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Biological evaluation of novel amidino substituted coumarin-benzazole hybrids as promising therapeutic agents†

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Herein we present the design and the synthesis of novel substituted coumarin–benzimidazole/benzothiazole hybrids bearing a cyclic amidino group on the benzazole core as biologically active agents. All prepared compounds were evaluated for their *in vitro* antiviral and antioxidative activity as well as for their *in vitro* antiproliferative activity against a panel of several human cancer cell lines. Coumarin–benzimidazole hybrid **10** (EC_{50} 9.0–43.8 μM) displayed the most promising broad spectrum antiviral activity, while two other coumarin–benzimidazole hybrids **13** and **14** showed the highest antioxidative capacity in the ABTS assay, superior to the reference standard BHT (IC_{50} 0.17 and 0.11 mM, respectively). Computational analysis supported these results and demonstrated that these hybrids benefit from the high C–H hydrogen atom releasing tendency of the cationic amidine unit, and the pronounced ease with which they can liberate an electron, promoted by the electron-donating diethylamine group on the coumarin core. The coumarin ring substitution at position 7 with a *N*,*N*-diethylamino group also caused a significant enhancement of the antiproliferative activity, with the most active compounds being derivatives with a 2-imidazolinyl amidine group **13** (IC_{50} 0.3–1.9 μM) and benzothiazole derivative with a hexacyclic amidine group **18** (IC_{50} 1.3–2.0 μM).

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Introduction

Molecular hybridization and the synthesis of hybrid molecules has been a major focus of medicinal chemists for over two decades.[1] The main purpose of combining two or more structurally different and biologically active scaffolds is to improve the activity and the affinity of newly synthesized compounds, to overcome drug resistance, to reduce side effects or to optimize various physico-chemical parameters including ADMET properties.[2] Usually these hybrid molecules contain a naturally active fragment combined with a synthetically prepared pharmacophoric group giving rise to new molecules with great and diverse biological potential.[3] Among all known heterocyclic derivatives, nitrogen heterocycles have been widely used in the rational design of novel molecules with improved biological properties.[4a,b,5a,b] The most important benzazole representatives, benzimidazole and benzothiazole derivatives, have been well established as principal structural motifs in medicinal and pharmaceutical chemistry.[6a,b,7,8] Numerous publications and reviews have described the biological potential and versatile properties of suchlike derivatives displaying prominent antitumoral, antimicrobial, antiviral, antioxidative, anti-inflammatory and antihistaminic activities.[9a,b,10,11] We have previously reported that the biological activity could significantly be improved by the incorporation of an amidine group in the cationic form, usually placed at the termini of the molecules, on the benzazole scaffold.[12–14] Consequently, the presence of this amidine group could improve the interaction with the putative biological target since the complex between the molecule and the target is further stabilized through both H-bonding and electrostatic interactions.[15,16] On the other hand, the flavonoid coumarin and its derivatives comprise an extensive class of both naturally occurring and synthetic compounds that also exhibit remarkable pharmacological activities combined with generally low toxicity.[17a,b,18a,b]

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Countless coumarin derivatives have been reported, and they still remain one of the most important substructures in rational drug design and discovery.[19a,b,20,21] It is well established that coumarins readily interact with diverse biological targets and active sites through the formation of noncovalent interactions, such as hydrophobic and electrostatic interactions, π–π interactions and/or by forming hydrogen or van der Waals bonds.[22,23] Their interesting chemical, physical and spectroscopic properties due to the structural planarity and the reactivity of both the benzene and the pyrone ring make coumarins compelling in a diverse array of research fields.[24,25]

Recently, several studies were published describing the design, synthesis and biological evaluation of coumarin–benzazole hybrids. A group of authors synthesized coumarin–benzimidazole hybrids, namely 3-(1*H*-benzo[*d*]imidazol-2-yl)-7-(substituted amino)-2*H*-chromen-2-one derivatives, which were tested for their *in vitro* antitumor activity against a panel of 60 cancer cell lines.[26] The 7-ethanolamino substituted derivative showed promising activity towards leukemia, colon and breast cancer cell lines. Coumarin–benzimidazole hybrids consisting of two series, namely derivatives with benzimidazole nuclei directly attached to the coumarin ring as well as derivatives with an amide bond connecting the coumarin and benzimidazole nuclei were synthesized to explore their antioxidative and anti-inflammatory potential.[27] Another paper described the synthesis of a coumarin–benzothiazole–chlorambucil conjugate for photocontrolled release of the anticancer drug chlorambucil. The study confirmed that this conjugate displayed improved cytotoxicity in comparison to unconjugated chlorambucil due to effective intracellular release of the drug.[28] A coumarin–benzothiazole ratiometric ATP probe has been designed and synthesized in order to detect ATP in aqueous solutions. This sensor showed high selectivity over other nucleotide polyphosphate (NPP) anions, which is promising for the development and application of suchlike molecules for various biological assays.[29] Benzothiazolyl substituted coumarin derivatives were tested for their *in vitro* antiproliferative activity against the MCF-7 cell line, while molecular docking was performed on receptor tyrosine kinases (RTKs) as a possible molecular target.[30] Another group of authors designed and synthesized benzimidazole–coumarin conjugates with a –SCH_2– linkage between these two heterocyclic moieties, with several conjugates displaying anti-Hepatitis C virus activity.[31]

Recently, we designed and synthesized coumarin–benzazole conjugates which were evaluated for their antiproliferative activity against several cancer cell lines as well as for their antioxidative activity.[32] The *N*,*N*-diethylamino substituted benzimidazole/imidazo[4,5-*b*]pyridine–coumarin hybrids (Fig. 1a) was the most promising derivative with potent and selective activity against CEM acute lymphoblastic leukemia cells. All synthesized compounds also showed moderate antioxidative potential. Encouraged by these findings, we have now designed prepared and evaluated novel coumarin–benzazole hybrids (Fig. 1b).

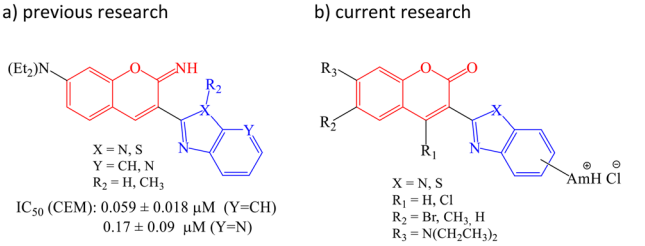


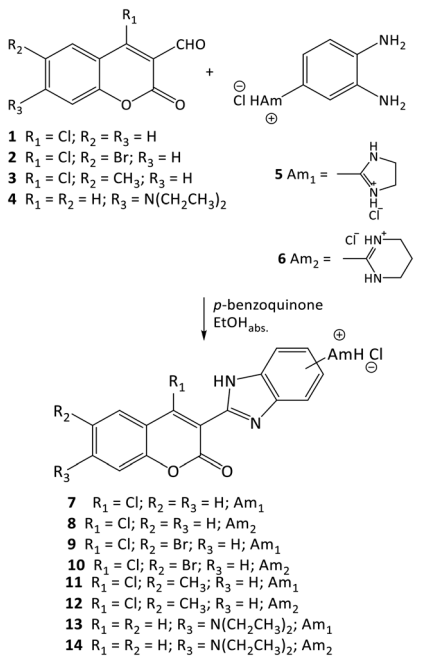
Fig. 1 Synthesized benzazole–coumarin conjugates in previous research (a) and current research (b).

