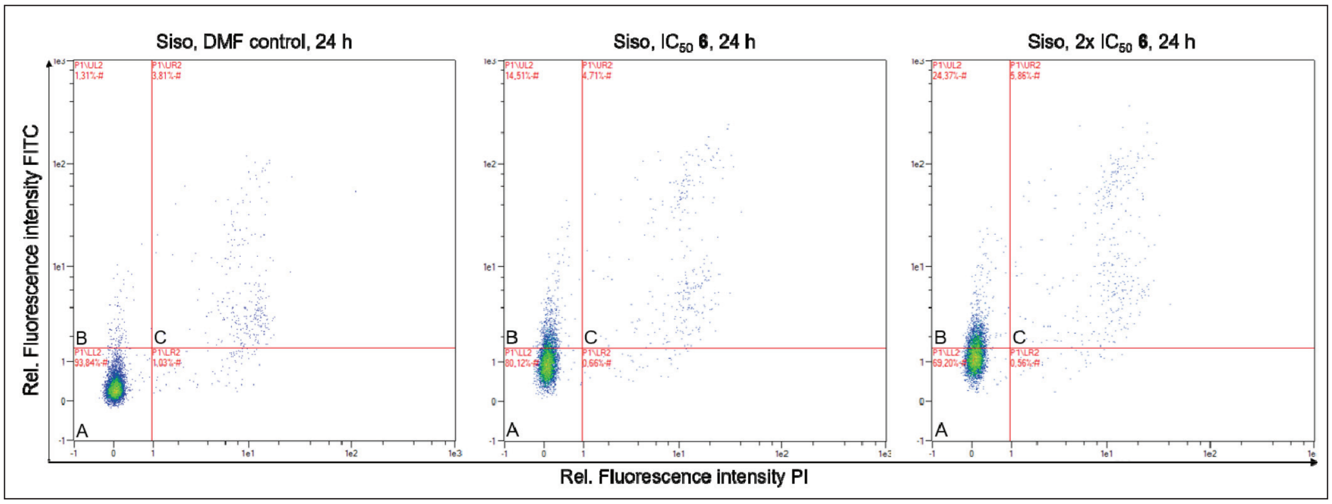


Fig. 4: Representative dot plots from flow cytometric analysis of the Annexin V-FITC/PI Assay in SISO cells after treatment with either DMF or the IC_{50} or doubled IC_{50} of **6** for 24 h. Displayed on x-axis: fluorescence intensity of propidium iodide (PI). Displayed on y-axis: fluorescence intensity of fluorescein isothiocyanate (FITC). Left: 24 h solvent control DMF. Centre: 24 h IC_{50} of **6**. Right: 24 h doubled IC_{50} of **6**. Analysis quadrants: A. viable cells (FITC/PI negative) B. early apoptotic cells (FITC positive/ PI negative) C. late apoptotic cells (FITC/PI positive).

7-diethylamino group as evidenced by compound **6**, the most active agent among all tested compounds. For this reason, we investigated whether **6** can induce apoptosis in two representative cell lines, SISO and A-427.

2.3. *Induction of apoptosis by compound 6*

Apoptosis is a key mechanism of programmed cell death induce by chemotherapeutic drugs such as cisplatin (Ouyang et al. 2012). A number of methods have been used to detect apoptosis in treated cancer cells, one of the most common is to double stain treated cells with the fluorescent labels Annexin-V-FITC and propidium iodide, which together distinguish cells as either normal, in early stage or late stage apoptosis (Vermes et al. 1995). The distribution of cells in the various stages is measured by fluorescent flow cytometry and the three fractions are quantified relative to each other by means of dot-plots (Fig. 4).

Figure 5 summarizes the average results of three independent experiments after treatment of cells for 24 h and 28 h at either the IC_{50} or double IC_{50} concentrations of compound **6**. After a 24 h treatment with the IC_{50} and doubled IC_{50} of **6** 14 % and 24 % of the SISO cells displayed signs of early apoptosis, respectively, while there was no significant increase of late apoptotic cells. An incubation period of 48 h resulted in a fraction of 75 % and 82 % early apoptotic SISO cells for the IC_{50} and doubled IC_{50} of **6**, respectively, and a significant increase to 10 % late apoptotic cells for the higher concentration of **6**.

In A-427 cells a 24 h treatment caused a portion of 22 % and 32 % of early apoptotic cells and 3 % and 5 % of late apoptotic cells for the IC_{50} and doubled IC_{50} of **6**, respectively. A prolonged incubation of 48 h resulted in a significant increase to 30 % and 43 % early apoptotic and 8 % and 12 % late apoptotic cells for the two applied concentrations of **6**.

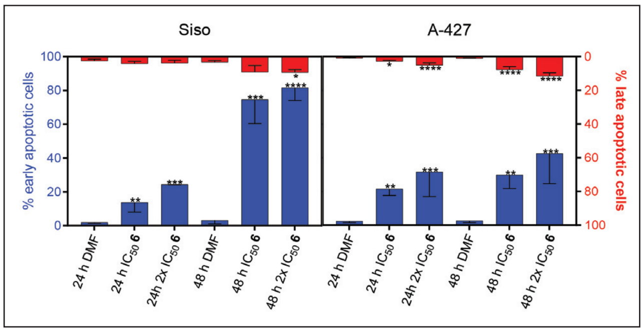


Fig. 5: Evaluation of apoptosis initiation by **6** in SISO and A-427 cells via flow cytometric analysis of Annexin V-FITC and PI staining**.** The fractions of early apoptotic cells are displayed via the left y-axis and the late apoptotic cells via the right y-axis. Apoptotic events were analysed after 24 h and 48 h after incubation of the cells with either the IC_{50} or the doubled IC_{50} of **6** or the solvent DMF. Mean values ± SD of ≥3 independent replicates are displayed. A One-way ANOVA with Dunnett's multiple comparisons Post-hoc-Test was performed relating the treatment to the solvent control with DMF for the respective incubation time; $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