The newly prepared derivatives were substituted at position 3, 4, 6 and/or 7 of the coumarin ring with benzazole (benzimidazole or benzothiazole) nuclei bearing amidine moieties. All newly synthesized compounds were evaluated for their *in vitro* antiviral activity, as well as for their antioxidative potential and antiproliferative activity on a diverse panel of cancer cell lines.

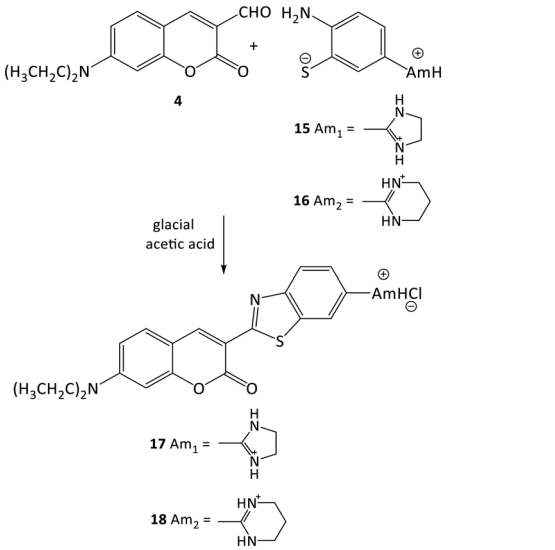
Results and discussion

Chemistry

Coumarin-benzimidazole/benzothiazole hybrids were synthesized by experimental procedures presented in Schemes 1 and 2. The main precursors necessary for the synthesis of the targeted amidino substituted benzimidazole derivatives, namely amidino substituted 1,2-phenylenediamines **5–6** and amidino substituted benzenethiolates **15–16** were synthesized according to previously published procedures that were optimized by our research group. Amidino substituted precursors **5–6** were prepared from the corresponding cyano substituted anilines,



Scheme 1 Synthesis of amidino-substituted coumarin-benzimidazole hybrids **7–14**.



Scheme 2 Synthesis of amidino-substituted coumarin-benzothiazole hybrids **17–18**.

while precursors **15–16** were both obtained through an acidic Pinner reaction from 6-cyanobenzothiazole in the form of zwitterions as monitored by IR spectroscopy. Targeted amidino substituted benzazole-coumarin conjugates were synthesized by using the reaction of cyclocondensation of corresponding precursors **5**, **6**, **15** and **16** with chosen commercially available substituted 2-oxo-2*H*-chromene-3-carbaldehydes **1–4**. Benzimidazole-coumarin hybrids **7–14** were synthesized in the cyclocondensation performed in absolute ethanol and by using *p*-benzoquinone as oxidants, in moderate reaction yields. Benzothiazole-coumarin hybrids obtained within the cyclocondensation in refluxing acetic acid followed by quenching with hydrochloric acid in moderate reaction yields. This method is direct condensation of aldehydes with amidino substituted 2-aminothiophenoles without using any catalyst or oxidant. Only 7-*N,N*-diethylamino substituted derivatives **17–18** were successfully synthesized while, the synthesis starting from halogeno substituted aldehydes **1–3** failed due to the competitive reaction of nucleophilic substitution.

All newly prepared amidino substituted coumarin-benzazole hybrids were structurally characterized with ^{1}H and ^{13}C NMR spectroscopy. NMR analysis was based on the values of H–H coupling constants in the ^{1}H spectra as well as chemical shifts in both ^{1}H and ^{13}C spectra, which were in consistence with the proposed structures. The signals for protons on N atoms on amidine groups were observed as bright signals in the region from 9–10 ppm. The signals for methylene protons of amidine group appeared in the aliphatic part of ^{1}H spectra as well as in the ^{13}C NMR spectra. The successful cyclocondensation and formation of benzimidazole nuclei was confirmed with the signal related to the proton of NH group on benzimidazole nuclei in the region around 13 ppm. IR spectroscopy was used for the monitoring of Pinner reaction due to the synthesis of main precursors.

The successful formation of imino-ester was confirmed within the disappearance of the signal for a CN group at around 2200 cm^{-1}.

Cytotoxic and antiviral activity

All synthesized coumarin-benzimidazole **7–14** and coumarin-benzothiazole **17–18** hybrids were tested for their antiviral activity.

The results for the cytotoxic and antiviral activity evaluation of the newly synthesized derivatives are summarized in Table 1.

For clarity, only compounds showing cytotoxic or antiviral activity are depicted. The results are expressed as CC_{50} (50% cytotoxic concentration) and EC_{50} (50% effective concentration) values. According to the obtained results we can conclude that the most promising antiviral activity was noted for coumarin-benzimidazole hybrid **10**, a 6-bromo-4-chloro substituted derivative bearing a hexacyclic amidine group (Fig. 2). This hybrid displayed broad spectrum antiviral activity with favorable EC_{50} values ranging from 9.0 μM to 51.0 μM against all tested influenza viruses (subtypes H1N1, H3N2 and B), promising activity towards flaviviruses (EC_{50} of 11.2 μM against YFV and 9.4 μM against zika virus), and more moderate activity against RSV (73.5 μM), sindbis virus (59.0 μM), and HCoV subtype OC43 (78.0 μM). Furthermore, hybrid **10** completely lacked cytotoxicity on all host cell lines, yielding encouraging selectivity indices. Benzimidazole derivatives **7–8** lacked antiviral activity, whereas the 6-bromo-4-chloro substituted derivative bearing a 2-imidazolinyl group **9** showed selective activity towards influenza A virus subtype H1N1. Interestingly, the *N*,*N*-diethylamino substituted benzimidazole hybrid **13** did not show any antiviral activity but proved markedly cytotoxic on all host cell lines. Among the synthesized benzothiazole derivatives, the *N*,*N*-diethylamino substituted derivative **18** bearing a hexacyclic amidine group displayed interesting activity against several respiratory viruses (EC_{50} values of 28.9 and 33.0 μM against HCoV OC43 and 229E, respectively, and 43.4 μM against RSV) as well as anti-HSV type 1 activity (25.5 μM), while the 2-imidazolinyl substituted derivative **17** showed only moderate activity against two of these viruses.