2.4. *Conclusion*

Two series of 3-(benzoxazol-2-yl)- and 3-(benzothiazol-2-yl)-2*H*-chromen-2-imines as well as the corresponding 3-(benzoxazol-2-yl)- and 3-(benzothiazol-2-yl)-2*H*-chromen-2-ones were synthesized for their evaluation as potential antitumor agents. Analysis of the structure-activity relationships of the cytotoxic activity on the human cancer cell lines of benzoxazole-iminocoumarin hybrids **3-9** (series **A-I**) or benzothiazole-iminocoumarin hybrids **10-16** (series **A-II**) revealed that the introduction of an electron-donating diethylamino substituent at position 7 of the imino-coumarin moiety improved the antiproliferative potency (compounds **6** and **13**), which is consistent with our previous studies of 2-imino-2*H*-chromen-3-yl-1,3,5-triazines (Makowska et al. 2018). In general, the transformation of benzoxazole-iminocoumarins **3-4** and **7-9** into the corresponding coumarins **17-21** (series **B-I**) led to loss in activity, while transformation of benzothiazole-iminocoumarins **10-16** into the benzothiazole-coumarin analogues **22-28** (series **B-II**) often resulted in compounds with high potency. Moreover, the cytotoxicity of coumarin derivatives of series **B-I** and **B-II** was enhanced by the presence of an electron-withdrawing F substituent at position 6 of the coumarin ring. From all of the compounds studied, 3-(benzo[*d*]oxazol-2-yl)-*N*,*N*-diethyl-2-imino-2*H*-chromen-7-amine (**6**) and 3-(benzo[*d*]thiazol-2-yl)-6-fluoro-2*H*-chromen-2-one (**26**) showed the most potent cytotoxic activity. Based on these findings one may conclude that the compounds incorporating both the benzoxazole or the benzothiazole moiety and imino-coumarin or coumarin ring system represent novel antitumor lead structures. Particularly, benzoxazole-iminocoumarin hybrid molecule **6** appears likely to be a useful heterocyclic scaffold for further structural optimization along with studies of the mechanism of cytotoxic activity. Preliminary results from the Annexin-V/propidium iodide assay indicate that compound **6** strongly induces apoptosis in human cancer cells.

3. Experimental

3.1. *Chemistry – General*

Melting points were determined with a Boëtius apparatus and are uncorrected. The infrared spectra were obtained on KBr pastilles using a Nicolet 380 FT-IR 1600 spectrophotometer. Magnetic resonance spectra (NMR) were recorded on a Varian Gemini 200, a Varian Unity 500, or a Bruker Avance III HD apparatus in a DMSO-d_6 solution. The residual peak of solvent was used as an internal standard. The chemical shift values (δ) are reported in ppm and coupling constants (*J*) in hertz (Hz). The elemental analyses for C, H and N were within 0.4% of the theoretical values. (1,3-Benzoxazol-2-yl)acetonitrile (**1**) (Sakamoto et al. 1995) and (1,3-benzothiazol-2-yl)acetonitrile (**2**) (Saito et al. 1983) were prepared according to published methods.

3.2. *General procedure for the preparation of 3-(benzoxazol-2-yl)-2H-chromen-2-imines 3-4, 8 and 3-(benzothiazol-2-yl)-2H-chromen-2-imines 10-11, 14-15*

To a stirred solution of (1,3-benzoxazol-2-yl)acetonitrile (**1**) (158 mg, 1.0 mmol) or (1,3-benzothiazol-2-yl)acetonitrile (**2**) (174 mg, 1.0 mmol) in 98 % ethanol (2 ml) was added the corresponding 2-hydroxybenzaldehyde (1.5 mmol) at 40 °C. After 1 min, three drops of triethylamine was added at 50 °C, and the reaction mixture was stirred at 40-50 °C for 3 min. The solid that precipitated was filtered, washed with anhydrous ethanol and crystallized from a mixture of ethanol and DMF in a ratio of 2:1. In this manner the following compounds were prepared.

3.2.1. *3-(Benzo[d]oxazol-2-yl)-8-chloro-2H-chromen-2-imine **(3)***

Starting from (1,3-benzoxazol-2-yl)acetonitrile (**1**) (158 mg, 1.0 mmol) and 3-chloro-2-hydroxybenzaldehyde (235 mg, 1.5 mmol). Yield 84 % as a yellow solid, m.p.: 216-217 °C. IR (KBr, ν/cm^{-1}): 3285, 3031, 1654, 1592, 1451, 1391, 1230, 1082, 992, 884, 759, 730. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 10.40 (br s, 1H, NH), 8.57 (s, 1H, 4-H), 7.92 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.86-7.82 (m, 1H, Ar-H), 7.79 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.75 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.54 (t, *J* = 7.3 Hz, 1H, Ar-H), 7.49 (t, *J* = 7.8 Hz, 1H, Ar-H), 7.29 (t, *J* = 7.8 Hz, 1H, Ar-H). Elemental analysis: calcd: C, 64.77 %, H, 3.06 %, N, 9.44 %, found: C, 64.65 %, H, 3.27 %, N, 9.28 %.

3.2.2. *3-(Benzo[d]oxazol-2-yl)-8-methoxy-2H-chromen-2-imine **(4)***

Starting from (1,3-benzoxazol-2-yl)acetonitrile (**1**) (158 mg, 1.0 mmol) and 2-hydroxy-3-methoxybenzaldehyde (228 mg, 1.5 mmol). Yield 83 % as a yellow solid, m.p.: 208-209 °C. IR (KBr, ν/cm^{-1}): 3272, 3040, 1654, 1602, 1474, 1453, 1391, 1269, 1239, 1108, 968, 883, 771, 722. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 10.54 (s, 1H, NH), 8.54 (s, 1H, 4-H), 7.92 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.83 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.55-7.47 (m, 2H, Ar-H), 7.38-7.29 (m, 2H, Ar-H), 7.22 (t, *J* = 7.3 Hz, 1H, Ar-H), 3.93 (s, 3H, OCH_3). Elemental analysis: calcd: C, 69.86 %, H, 4.14 %, N, 9.58 %, found: C, 69.95 %, H, 3.93 %, N, 9.67 %.