Antioxidative activity *in vitro*

The antioxidant activity of the newly prepared coumarin-benzazole hybrids **7–14** and **17–18** is presented in Table 2. Reference compound BHT (butylated hydroxytoluene) was included in all assays and the results are presented as IC_{50} values (for DPPH and ABTS) and $mmolFe^{2+}/mmol_C$ (for FRAP) in Table 2. The antioxidant capacity is measured as the ability of pure compounds to decrease the assay color reacting directly with the $ABTS^{\bullet+}$ radicals which could be used to evaluate hydrophilic and lipophilic compounds as well.

Obtained results revealed that two coumarin-benzimidazole hybrids substituted with *N*,*N*-diethylamino group at the position 7 bearing either 2-imidazolinyl **13** and

Table 1 Cytotoxicity and antiviral activity of hybrids 7–18

	Cytotoxicity (CC_{50}/μM)			Antiviral activity (EC_{50}/μM)											
Cpd	HEL 229	Huh7	MDCK	HCoV 229E HEL 299	HCoV OC43 HEL 299	HCoV NL63 Huh7	Influenza H1N1 MDCK	Influenza H3N2 MDCK	Influenza B MDCK	RSV Along HEL 299	HSV-1 KOS HEL 299	Yellow fewer 17D Huh7	Zika Mr776 Huh7	Sindbis Huh7
7	99.5	85	92.1	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
8	87.4	61.7	81.7	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
9	51.6	2.3	59.0	>100	>100	>100	16.2	>100	>100	>100	>100	>100	>100	>100
10	>100	>100	>100	>100	78.0	>100	43.8	51.0	9.0	73.5	>100	11.2	9.4	59
11	77	<0.8	10.0	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
12	>100	78.7	77.1	>100	74.2	>100	>100	>100	>100	64.1	>100	>100	>100	>100
13	5.1	<0.8	<0.8	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
14	>100	>100	43.4	>100	>100	>100	>100	>100	>100	73.3	>100	72.6	77.7	>100
17	>100	54.2	64.2	>100	69.7	>100	>100	>100	>100	>100	47.9	>100	>100	>100
18	>100	1.8	4.2	33.0	28.9	>100	>100	>100	>100	43.4	25.5	>100	>100	>100
Rem	>10	>10	—	0.06	0.06	0.03	—	—	—	0.03	—	6.2	0.7	>10
Rib	>250	8.9	67.0	82.6	170.1	>250	10.5	4.0	2.8	10.8	—	>250	>250	148.1
Zan	—	—	>100	—	—	—	0.1	16.8	0.05	—	—	—	—	—
Rim	—	—	>100	—	—	—	5.0	0.05	>100	—	—	—	—	—
BVDU	>100	—	—	—	—	—	—	—	—	—	0.05	—	—	—

hexacyclic amidine group **14** have shown the strongest antioxidative capacity, improved in comparison to the standard BHT (IC_{50} 0.17 and 0.11 mM, respectively). All other benzimidazole derivatives have shown moderate antioxidative capacity. Coumarin-benzothiazole hybrids **17–18** have proven to be less active in comparison to the benzimidazole analogues with the most active derivative bearing hexacyclic amidine group **18**. The radical scavenging method using DPPH stable radical indicates the ability of tested species to donate proton/electron. From the presented results, it can be concluded that all tested coumarin-benzazole hybrids were less active when compared to the standard BHT. The best activity was displayed by 4-chloro substituted benzimidazole derivative bearing hexacyclic amidine group **8** (IC_{50} 0.07 mM) as well as 6-bromine-4-chloro substituted benzimidazole derivative **13** bearing hexacyclic amidine group (IC_{50} 0.11 mM). Benzothiazole derivatives **17–18** did not showed any results within this assay due to precipitation. The reducing power of tested compounds refers to their antioxidant activity and FRAP assay was used for its determination. The FRAP values showed that all tested compounds are significantly less active relative to the standard BHT. The best activity in this assay was shown by 6-bromine-4-chloro substituted benzimidazole derivative bearing 2-imidazolinyl group **9**. Benzothiazole derivatives **17–18** were less active when compared to the benzimidazole analogues. Thus, to conclude, the most promising antioxidative capacity (ABTS assay) was observed for benzimidazole derivatives **13** and **14**

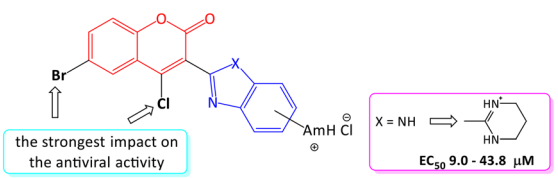


Fig. 2 Structure–activity relationship regarding the antiviral activity.

(Fig. 3), both substituted with *N*,*N*-diethylamino group at the position 7 being more active in comparison to BHT standard.

Scavenging the DPPH/ABTS/FRAP radicals by prepared compounds resulted in a decrease in absorption readings over time; the extent of decrease in DPPH/ABTS/FRAP absorption being proportional to the concentration of radicals that are being scavenged. It is well known that antioxidant capacity assays may be classified as electron transfer (SET) and hydrogen atom transfer (HAT)-based assays and that is difficult to distinguish between them. In most situations, these two reactions take place simultaneously. The results in our study emphasize that protentional antioxidant capacity of all prepared compounds is based on predominantly on SET mechanism in all three assays.

Computational analysis of the antioxidative activity

Computational analysis was employed to offer additional insights into the structure and properties of studied systems, and to elucidate relevant processes responsible for their antioxidant features. Given a large number of structurally similar systems inspected here, we decided to address a selected number of derivatives, involving **7**, **9** and the most potent **13** and **14**, together with several model systems **m1–m12** (Fig. 4). The latter are selected to allow enough structural and electronic information about the studied compounds, and aid in designing even more potent antioxidants based on the employed synthetic strategy. Data in Table 3 show that each system is characterized by its single-electron ionization energy (IE) and electron affinity (EA), together with the bond dissociation energy (BDE) required to homolytically cleave the hydrogen atom ($H^{\bullet}$) in the thermodynamically most favourable way. In some cases, BDE values for other bonds C–X (X = Cl, Br, NEt_2) are also computed, while all data are placed within a context by repeating the analysis for reference systems used in

Table 2 Antioxidative activity *in vitro* of tested compounds **7**–**14** and **17**–**18**[a]

Compound	ABTS (IC_{50}/mM)	DPPH (IC_{50}/mM)	FRAP ($mmolFe^{2+}/mmol_C$)
7	0.37 ± 0.04	0.44 ± 0.01	35.17 ± 0.88
8	0.29 ± 0.03	0.07 ± 0.01	38.37 ± 0.21
9	0.26 ± 0.01	0.13 ± 0.01	130.55 ± 3.54
10	0.36 ± 0.01	0.11 ± 0.00	95.25 ± 1.44
11	0.35 ± 0.02	0.19 ± 0.03	47.13 ± 0.23
12	0.46 ± 0.05	0.52 ± 0.08	47.87 ± 0.25
13	0.17 ± 0.01	0.14 ± 0.02	28.44 ± 0.75
14	0.11 ± 0.01	0.61 ± 0.04	28.83 ± 0.75
17	2.19 ± 0.26	—	2.84 ± 0.71
18	0.98 ± 0.09	—	3.29 ± 0.21
BHT	0.18 ± 0.02	0.025 ± 4.2	2089.34 ± 55.98