3.2.3. *3-(Benzo[d]oxazol-2-yl)-6-methyl-2H-chromen-2-imine **(8)***

Starting from (1,3-benzoxazol-2-yl)acetonitrile (**1**) (158 mg, 1.0 mmol) and 2-hydroxy-5-methylbenzaldehyde (204 mg, 1.5 mmol). Yield 81 % as a yellow solid, m.p.: 220-222 °C. IR (KBr, ν/cm^{-1}): 3286, 3045, 1684, 1575, 1488, 1454, 1392, 1243, 1084, 986, 890, 821, 731. ^{1}H NMR (DMSO-d_6, 200 MHz) δ/ppm: 10.13 (s, 1H, NH), 8.50 (s, 1H, 4-H), 7.90-7.81 (m, 2H, Ar-H), 7.58-7.56 (m, 1H, Ar-H), 7.53-7.41 (m, 3H, Ar-H), 7.23 (s, 1H, Ar-H), 2.34 (s, 3H, CH_3). Elemental analysis: calcd: C, 73.90 %, H, 4.38 %, N, 10.14 %, found: C, 73.79 %, H, 4.65 %, N, 10.23 %.

3.2.4. *3-(Benzo[d]thiazol-2-yl)-8-chloro-2H-chromen-2-imine **(10)***

Starting from (1,3-benzothiazol-2-yl)acetonitrile (**2**) (174 mg, 1.0 mmol) and 3-chloro-2-hydroxybenzaldehyde (235 mg, 1.5 mmol). Yield 96 % as a yellow solid, m.p.: 215-216 °C. IR (KBr, ν/cm^{-1}): 3245, 3051, 1671, 1594, 1557, 1474, 1447, 1317, 1303, 1217, 1202, 1034, 925, 859, 757, 734, 722. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 9.46 (s, 1H, NH), 8.78 (s, 1H, 4-H), 8.17 (d, J = 6.8 Hz, 1H, Ar-H), 8.08 (d, J = 7.3 Hz, 1H, Ar-H), 7.85 (d, J = 6.3 Hz, 1H, Ar-H), 7.69 (d, J = 6.8 Hz, 1H, Ar-H), 7.62-7.56 (m, 1H, Ar-H), 7.50-7.44 (m, 1H, Ar-H), 7.29 (t, J = 7.3 Hz, 1H, Ar-H). Elemental analysis: calcd: C, 61.44 %, H, 2.90 %, N, 8.96 %, found: C, 61.27 %, H, 3.07 %, N, 8.89 %.

3.2.5. *3-(Benzo[d]thiazol-2-yl)-8-methoxy-2H-chromen-2-imine **(11)***

Starting from (1,3-benzothiazol-2-yl)acetonitrile (**2**) (174 mg, 1.0 mmol) and 2-hydroxy-3-methoxybenzaldehyde (228 mg, 1.5 mmol). Yield 89 % as a yellow solid, m.p.: 188-189 °C (mp: 187-188°C, Dryanska 1991). IR (KBr, ν/cm^{-1}): 3271, 3051, 1667, 1602, 1571, 1482, 1306, 1270, 1206, 1110, 1038, 935, 840, 777, 757, 728. ^{1}H NMR (DMSO-d_6, 200 MHz) δ/ppm: 9.21 (s, 1H, NH), 8.72 (s, 1H, 4-H), 8.13 (d, J = 7.6 Hz, 1H, Ar-H), 8.06 (d, J = 7.8 Hz, 1H, Ar-H), 7.59-7.36 (m, 3H, Ar-H), 7.28-7.16 (m, 2H, Ar-H), 3.90 (s, 3H, OCH$_3$). Elemental analysis: calcd: C, 66.22 %, H, 3.92 %, N, 9.08 %, found: C, 66.35 %, H, 3.77 %, N, 8.97 %.

3.2.6. *3-(Benzo[d]thiazol-2-yl)-6-fluoro-2H-chromen-2-imine **(14)***

Starting from (1,3-benzothiazol-2-yl)acetonitrile (**2**) (174 mg, 1.0 mmol) and 5-fluoro-2-hydroxybenzaldehyde (210 mg, 1.5 mmol). Yield 93 % as a yellow solid, m.p.: 259-260 °C. IR (KBr, ν/cm^{-1}): 3230, 3053, 1662, 1611, 1569, 1488, 1428, 1367, 1318, 1219, 1141, 1052, 971, 947, 928, 860, 804, 752, 721. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 9.21 (s, 1H, NH), 8.78 (s, 1H, 4-H), 8.17 (d, J = 7.8 Hz, 1H, Ar-H), 8.08 (d, J = 8.3 Hz, 1H, Ar-H), 7.80-7.78 (m, 1H, Ar-H), 7.58 (t, J = 7.3 Hz, 1H, Ar-H), 7.48 (t, J = 7.8 Hz, 1H, Ar-H), 7.43-7.40 (m, 1H, Ar-H), 7.32-7.29 (m, 1H, Ar-H). Elemental analysis: calcd: C, 64.85 %, H, 3.06 %, N, 9.45 %, found: C, 64.77 %, H, 2.95 %, N, 9.57 %.

3.2.7. *3-(Benzo[d]thiazol-2-yl)-6-methyl-2H-chromen-2-imine **(15)***

Starting from (1,3-benzothiazol-2-yl)acetonitrile (**2**) (174 mg, 1.0 mmol) and 2-hydroxy-5-methylbenzaldehyde (204 mg, 1.5 mmol). Yield 71 % as a yellow solid, m.p.: 176-177 °C. IR (KBr, ν/cm^{-1}): 3216, 3052, 2911, 1662, 1573, 1492, 1433, 1375, 1318, 1223, 1126, 1052, 965, 933, 874, 817, 749, 719. ^{1}H NMR (DMSO-d_6, 200 MHz) δ/ppm: 9.04 (s, 1H, NH), 8.68 (s, 1H, 4-H), 8.16-8.05 (m, 2H, Ar-H), 7.62-7.33 (m, 4H, Ar-H), 7.15 (d, J = 8.4 Hz, 1H, Ar-H), 2.34 (s, 3H, CH$_3$). Elemental analysis: calcd: C, 69.84 %, H, 4.14 %, N, 9.58 %, found: C, 69.65 %, H, 4.27 %, N, 9.69 %.

3.3. *General procedure for the preparation of 3-(benzoxazol-2-yl)-2H-chromen-2-imines 5, 7, 9 and 3-(benzothiazol-2-yl)-2H-chromen-2-imines 12, 16*

To a stirred solution of (1,3-benzoxazol-2-yl)acetonitrile (**1**) (158 mg, 1.0 mmol) or (1,3-benzothiazol-2-yl)acetonitrile (**2**) (174 mg, 1.0 mmol) in 98 % ethanol (2 ml) was added the corresponding 2-hydroxybenzaldehyde (1.5 mmol) at 40 °C and after 1 min, three drops of triethylamine was added. Then reaction mixture was stirred at ambient temperature (20-20 °C) for 15 min. The solid that precipitated was filtered, washed with anhydrous ethanol and crystallized from a mixture of ethanol and DMF in a ratio of 2:1. In this manner the following compounds were prepared.

3.3.1. *3-(Benzo[d]xazol-2-yl)-8-bromo-2H-chromen-2-imine **(5)***

Starting from (1,3-benzoxazol-2-yl)acetonitrile (**1**) (158 mg, 1.0 mmol) and 3-bromo-2-hydroxybenzaldehyde (302 mg, 1.5 mmol). Yield 71 % as a yellow solid, m.p.: 206-207 °C. IR (KBr, ν/cm^{-1}): 3275, 3036, 1660, 1591, 1445, 1399, 1254, 1234, 1080, 982, 876, 770, 725. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 10.37 (br s, 1H, NH), 8.55 (s, 1H, 4-H), 7.93 (d, J = 7.8 Hz, 1H, Ar-H), 7.88 (d, J = 7.8 Hz, 1H, Ar-H), 7.84-7.81 (m, 2H, Ar-H), 7.54 (t, J = 7.3 Hz, 1H, Ar-H), 7.51-7.46 (m, 1H, Ar-H), 7.23 (t, J = 7.3 Hz, 1H, Ar-H). Elemental analysis: calcd: C, 56.33 %, H, 2.66 %, N, 8.21 %, found: C, 56.55 %, H, 2.43 %, N, 7.93 %.

3.3.2. *3-(Benzo[d]oxazol-2-yl)-6-fluoro-2H-chromen-2-imine **(7)***

Starting from (1,3-benzoxazol-2-yl)acetonitrile (**1**) (158 mg, 1.0 mmol) and 5-fluoro-2-hydroxybenzaldehyde (210 mg, 1.5 mmol). Yield 92 % as a yellow solid, m.p.: 256-257 C. IR (KBr, ν/cm^{-1}): 3291, 3039, 1663, 1608, 1576, 1489, 1426, 1396, 1245, 1082, 868, 815, 726. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 10.24 (s, 1H, NH), 8.56 (s, 1H, 4-H), 7.94 (d, J = 7.3 Hz, 1H, Ar-H), 7.85 (d, J = 7.8 Hz, 1H, Ar-H), 7.72 (dd, J_1 = 2.9 Hz, J_2 = 8.3 Hz, 1H, Ar-H), 7.56 (t, J = 7.3 Hz, 1H, Ar-H), 7.52-7.49 (m, 2H, Ar-H), 7.39-7.35 (m, 1H, Ar-H). Elemental analysis: calcd: C, 68.57 %, H, 3.24 %, N, 10.00 %, found: C, 68.67 %, H, 3.43 %, N, 9.83 %.

3.3.3. *3-(Benzo[d]oxazol-2-yl)-6-chloro-2H-chromen-2-imine **(9)***

Starting from (1,3-benzoxazol-2-yl)acetonitrile (**1**) (158 mg, 1.0 mmol) and 5-chloro-2-hydroxybenzaldehyde (235 mg, 1.5 mmol). Yield 73 % as a yellow solid, m.p.: 259-260 °C. IR (KBr, ν/cm^{-1}): 3289, 3023, 1661, 1595, 1566, 1480, 1452, 1393, 1245, 1071, 956, 875, 819, 732. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 10.29 (s, 1H, NH), 8.55 (s, 1H, 4-H), 7.94 (d, J = 7.8 Hz, 1H, Ar-H), 7.86 (d, J = 7.8 Hz, 1H, Ar-H), 7.66 (d, J = 8.3 Hz, 1H, Ar-H), 7.56 (t, J = 7.3 Hz, 1H, Ar-H), 7.52-7.46 (m, 2H, Ar-H), 7.42 (d, J = 8.8 Hz, 1H, Ar-H). Elemental analysis: calcd: C, 64.77 %, H, 3.06 %, N, 9.44 %, found: C, 64.55 %, H, 3.27 %, N, 9.35 %.

3.3.4. *3-(Benzo[d]thiazol-2-yl)-8-bromo-2H-chromen-2-imine **(12)***

Starting from (1,3-benzothiazol-2-yl)acetonitrile (**2**) (174 mg, 1.0 mmol) and 3-bromo-2-hydroxybenzaldehyde (302 mg, 1.5 mmol). Yield 93 % as a yellow solid, m.p.: 218-219 °C. IR (KBr, ν/cm^{-1}): 3302, 3051, 2925, 1663, 1601, 1587, 1554, 1475, 1441, 1301, 1197, 1027, 972, 916, 835, 756, 734. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 9.42 (s, 1H, NH), 8.76 (s, 1H, 4-H), 8.16 (d, J = 8.3 Hz, 1H, Ar-H), 8.08 (d, J = 7.8 Hz, 1H, Ar-H), 7.88 (d, J = 7.3 Hz, 1H, Ar-H), 7.83 (d, J = 7.8 Hz, 1H, Ar-H), 7.58 (t, J = 7.3 Hz, 1H, Ar-H), 7.48 (t, J = 7.3 Hz, 1H, Ar-H), 7.23 (t, J = 7.8 Hz, 1H, Ar-H). Elemental analysis: calcd: C, 53.80 %, H, 2.54 %, N, 7.84 %, found: C, 53.93 %, H, 2.37 %, N, 7.75 %.