[a] Values are presented as means ± standard deviation.

experimental assays, namely $ABTS^{\bullet+}$, FRAP and $DPPH^{\bullet}$ (Table 4). All molecules with the amidine unit were considered as protonated monocations (Fig. 5), based on a typically high basicity of amidine ($pK_\alpha \approx 11$–13), which surpasses the basicity of benzimidazole ($pK_\alpha \approx 5$–6), benzothiazole ($pK_\alpha \approx 1$–2), coumarin ($pK_\alpha < 0$), and diethylaniline fragments ($pK_\alpha \approx 6$–7).[33]

Starting with the reference compounds (Table 4), FRAP is seemingly the simplest system, where it is clear that its radical scavenging ability relies on the electron-accepting SET mechanism that reduces a ferric Fe(III) complex into an ferrous Fe(II) analogue. According to the literature and our data, FRAP is an excellent radical scavenger, evident in a very high electron affinity EA = 147.0 kcal mol^{-1}. The latter surpasses ionization energies of all investigated systems (Table 3), thereby supporting the thermodynamic feasibility of the electron-transfer process, and confirming its reported non-selective nature for a broad array of antioxidants.[34] The situation with other reagents, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$, is less obvious, since their EA values cluster around 125 kcal mol^{-1}, which leaves only a modest (if any) thermodynamically favorable nature to exchange electrons with studied systems. Yet, we must emphasize that all of our systems have ionization energies that are either lower or close to 125 kcal mol^{-1}, which permits a certain feasibility of the SET mechanism even in these two assays. Moreover, when one relates the amount of the released energy following the attachment on the hydrogen atom onto the reference $ABTS^{\bullet+}$ (−36.6 kcal mol^{-1}) and $DPPH^{\bullet}$ (−66.7 kcal mol^{-1}) with the fact that all BDE values in **7**, **9**, **13** and **14** cluster around 80 kcal mol^{-1}, it leads to a conclusion that the HAT mechanism is highly unlikely with $ABTS^{\bullet+}$, and only moderately feasible with $DPPH^{\bullet}$. We note in passing that the latter value appears in the right range, given that the value experimentally determined in the more polar aqueous solution is expectedly higher at −78.9 kcal mol^{-1}.[35] Therefore, in concluding this section we can emphasize that the SET mechanism likely dominates in determining the antioxidant capacity of our compounds in all three assays, with some relevance of the HAT mechanism within the $DPPH^{\bullet}$ assay, in line with similar reports in the literature.[36]

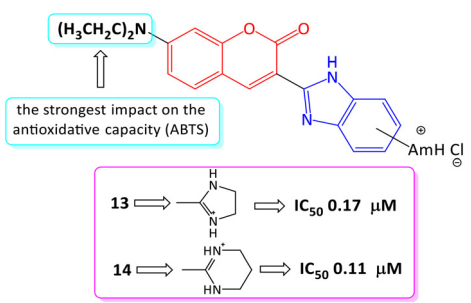


Fig. 3 Structure–activity relationship regarding the antioxidative capacity.

It is in this context that we will interpret the observed trends in the measured antioxidant capacities. Our designed systems consist of three distinct parts, namely coumarin, benzazole and amidine fragments. According to our analysis, each of them has its own role in governing the antioxidant features of the prepared hybrids. First of all, benzimidazole **m7** appears to be a better building block over benzothiazole **m8**, since it exhibits significantly lower IE (by 8 kcal mol^{-1}) and BDE values (by 16 kcal mol^{-1}), the latter pertaining to its ability to cleave the N–H bond relative to that from the C–H group. This is found in line with the fact that, in general, both benzothiazole hybrids **17** and **18** demonstrate significantly lower antioxidant features over their benzimidazole analogues **13** and **14**, and thus are not considered further. On the other hand, amidines seem to worsen the electron-releasing tendency, since their IE values in **m9** and **m10** extend to around 150 kcal mol^{-1}, which stretches even beyond the electron-accepting ability of the FRAP assay, whose EA = 147 kcal mol^{-1}. As an illustration, the IE value is reduced by 8.5 kcal mol^{-1} on going from IE = 122.2 kcal mol^{-1} in m11 to 113.7 kcal mol^{-1}, once the pentacyclic amidine is removed from the structure. Yet, their benefit is evident in their ability to facilitate the HAT mechanism, as the corresponding BDE values in **m9** and **m10** are lowered to 83.3 and 82.5 kcal mol^{-1}, respectively, which bears some significance for their response in the $DPPH^{\bullet}$ assay. Interestingly, if one considers unionized amidines, it turns out that in the pentacyclic version, both IE and BDE values are further reduced to 108.0 and 75.0 kcal mol^{-1}.

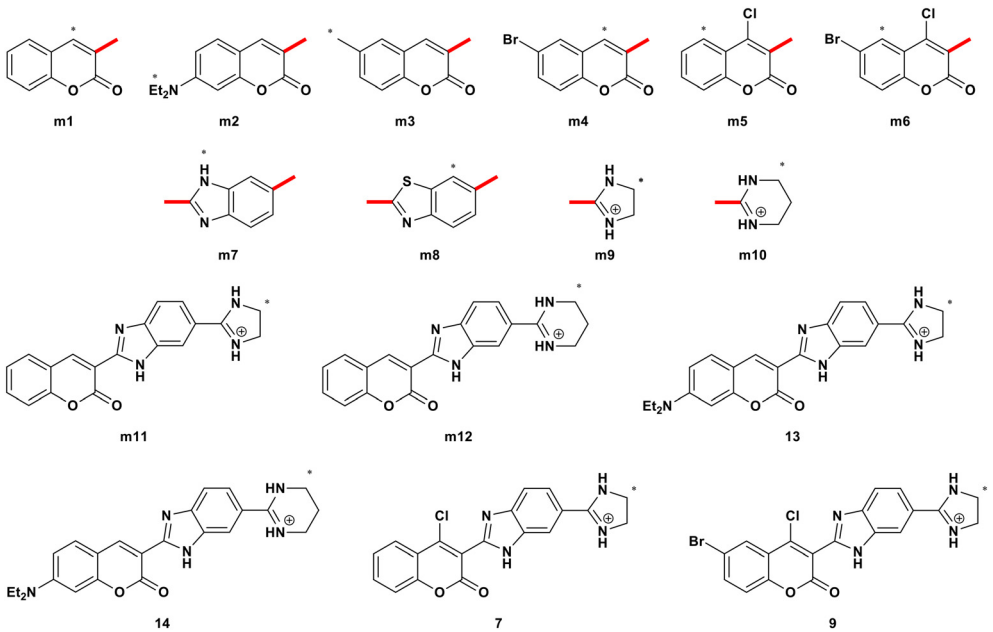


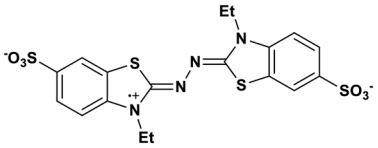
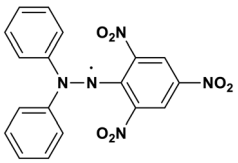
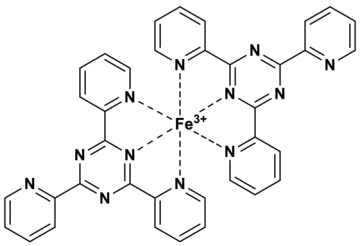
Fig. 4 Chemical structures of systems evaluated computationally. Asterisk (*) denotes thermodynamically most favourable hydrogen atom abstraction site elucidated from all X–H bonds except those from the junction methyl moieties marked in red.