3.3.5. *3-(Benzo[d]thiazol-2-yl)-6-chloro-2H-chromen-2-imine **(16)***

Starting from (1,3-benzothiazol-2-yl)acetonitrile (**2**) (174 mg, 1.0 mmol) and 5-chloro-2-hydroxybenzaldehyde (235 mg, 1.5 mmol). Yield 88 % as a yellow solid, m.p.: 246-247 °C. IR (KBr, ν/cm^{-1}): 3252, 3051, 1658, 1597, 1557, 1478, 1434, 1367, 1319, 1215, 1205, 1087, 1048, 959, 923, 869, 840, 803, 751, 725. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 9.29 (s, 1H, NH), 8.76 (s, 1H, 4-H), 8.17 (d, J = 7.8 Hz, 1H, Ar-H), 8.09 (d, J = 7.8 Hz, 1H, Ar-H), 8.01 (d, J = 2.4 Hz, 1H, Ar-H), 7.59-7.56 (m, 2H, Ar-H), 7.52-7.47 (m, 1H, Ar-H), 7.29 (d, J = 8.8 Hz, 1H, Ar-H). Elemental analysis: calcd: C, 61.44 %, H, 2.90 %, N, 8.96 %, found: C, 61.51 %, H, 3.01 %, N, 9.07 %.

3.4. *General procedure for the preparation of 6 and 13*

To a stirred solution of (1,3-benzoxazol-2-yl)acetonitrile (**1**) (158 mg, 1.0 mmol) or (1,3-benzothiazol-2-yl)acetonitrile (**2**) (174 mg, 1.0 mmol) in 98 % ethanol (2 ml) was added 4-(diethylamino)-2-hydroxybenzaldehyde (290 mg, 1.5 mmol) and three drops of triethylamine. Stirring was continued at 50 °C for 15 min and then at ambient temperature (20-22 °C) for 18 h. The precipitate thus obtained was collected by vacuum filtration, washed with anhydrous ethanol and purified by crystallization from a mixture of ethanol and DMF in a ratio of 2:1. In this manner the following compounds were prepared.

3.4.1. *3-(Benzo[d]oxazol-2-yl)-N,N-diethyl-2-imino-2H-chromen-7-amine **(6)***

Starting from (1,3-benzoxazol-2-yl)acetonitrile (**1**) (158 mg, 1.0 mmol). Yield 68 % as an orange solid, m.p.: 219-220 C. IR (KBr, ν/cm^{-1}): 3279, 3009, 2970, 1655, 1602, 1539, 1452, 1411, 1279, 1235, 1137, 1074, 883, 821, 740. ^{1}H NMR (DMSO-d_6, 200 MHz) δ/ppm: 9.84 (s, 1H, NH), 8.34 (s, 1H, 4-H), 7.83-7.72 (m, 2H, Ar-H), 7.54-7.40 (m, 3H, Ar-H), 6.66-6.61 (m, 1H, Ar-H), 6.47 (s, 1H, Ar-H), 3.48 (q, J = 6.8 Hz, 4H, 2xCH$_2$), 1.14 (t, J = 7.8 Hz, 6H, 2xCH$_3$). Elemental analysis: calcd: C, 72.05 %, H, 5.74 %, N, 12.60 %, found: C, 71.93 %, H, 5.67 %, N, 12.71 %.

3.4.2. *3-(Benzo[d]thiazol-2-yl)-N,N-diethyl-2-imino-2H-chromen-7-amine **(13)***

Starting from (1,3-benzothiazol-2-yl)acetonitrile (**2**) (174 mg, 1.0 mmol). Yield 38 % as an orange solid, m.p.: 183-184 °C (Komatsu et al. 2007). IR (KBr, ν/cm^{-1}): 3213, 3051, 2971, 1664, 1607, 1585, 1515, 1470, 1411, 1356, 1203, 1129, 1076, 952, 881, 821, 801, 757, 729. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 8.62 (s, 1H, NH), 8.54 (s, 1H, 4-H), 8.08 (d, J = 7.3 Hz, 1H, Ar-H), 7.97 (t, J = 7.3 Hz, 1H, Ar-H), 7.59 (d, J = 8.3 Hz, 1H, Ar-H), 7.52-7.50 (m, 1H, Ar-H), 7.43-7.34 (m, 1H, Ar-H), 6.65 (d, J = 8.3 Hz, 1H, Ar-H), 6.41 (s, 1H, Ar-H), 3.47-3.43 (m, 4H, 2xCH$_2$), 1.16 (t, J = 5.4 Hz, 6H, 2xCH$_3$). Elemental analysis: calcd: C, 68.74 %, H, 5.48 %, N, 12.02 %; found: C, 68.65 %, H, 5.35 %, N, 12.15 %.

3.5. *General procedure for the preparation of 3-(benzoxazol-2-yl)-2H-chromen-2-ones 17-21 and 3-(benzothiazol-2-yl)-2H-chromen-2-ones 22-28*

An appropriate 3-(benzoxazol-2-yl)-2*H*-chromen-2-imine **3-4**, **7-9** (1.0 mmol) or 3-(benzothiazol-2-yl)-2*H*-chromen-2-imine **10-16** (1.0 mmol) was dissolved in DMF (2-4 ml) containing 10% water. Then the solution was slowly heated with stirring to boiling for 10 min (in the case of **3-4** and **7-9**) or 30 min (in the case of **10-16**), before being cooled to ambient temperature (20-22 °C). Stirring was continued at room temperature (20-22 °C) and crushed ice was added until precipitate was formed. The product was filtered, washed with water, dried and crystallized from DMF. In this manner the following compounds were prepared.

3.5.1. *3-(Benzo[d]oxazol-2-yl)-8-chloro-2H-chromen-2-one **(17)***

Starting from 3-(benzo[*d*]oxazol-2-yl)-8-chloro-2*H*-chromen-2-imine (**3**) (297 mg, 1 mmol). Yield 84% as a yellow solid, m.p.: 203-204 °C. IR (KBr, ν/cm^{-1}): 3068, 2927, 1759, 1598, 1560, 1448, 1224, 1182, 1003, 976, 950, 771, 730. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 9.10 (s, 1H, 4-H), 7.98 (d, J = 7.8 Hz, 1H, Ar-H), 7.91-7.86 (m, 2H, Ar-H), 7.83 (d, J = 7.8 Hz, 1H, Ar-H), 7.51-7.43 (m, 3H, Ar-H). Elemental analysis: calcd: C, 64.55 %, H, 2.71 %, N, 4.71 %, found: C, 64.45 %, H, 2.87 % N, 4.79 %.

3.5.2. *3-(Benzo[d]oxazol-2-yl)-8-methoxy-2H-chromen-2-one **(18)***

Starting from 3-(benzo[*d*]oxazol-2-yl)-8-methoxy-2*H*-chromen-2-imine (**4**) (292 mg, 1 mmol). Yield 80 % as a yellow solid, m.p.: 229-230 C (Lack et al. 2011). IR (KBr, ν/cm^{-1}): 3058, 2968, 2938, 1731, 1611, 1576, 1541, 1471, 1271, 1182, 1092, 956, 874, 773, 750, 721. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 9.07 (s, 1H, 4-H), 7.88-7.83 (m, 2H, Ar-H), 7.56 (d, J = 7.8 Hz, 1H, Ar-H), 7.52-7.45 (m, 2H, Ar-H), 7.41-7.38 (m, 2H, Ar-H), 3.97 (s, 3H, OCH$_3$). Elemental analysis: calcd: C, 69.62 %, H, 3.78 %, N, 4.78 %, found: C, 69.57 %, H, 3.57 %, N, 4.85 %.

3.5.3. *3-(Benzo[d]oxazol-2-yl)-6-fluoro-2H-chromen-2-one* **(19)**

Starting from 3-(benzo[*d*]oxazol-2-yl)-6-fluoro-2*H*-chromen-2-imine (**7**) (280 mg, 1 mmol). Yield 89 % as a yellow solid, m.p.: 291-292 C. IR (KBr, n/cm^{-1}): 3048, 2920, 1745, 1570, 1488, 1422, 1383, 1285, 1248, 1186, 1117, 959, 924, 837, 773, 745. ^{1}H NMR (DMSO-d_6, 200 MHz) δ/ppm: 9.05 (s, 1H, 4-H), 7.97-7.81 (m, 3H, Ar-H), 7.71-7.46 (m, 4H, Ar-H). Elemental analysis: calcd: C, 68.33 %, H, 2.87 %, N, 4.98 %, found: C, 68.21 %, H, 2.95 %, N, 4.85 %.

3.5.4. *3-(Benzo[d]oxazol-2-yl)-6-methyl-2H-chromen-2-one* **(20)**

Starting from 3-(benzo[*d*]oxazol-2-yl)-6-methyl-2*H*-chromen-2-imine (**8**) (276 mg, 1 mmol). Yield 85 % as a yellow solid, m.p.: 208-209 C (mp: 208.5-209.5 °C [Ciba Ltd. 1963]). IR (KBr, n/cm^{-1}): 3052, 2923, 1745, 1620, 1575, 1497, 1451, 1211, 1190, 1021, 819, 774, 745. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 8.99 (s, 1H, 4-H), 7.85 (d, J = 7.8 Hz, 1H, Ar-H), 7.82 (d, J = 7.3 Hz, 1H, Ar-H), 7.77 (s, 1H, Ar-H), 7.57 (dd, J_1 = 2.0 Hz, J_2 = 8.8 Hz, 1H, Ar-H), 7.49-7.44 (m, 2H, Ar-H), 7.40 (d, J = 8.8 Hz, 1H, Ar-H), 2.40 (s, 3H, CH$_3$). Elemental analysis: calcd: C, 73.64%, H, 4.00%, N, 5.05%, found: C, 73.47%, H, 4.15%, N, 4.85%.

3.5.5. *3-(Benzo[d]oxazol-2-yl)-6-chloro-2H-chromen-2-one* **(21)**

Starting from 3-(benzo[*d*]oxazol-2-yl)-6-chloro-2*H*-chromen-2-imine (**9**) (297 mg, 1 mmol). Yield 89 % as a yellow solid, m.p.: 263-264 C (mp: 237-239 °C [Jiang et al. 2016]). IR (KBr, n/cm^{-1}): 3050, 1747, 1605, 1569, 1480, 1453, 1386, 1250, 1120, 1078, 973, 927, 868, 814, 774, 745. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 9.04 (s, 1H, 4-H), 8.14 (d, J = 2.4 Hz, 1H, Ar-H), 7.89-7.84 (m, 2H, Ar-H), 7.80 (dd, J_1 = 2.4 Hz, J_2 = 8.8 Hz, 1H, Ar-H), 7.55 (d, J = 8.8 Hz, 1H, Ar-H), 7.53-7.46 (m, 2H, Ar-H). Elemental analysis: calcd: C, 64.55 %, H, 2.71 %, N, 4.71 %, found: C, 64.37 %, H, 2.67 %, N, 4.89 %.