This indicates that all prepared compounds could demonstrate even better antioxidative features under, for example, more basic conditions or less polar environments, which would disfavour amidine protonation, thereby leaving it unionized and promoting radical scavenging properties. Finally, the essential coumarin core does not appear to be particularly suitable for designing potent antioxidants, as **m1** is linked with relatively unfavorable parameters, IE = 124.5 kcal mol^{-1} and BDE = 102.7 kcal mol^{-1}. Still, due to its electron-conjugated nature, our synthetic strategy allows for a specific tuning of the antioxidant features by placing appropriate substituents at strategic places on the coumarin unit. This is only moderately observed with halogen atoms, as bromide in **m4** and chloride in **m5**, or their combination in **m6**, do not reveal any notable effect on the calculated parameters; yet only making these compounds even a bit less potent over **m1**. Still, a very interesting feature of all halogen-containing systems is that they can more easily liberate halogen radicals than any hydrogen atom within their structure. The matching BDEs for C–X (X = Cl, Br) bonds in **m4–m6** cluster between 75–78 kcal mol^{-1}, and are significantly lower than those for the most feasible hydrogen atom abstractions, for which BDEs exceed 100 kcal mol^{-1}. However, this is only one part of the picture, as one needs to look at the eventual ability of the reference assays to capture the liberated halogen radicals. Data in Table 4 reveal that neither $ABTS^{\bullet+}$ or $DPPH^{\bullet}$ are able to efficiently bind $Cl^{\bullet}$ and $Br^{\bullet}$ radicals; instead only moderately stable non-bonding complexes are formed, which appears insufficient for the expected assay response. With this in mind, we can eliminate homolytic carbon–halogen bond cleavage as responsible for the observed antioxidant features in halogen-containing derivatives, which unfortunately opposes their favorable effect for the antiviral activity expounded earlier. In contrast, the electron-donating methyl (**m3**) and diethylamine in particular (**m2**), offer significant antioxidative improvements. They both reduce the BDE values to around 80 kcal mol^{-1}, thereby precisely matching the described effect of the amidine unit, while the $-NEt_2$ group additionally lowers the IE value to 100.1 kcal mol^{-1} in **m2**. Because of that, the effect of the diethylamine moiety on the coumarin core appears as most efficient in promoting the potency of these compounds for both HAT and SET mechanisms, which is clearly seen in all three assays employed here. We note in passing that, due

Table 3 Ionization energies (IEs), electron affinities (EAs), and X–H bond dissociation energies (BDEs) in ethanol calculated by the (SMD)/B3LYP/6–31+G(d) model (in kcal mol^{-1}). The position of the X–H hydrogen atom abstraction is marked with an asterisk on Fig. 5

System	IE	EA	X–H BDE	Alternative BDE
m1	124.5	76.4	102.7	
m2	100.1	71.0	80.7	70.2 (C–NEt_2 bond)
m3	121.8	76.3	80.6	87.4 (C–CH_3 bond)
m4	127.2	79.5	103.2	78.0 (C–Br bond)
m5	128.6	80.9	103.2	75.2 (C–Cl bond)
m6	130.4	83.5	104.2	75.4 (C–Cl bond); 77.9 (C–Br bond)
m7	112.3	44.3	86.6	
m8	120.4	55.9	102.7	
m9	146.8	63.1	83.3	
m10	151.4	56.6	82.5	
m11	122.2	91.9	80.5	
m12	121.9	90.7	80.0	
7	129.1	91.3	78.5	70.5 (C–Cl bond)
9	125.9	97.0	80.2	70.7 (C–Cl bond); 77.8 (C–Br bond)
13	104.9	84.6	80.4	73.4 (C–NEt_2 bond)
14	106.0	83.6	80.9	73.4 (C–NEt_2 bond)

Table 4 Ionization energies (IEs), electron affinities (EAs), and N–X bond association energies (BAEs) in ethanol solution for reference systems used in experimental assays as obtained by the (SMD)/B3LYP/6–31+G(d) model (in kcal mol^{-1})

System	Structure	IE	EA	Bond	BAE	*d*(N–X)
ABTS$^{\bullet+}$		111.2	125.3	N–H	−36.6	1.03 Å
				N–cl	−16.4 (no bonding)	3.54 Å
				N–Br	−9.5 (no bonding)	3.36 Å
				N–NEt_2	+31.6	1.54 Å
DPPH$^{\bullet}$		104.5	124.5	N–H	−66.7	1.02 Å
				N–Cl	−25.3 (no bonding)	4.85 Å
				N–Br	−19.5 (no bonding)	3.05 Å
				N–NEt_2	+4.8	1.40 Å
FRAP			147.0			

to the high stability of the N-centered radicals, such as, for example, DPPH$^{\bullet}$, the energy required to cleave the C–NEt_2 bond in **m2** is very small, BDE = 70.2 kcal mol^{-1}, yet this turns irrelevant since none of the assays is able to bind the liberated $Et_2N^{\bullet}$ radical in a thermodynamically favorable way (Table 4). When structures containing all three building blocks are considered (**m11** and **m12**), thereby more closely resembling the prepared hybrids, it appears that they retain the low electron-donating ability of the coumarin core (IEs ≈ 122 kcal mol^{-1}), and the high hydrogen atom donating tendency of the amidine groups (BDEs ≈ 80 kcal mol^{-1}); the latter making them very potent for the DPPH$^{\bullet}$ assay. To further confirm that, let us mention that if **m11** is derived from the amidine moiety, its HAT ability is decreased to BDE = 102.8 kcal mol^{-1}, while its SET capacity is enhanced to IE = 113.7 kcal mol^{-1}. Yet, the full potential of our synthetic strategy becomes evident in compounds dressed up with the –NEt_2 group on the coumarin core, which does not impact the favorable hydrogen atom releasing ability of a distant five (**13**, BDE = 80.4 kcal mol^{-1}) or six-membered amidine (**14**, BDE = 80.9 kcal mol^{-1}), while significantly improving the feasibility of the electron ejection to around 105 kcal mol^{-1}, being lowest of all examined systems. This feature is evident in all three employed assays, promoting systems **13–14** as starting points for further derivatization towards even more efficient antioxidants.