3.5.6. *3-(Benzo[d]thiazol-2-yl)-8-chloro-2H-chromen-2-one* **(22)**

Starting from 3-(benzo[*d*]thiazol-2-yl)-8-chloro-2*H*-chromen-2-imine (**10**) (313 mg, 1 mmol). Yield 85 % as a yellow solid, m.p.: 229-230 C. IR (KBr, n/cm^{-1}): 3058, 1731, 1599, 1556, 1480, 1446, 1316, 1250, 1196, 1064, 985, 938, 765, 755, 726. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 9.25 (s, 1H, 4-H), 8.19 (d, J = 7.3 Hz, 1H, Ar-H), 8.10-8.05 (m, 2H, Ar-H), 7.89 (d, J = 7.3 Hz, 1H, Ar-H), 7.58 (t, J = 7.3 Hz, 1H, Ar-H), 7.51-7.45 (m, 2H, Ar-H). Elemental analysis: calcd: C, 61.25 %, H, 2.57 %, N, 4.46 %, found: C, 61.37 %, H, 2.35 %, N, 4.57 %.

3.5.7. *3-(Benzo[d]thiazol-2-yl)-8-methoxy-2H-chromen-2-one* **(23)**

Starting from 3-(benzo[*d*]thiazol-2-yl)-8-methoxy-2*H*-chromen-2-imine (**11**) (308 mg, 1 mmol). Yield 73 % as a yellow solid, m.p.: 246-249 C (mp: 239-240 °C [Chao et al. 2010]). IR (KBr, /cm^{-1}): 3059, 2938, 2839, 1742, 1586, 1438, 1435, 1348, 1276, 1213, 1196, 1105, 927, 765, 729. ^{1}H NMR (, DMSO-d_6, 500 MHz) δ/ppm: 9.25 (s, 1H, 4-H), 8.22 (d, J = 8.3 Hz, 1H, Ar-H), 8.11 (d, J = 8.3 Hz, 1H, Ar-H), 7.65 (d, J = 7.8 Hz, 1H, Ar-H), 7.61 (t, J = 7.8 Hz, 1H, Ar-H), 7.51 (t, J = 7.3 Hz, 1H, Ar-H), 7.47-7.41 (m, 2H, Ar-H), 3.98 (s, 3H, OCH$_3$). Elemental analysis: calcd: C, 66.01 %, H, 3.58 %, N, 4.53 %, found: C, 65.93 %, H, 3.65 %, N, 4.37 %.

3.5.8. *3-(Benzo[d]thiazol-2-yl)-8-bromo-2H-chromen-2-one* **(24)**

Starting from 3-(benzo[*d*]thiazol-2-yl)-8-bromo-2*H*-chromen-2-imine (**12**) (357 mg, 1 mmol). Yield 90 % as a yellow solid, m.p.: 276-277 C. IR (KBr, n/cm^{-1}): 3055, 2922, 1727, 1661, 1595, 1481, 1441, 1192, 1068, 972, 930, 758, 728. ^{1}H NMR (DMSO-d_6, 400 MHz) δ/ppm: 9.25 (s, 1H, 4-H), 8.22 (d, J = 8.0 Hz, 1H, Ar-H), 8.13-8.10 (m, 1H, Ar-H), 8.04 (dd, J_1 = 1.6 Hz, J_2 = 8.2 Hz, 1H, Ar-H), 7.63-7.59 (m, 1H, Ar-H), 7.50-7.53 (m, 1H, Ar-H), 7.42 (t, J = 8.1 Hz, 1H, Ar-H). Elemental analysis: calcd: C, 53.65 %, H, 2.25 %, N, 3.91 %, found: C, 53.47 %, H, 2.55 %, N, 4.01 %.

3.5.9. *3-(Benzo[d]thiazol-2-yl)-7-(diethylamino)-2H-chromen-2-one* **(25)**

Starting from 3-(benzo[*d*]thiazol-2-yl)-*N*,*N*-diethyl-2-imino-2*H*-chromen-7-amine (**13**) (349 mg, 1 mmol). Yield 70 % as a brownish solid, m.p.: 213-214 °C (mp: 213-215 °C [Min et al. 2012]). IR (KBr, n/cm^{-1}): 2972, 2929, 1715, 1617, 1578, 1510, 1474, 1349, 1263, 1197, 1133, 1078, 944, 757. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 9.05 (s, 1H, 4-H), 8.13 (d, J = 7.8 Hz, 1H, Ar-H), 8.00 (d, J = 8.3 Hz, 1H, Ar-H), 7.81 (d, J = 8.8 Hz, 1H, Ar-H), 7.54 (t, J = 9.3 Hz, 1H, Ar-H), 7.42 (t, J = 7.8 Hz, 1H, Ar-H), 6.87 (dd, J_1 = 2.4 Hz , J_2 = 9.3 Hz, 1H, Ar-H), 6.70 (d, J = 2.0 Hz, 1H, Ar-H), 3.53 (q, J = 6.8 Hz, 4H, 2xCH$_2$), 1.18 (t, J = 6.8 Hz, 6H, 2xCH$_3$). Elemental analysis: calcd: C, 68.55 %, H, 5.18 %, N, 7.99 %, found: C, 68.67 %, H, 5.07 %, N, 7.85 %.

3.5.10. *3-(Benzo[d]thiazol-2-yl)-6-fluoro-2H-chromen-2-one* **(26)**

Starting from 3-(benzo[*d*]thiazol-2-yl)-6-fluoro-2*H*-chromen-2-imine (**14**) (296 mg, 1 mmol). Yield 93 % as a yellow solid, m.p.: 266-267 C. IR (KBr, n/cm^{-1}): 3047, 2922, 1724, 1566, 1490, 1205, 969, 954, 818, 755, 722. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 9.26 (s, 1H, 4-H), 8.22 (d, J = 7.8 Hz, 1H, Ar-H), 8.12 (d, J = 8.3 Hz, 1H, Ar-H), 8.01-7.99 (m, 1H, Ar-H), 7.53-7.50 (m, 1H, Ar-H), 7.66-7.59 (m, 3H, Ar-H). Elemental analysis: calcd: C, 64.64 %, H, 2.71 %, N, 4.71 %, found: C, 64.47 %, H, 2.93 %, N, 4.67 %.