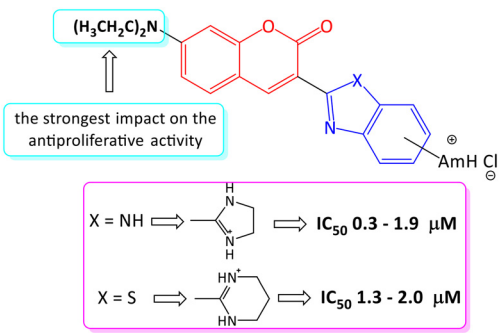


Fig. 5 Structure–activity relationship regarding the antiproliferative activity.

In vitro antiproliferative activity

All synthesized coumarin-benzimidazole **7–14** and coumarin-benzothiazole **17–18** hybrids were tested for their *in vitro* antiproliferative activity on the following panel of cancer cell lines: LN-229 – glioblastoma, Capan-1 – pancreatic adenocarcinoma, HCT-116 – colorectal carcinoma, NCI-H460 – lung carcinoma, DND-41 – acute lymphoblastic leukemia, HL-60 – acute myeloid leukemia, K-562 – chronic myeloid leukemia and Z-138 – non-Hodgkin lymphoma. Two standard anticancer drugs used in medical treatment, etoposide and nocodazole, were included as reference compounds. The obtained results are presented in Table 5 as IC_{50} values (50% inhibitory concentration). Based on the obtained results, we can conclude that the majority of the tested hybrids showed low or moderate activity, while four derivatives displayed a pronounced antiproliferative effect on either a selection of cancer cell lines or on the whole panel. The *N*,*N*-diethylamino coumarin-benzimidazole hybrid bearing a cyclic 2-imidazolinyl amidine group **13** was the most promising hybrid yielding IC_{50} values ranging from 0.3 to 2.1 μM on all the different cancer types. Derivative **11**, a 4-chloro-6-methyl substituted benzimidazole hybrid substituted with a

Table 5 Antiproliferative activity *in vitro* of tested compounds **7**–**14** and **17**–**18** against a broad panel of cancer cell types

	IC_{50}/μM							
	Cell line							
Cpd	LN-229	Capan-1	HCT-116	NCI-H460	DND-41	HL-60	K-562	Z-138
7	>100	99.3	>100	>100	36.5	55.7	>100	50.5
8	>100	69.2	>100	>100	19.1	53.5	>100	44.5
9	40.9	29.9	50.2	>100	6.2	49.6	9.7	6.4
10	>100	54.2	>100	>100	49.2	>100	59.5	52.1
11	9.1	10.2	34.4	31.7	5.8	21.5	9.9	6.4
12	>100	71.9	>100	>100	16.9	64.2	>100	48.1
13	1.4	1.7	1.9	1.8	0.3	2.1	1.9	1.5
14	97.0	66.7	>100	>100	10.8	63.8	63.5	67.3
17	>100	80.6	>100	>100	10.2	>100	65.5	11.3
18	1.8	2.0	>100	>100	1.3	>100	>100	1.5
Etoposide	0.43	1.45	2.40	3.65	2.80	0.42	1.77	0.85
Nocodazole	0.10	0.13	0.29	0.25	0.47	0.10	0.07	0.31

2-imidazolinyl amidine group, also showed marked antiproliferative activity with selectivity towards four cancer cell types (IC_{50} 5.8–9.9 μM). The benzimidazole derivative **9** substituted with bromine, chlorine and a 2-imidazolinyl group displayed a similar pattern with inhibitory concentrations in the range of 6.2 to 9.7 μM against three cancer cell types. When comparing all coumarin-benzimidazole hybrids, we can conclude that the presence of a pentacyclic 2-imidazolinyl amidine group (derivatives **7**, **9**, **11** and **13**) significantly enhanced the antiproliferative activity when compared to derivatives bearing a hexacyclic amidine group. Additionally, the introduction of a methyl group instead of bromine at position 6 on the coumarin ring slightly improved the antiproliferative activity. The introduction of a *N*,*N*-diethylamino group placed at position 7 on the coumarin ring enhanced the antiproliferative activity to a greater extend when compared to a chlorine at position 4 or a bromine and methyl at position 6. Regarding the coumarin-benzothiazole hybrids, the *N*,*N*-diethyl-amino substituted benzothiazole derivative bearing a hexacyclic amidine group **18** proved most potent with favorable inhibitory concentrations ranging from 1.3 to 2.0 μM against four different cancer cell types.

In general, we might conclude that out of all synthesized coumarin-benzazoles the hybrids substituted with a *N*,*N*-diethylamino group at position 7 of the coumarin ring (Fig. 5) show the most promising antitumor profile.

Subcellular distribution of compounds 13 and 18

Since the newly synthesized derivatives **13** and **18** display strong autofluorescent properties, their subcellular distribution can be investigated by fluorescence microscopy. After an incubation period of 3 hours to allow for cellular uptake, imaging was performed on Hep-2 cells treated with 10 μM of compound **13** or **18** (Fig. 6). For both derivatives, marked localization towards the nucleoli and nuclear membrane is noted. Derivative **18** is also distributed throughout the cytoplasm in a pattern strongly resembling ER localization as shown in Fig. 6. Hep-2 cells were obtained from ATCC (CCL-23).

Toxicity of compounds 13 and 18 to normal cells

The ratio between the concentrations of drug required for efficacy and the concentration that causes toxicity is referred to as the therapeutic window. At the highest dose tested (100 μM), both derivatives **13** and **18** show some toxicity towards normal PBMC from healthy donors (Fig. 7), although more pronounced for compound **13**. While 20 μM of derivative **13** yielded an average of 60% viability, all other tested concentrations for both compounds resulted in viability percentages above 80% relative to the untreated PBMC control. Since both molecules are active at low micromolar concentrations (average IC50 1.6 μM for **13** and 1.7 μM for **18**), we can conclude that there is good selectivity towards cancer cells (Fig. 7).

Conclusions

In this work, we have used molecular hybridization approach to design and synthesize amidino substituted coumarin-benzazole hybrids. All newly synthesized compounds were substituted at position 3 of the coumarin ring with benzazole nuclei bearing either an unsubstituted or a cyclic amidino group, as well as positions 4, 6 and 7 of the coumarin ring

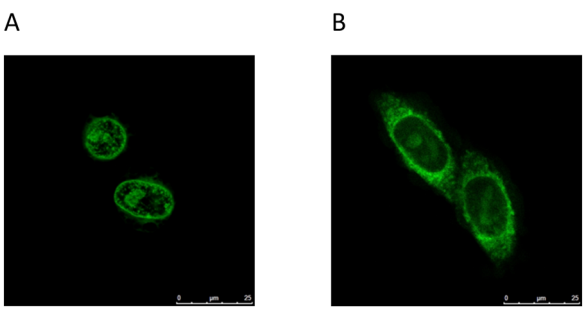


Fig. 6 Fluorescence imaging was performed to detect the subcellular localization of **13** (panel A) and **18** (panel B).

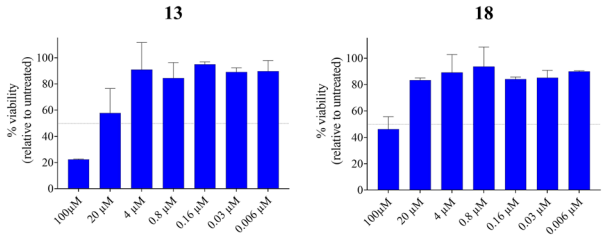


Fig. 7 Cell viability of PBMC treated with **13** and **18** for 72 hours (percentage relative to the untreated control).

with halogen, methyl of *N*,*N*-diethylamino groups. For the synthesis we have used previously published and well optimized synthetic procedures.

All newly synthesized compounds were evaluated for their biological activities including *in vitro* antiviral, antioxidative and antiproliferative activity. Antiviral activity was evaluated against a diverse panel of viruses and the obtained results revealed that the coumarin-benzimidazole hybrid **10** with a hexacyclic amidine group and bromine and chlorine at positions 6 and 4, respectively, displayed the most promising broad antiviral activity with EC_{50} values ranging from 9.0 to 43.8 μM. Additionally, the 2-imidazolinyl substituted benzimidazole derivative **9** showed selective activity towards influenza A virus subtype H1N1. Benzothiazole hybrids were less active in comparison to benzimidazoles. Regarding the antioxidative activities, the most promising results were observed for two coumarin-benzimidazole derivatives **13** and **14**, whose high radical-scavenging ability is evident in all three assays, while even surpassing that of the standard BHT in the ABTS assay with the IC_{50} values of 0.17 and 0.11 mM, respectively. Computational analysis supported these results and demonstrated that these hybrids benefit from the high C–H hydrogen atom releasing tendency of the cationic amidine unit, and the pronounced ease with which they can liberate an electron, promoted by the electron-donating diethylamine group on the coumarin core. Good antioxidative activity was also observed in DPPH assay with 4-chloro substituted benzimidazole derivative bearing a hexacyclic amidine group **7** being the most active one (IC_{50} 0.07 mM). In FRAP assay, the majority of the tested compounds were significantly less active in comparison to the included reference BHT. Antiproliferative activity was tested on a diverse panel of human cancer cell lines and the obtained results revealed that introducing a *N*,*N*-diethylamino group at position 7 of the coumarin ring significantly improved the antitumoral activity. The most pronounced activity was noted for the 2-imidazolinyl substituted benzimidazole derivative **13** (IC_{50} 0.3–1.9 μM) and the benzothiazole derivative with a hexacyclic amidine group **18** (IC_{50} 1.3–2.0 μM). We can thus conclude that the pentacyclic 2-imidazolinyl amidine group had the most significant impact on the enhancement of the antiproliferative activity. To summarize, the newly prepared coumarin-benzazole hybrids bearing an amidine group at the benzazole nuclei show considerable biological potential with pronounced antiproliferative, antiviral and antioxidant activities, thus representing a promising scaffold for further design and optimization of promising therapeutic agents.

Experimental part

Chemistry

General methods. Melting points were recorded on SMP11 Bibby and Büchi 535 apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 or Varian Gemini 600 spectrophotometer using TMS as an internal standard in DMSO-d6. Chemical shifts are reported in ppm (δ) relative to TMS. All compounds were routinely checked by thin layer chromatography (TLC) using precoated Merck silica gel 60F-254 plates and the spots were detected under UV light (254 nm). Elemental analysis for C, H and N were performed on a Perkin-Elmer 2400 elemental analyzer where analyses are indicated only as symbols of elements, analytical results obtained are within 0.4% of the theoretical value.

Synthesis. Synthesis of main precursors necessary for the synthesis of targeted coumarin-benzothiazole and coumarin-benzimidazole hybrids, namely 2-(3,4-diaminophenyl)-4,5-dihydro-1*H*-imidazol-3-ium chloride,[35] 2-(3,4-diaminophenyl)-3,4,5,6-tetrahydropyrimidin-1-ium chloride,[37,38] 2-amino-5-(4,5-dihydro-1*H*-imidazol-3-ium-2-yl)-benzenethiolate hydrate[14] or 2-amino-5-(3,4,5,6-tetrahydropyrimidin-1-ium-2-yl)-benzenethiolate[14] was carried out according to the previously published experimental procedures.

General method for the synthesis of amidino-substituted coumarin-benzimidazole hybrids 7–14. A mixture of equivalent amounts of corresponding aldehyde, 2-(3,4-diaminophenyl)-4,5-dihydro-1*H*-imidazol-3-ium chloride **5** or 2-(3,4-diaminophenyl)-3,4,5,6-tetrahydropyrimidin-1-ium chloride **6** in apsolute ethanol and *p*-benzoquinone was stirred at reflux for 4 hours. The reaction mixture was cooled to room temperature and resulting product was filtered off and washed with diethyl-ether.

2-(2-(4-chloro-2-oxo-2H-chromen-3-yl)-1H-benzo[d]imidazol-5-yl)-4,5-dihydro-1H-imidazol-3-ium chloride 7. The compound **7** was prepared following the general method from 4-chloro-2-oxo-2*H*-chromene-3-carbaldehyde **1** (0.10 g, 0.5 mmol), 2-(3,4-diaminophenyl)-4,5-dihydro-1*H*-imidazol-3-ium chloride **5** (0.10 g, 0.5 mmol) and *p*-benzoquinone (0.05 g, 0.5 mmol) in apsolute ethanol (3.5 mL) to obtain 0.09 g (45%) of brown powder. m.p. > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) (δ/ppm): 13.68 (bs, 1H, NH_{benz}), 13.63 (bs, 1H, NH_{benz}), 10.18 (s, 2H, $NH_{amidine}$), 8.11 (d, 1H, J = 1.19 Hz, H_{arom}), 8.07 (dd, 1H, J_1 = 8.07, J_2 = 1.59 Hz, H_{arom}), 7.94 (d, 1H, J = 8.47 Hz, H_{arom}), 7.72–7.67 (m, 2H, H_{arom}), 7.40–7.37 (m, 2H, H_{arom}), 2.11–1.92 (m, 4H, CH_2); ^{13}C NMR (151 MHz, DMSO-d_6) (δ/ppm): 176.1, 162.3, 159.5, 153.3, 150.9, 133.6, 133.0, 129.8, 125.2, 124.3, 123.9, 123.7, 120.5, 116.7, 115.6, 113.6, 113.0, 85.5, 17.7 (2C); anal. calcd. for $C_{20}H_{18}Cl_2N_4O_2$: C, 57.57; H, 4.35; Cl, 16.99; N, 13.43; O, 7.67. Found: C, 57.49; H, 4.27; Cl, 16.91; N, 13.39; O, 7.73%.