3.5.11. *3-(Benzo[d]thiazol-2-yl)-6-methyl-2H-chromen-2-one* **(27)**

Starting from 3-(benzo[*d*]thiazol-2-yl)-6-methyl-2*H*-chromen-2-imine (**15**) (292 mg, 1 mmol). Yield 78 % as a yellow solid, m.p.: 258-259 C (mp: 230-232 °C [Min et al. 2012]). IR (KBr, n/cm^{-1}): 3063, 2927, 1711, 1619, 1569, 1491, 1317, 1204, 1162, 965, 942, 815, 764. ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 9.20 (s, 1H, 4-H), 8.21 (d, J = 7.8 Hz, 1H, Ar-H), 7.99 (d, J = 8.3 Hz, 1H, Ar-H), 7.88 (s, 1H, Ar-H), 7.62-7.59 (m, 2H, Ar-H), 7.52-7.46 (m, 2H, Ar-H), 2.43 (s, 3H, CH$_3$). Elemental analysis: calcd: C, 69.61 %, H, 3.78 %, N, 4.77 %, found: C, 69.55 %, H, 3.67 %, N, 4.87 %.

3.5.12. *3-(Benzo[d]thiazol-2-yl)-6-chloro-2H-chromen-2-one* **(28)**

Starting from 3-(benzo[*d*]thiazol-2-yl)-6-chloro-2*H*-chromen-2-imine (**16**) (313 mg, 1 mmol).Yield 85 % as a yellow solid, m.p.: 249-251 °C (mp: 250-252 °C [Min et al. 2012]). IR (KBr, n/cm^{-1}): 3097, 3045, 1714, 1603, 1556, 1482, 1317, 1202, 1129, 1068, 967, 834, 758. ^{1}H NMR (DMSO-d_6, 200 MHz) δ/ppm: 9.24 (s, 1H, 4-H), 8.24-8.20 (m, 2H, Ar-H), 8.11 (d, J = 8.1 Hz, 1H, Ar-H), 7.79 (dd, J_1 = 2.3 Hz, J_2 = 8.9 Hz, 1H, Ar-H), 7.64-7.51 (m, 3H, Ar-H). Elemental analysis: calcd: C, 61.25 %, H, 2.57 %, N, 4.46 %, found: C, 61.35 %, H, 2.45 %, N, 4.35 %.

3.6. *In vitro cytotoxicity studies*

All cell culture reagents were purchased from Sigma (Deisenhofen, FRG). The cancer cell lines included: human pancreas cell adenocarcinoma (DAN-G), human lung carcinoma (A-427), human large cell lung carcinoma (LCLC-103H), human bladder cell carcinoma (5637 and RT-4) and human uterine cervical adenocarcinoma (SISO) were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, FRG). The culture medium for cell lines was RPMI-1640 medium containing 2 g/l HCO$_3$, and 10 % fetal calf serum (FCS). Cells were grown in 75 cm^2 plastic culture flasks (Sarstedt, Nümbrecht, FRG) in a humid atmosphere of 5 % CO$_2$ at 37 °C and were passaged shortly before becoming confluent. Cytotoxicity studies were performed with a well-established microtiter assay based on the staining of adherent cells with crystal violet and performed as previously described (Bracht et al. 2006). Briefly, a volume of 100 ml of a cell suspension were seeded into 96-well microtiter plates (Sarstedt) at a density of 1000 cell per well expect for the LCLC-103H cell line, which was plated out at 250 cells per well. Twenty-four hours later, cells were exposed to the substance at five concentrations per compound. The 1000-fold concentrated stock solutions in DMF or DMSO were serially diluted by 50 % in DMF or DMSO to give the feed solutions, which were diluted by 500-fold in the culture medium. The controls received DMF or DMSO. Each concentration was tested in eight wells, with each well receiving 100 ml of the medium containing the substance. The concentration ranges were chosen to bracket the expected IC$_{50}$ values as best as possible. Cells were then incubated for 96 h. After this, the medium was removed and replaced with 1 % glutaraldehyde/PBS. The cells were then stained with crystal violet and the optical density (OD) was measured at λ = 570 nm by use of a Sunrise plate reader (Tecan, Männedorf, Switzerland). The corrected percent growth values [T/C_{corr}(%)] were calculated by the following equation:

$$T/C_{corr}(\%) = (\mathrm{OD_T} - \mathrm{OD_{c,0}})/(\mathrm{OD_c} - \mathrm{OD_{c,0}}) \times 100$$

where OD$_\mathrm{T}$ is the mean absorbance of the treated cells, OD$_\mathrm{c}$ is the mean absorbance of the controls, and OD$_\mathrm{c,0}$ is the mean absorbance at the time the drug was added. The IC$_{50}$ values were estimated by a linear least-squares regression of the T/C_{corr} values *versus* the logarithm of the substance concentration; only concentrations that yielded T/C_{corr} values between 10% and 90% were used in the calculation. The reported IC$_{50}$ values are the averages of three independent experiments.

3.7. *Annex-V-FITC/Propidium iodide assay for apoptosis*

For this assay, 125,000 cells per well were seeded into 6-well culture plates, allowed to adhere for 24 h and incubated with the IC$_{50}$ (Siso: 0.285 μM; A-427: 0.119 μM), the doubled IC$_{50}$ of **6** or the solvent DMF for 24 and 48 h, respectively. Afterwards, the cells were collected by trypsinisation and the Annexin-V-FITC/propidium iodide assay was performed with an Annexin V-FITC Kit according to the manufacturer's instructions (Miltenyi Biotec, Bergisch-Gladbach, Germany). Finally, the cells were analysed by using the B1 (λ_{ex}= 488 nm; λ_{em}= 525-550 nm) and B3 (λ_{ex}= 488 nm; λ_{em}= 655-730 nm) channels of a MACSquant Analyzer 10 flow cytometer and data evaluated with MACSquantify™ software. Data display and statistical analysis was performed with the software GraphPad Prism7.

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