2-(2-(4-chloro-2-oxo-2H-chromen-3-yl)-1H-benzo[d]imidazol-5-yl)-3,4,5,6-tetrahydropyrimidin-1-ium chloride 8. The compound

8 was prepared following the general method from 4-chloro-2-oxo-2*H*-chromene-3-carbaldehyde **1** (0.10 g, 0.5 mmol), 2-(3,4-diaminophenyl)-3,4,5,6-tetrahydropyrimidin-1-ium chloride **6** (0.11 g, 0.5 mmol) and *p*-benzoquinone (0.05 g, 0.5 mmol) in apsolute ethanol (2 mL) to obtain 0.14 g (64%) of green powder. m.p. > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) (δ/ppm): 13.68 (bs, 1H, NH_{benz}), 13.62 (bs, 1H, NH_{benz}), 10.17 (s, 2H, $NH_{amidine}$), 8.11 (s, 1H, H_{arom}), 8.07 (dd, 1H, J_1 = 8.08, J_2 = 1.69 Hz, H_{arom}), 7.94 (d, 1H, J = 8.47 Hz, H_{arom}), 7.71–7.66 (m, 2H, H_{arom}), 7.40–7.36 (m, 2H, H_{arom}), 3.53 (s, 4H, CH_2), 2.05–1.99 (m, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) (δ/ppm): 176.6, 162.8, 160.0, 153.8, 151.4, 150.2, 134.1, 133.5, 130.3, 125.7, 124.8, 124.3, 121.0, 117.2, 116.1, 114.1, 113.5, 86.0, 18.2 (2C); anal. calcd. for $C_{21}H_{20}Cl_2N_4O_2$: C, 58.48; H, 4.67; Cl, 16.44; N, 12.99; O, 7.42. Found: C, 58.49; H, 4.63; Cl, 16.49; N, 13.09; O, 7.50%.

2-(2-(6-bromo-4-chloro-2-oxo-2H-chromen-3-yl)-1H-benzo[d] imidazol-5-yl)-4,5-dihydro-1H-imidazol-3-ium chloride ***9***. The compound **9** was prepared following the general method from 6-bromo-4-chloro-2-oxo-2*H*-chromene-3-carbaldehyde **2** (0.10 g, 0.3 mmol), 2-(3,4-diaminophenyl)-4,5-dihydro-1*H*-imidazol-3-ium chloride **5** (0.07 g, 0.3 mmol) and *p*-benzoquinone (0.04 g, 0.3 mmol) in apsolute ethanol (3 mL) to obtain 0.11 g (67%) of green powder. m.p. > 300 °C; ^{1}H NMR (600 MHz, DMSO-d_6) (δ/ppm): 13.67 (bs, 1H, H_{benz}), 13.61 (bs, 1H, H_{benz}), 10.15 (s, 2H, $H_{amidine}$), 8.10 (s, 1H, H_{arom}), 8.09 (d, 1H, J = 2.18 Hz, H_{arom}), 7.94 (d, 1H, J = 8.45 Hz, H_{arom}), 7.83 (dd, 1H, J_1 = 8.67, J_2 = 2.49 Hz, H_{arom}), 7.69 (d, 1H, J = 8.37 Hz, H_{arom}), 7.38 (d, 1H, J = 8.68 Hz, H_{arom}), 2.05–1.97 (m, 4H, H_{arom}); ^{13}C NMR (151 MHz, DMSO-d_6) (δ/ppm): 174.6, 165.3, 161.8, 152.3, 150.9, 136.0, 134.0, 130.0, 127.2, 124.5, 122.3, 119.3, 117.8, 115.9, 115.6, 113.9, 85.7, 44.4 (2C); anal. calcd. for $C_{20}H_{17}BrCl_2N_4O_2$: C, 48.41; H, 3.45; Br, 16.10; Cl, 14.29; N, 11.29; O, 6.45. Found: C, 48.46; H, 3.40; Br, 16.05; Cl, 14.22; N, 11.23; O, 6.40%.

2-(2-(6-bromo-4-chloro-2-oxo-2H-chromen-3-yl)-1H-benzo[d] imidazol-5-yl)-3,4,5,6-tetrahydropyrimidin-1-ium chloride ***10***. The compound **10** was prepared following the general method from 6-bromo-4-chloro-2-oxo-2*H*-chromene-3-carbaldehyde **2** (0.10 g, 0.3 mmol), 2-(3,4-diaminophenyl)-3,4,5,6-tetrahydropyrimidin-1-ium chloride **6** (0.08 g, 0.3 mmol) and *p*-benzoquinone (0.04 g, 0.3 mmol) in apsolute ethanol (3 mL) to obtain 0.09 g (54%) of green powder. m.p. > 300 °C; ^{1}H NMR (600 MHz, DMSO-d_6) (δ/ppm): 13.67 (bs, 1H, H_{benz}), 13.61 (bs, 1H, H_{benz}), 10.16 (s, 2H, $H_{amidine}$), 8.10 (s, 1H, H_{arom}), 8.09 (d, 1H, J = 2.48 Hz, H_{arom}), 7.94 (d, 1H, J = 8.49 Hz, H_{arom}), 7.83 (dd, 1H, J_1 = 8.69, J_2 = 2.48 Hz, H_{arom}), 7.69 (dd, 1H, J_1 = 8.48, J_2 = 1.45 Hz, H_{arom}), 7.38 (d, 1H, J = 8.68 Hz, H_{arom}), 3.52 (s, 4H, CH_2), 2.04–2.00 (m, 2H, CH_2); ^{13}C NMR (151 MHz, DMSO-d_6) (δ/ppm): 162.8, 160.0, 153.8, 151.4, 150.2, 134.1, 133.5, 130.3, 125.7, 124.7, 124.3, 124.2, 121.0, 117.2, 116.1, 114.1, 113.5, 86.0, 18.2; anal. calcd. for $C_{21}H_{19}BrCl_2N_4O_2$: C, 49.44; H, 3.75; Br, 15.66; Cl, 13.90; N, 10.98; O, 6.27. Found: C, 49.46; H, 3.71; Br, 15.72; Cl, 13.81; N, 11.04; O, 6.21%.

2-(2-(4-chloro-6-methyl-2-oxo-2H-chromen-3-yl)-1H-benzo[d] imidazol-5-yl)-4,5-dihydro-1H-imidazol-3-ium chloride ***11***. The compound **11** was prepared following the general method from 4-chloro-6-methyl-2-oxo-2*H*-chromene-3-carbaldehyde **3** (0.10 g, 0.4 mmol), 2-(3,4-diaminophenyl)-4,5-dihydro-1*H*-imidazol-3-ium chloride **5** (0.09 g, 0.4 mmol) and *p*-benzoquinone (0.05 g, 0.4 mmol) in apsolute ethanol (2.5 mL) to obtain 0.12 g (69%) of green powder. m.p. > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) (δ/ppm): 13.70 (bs, 1H, H_{benz}), 13.65 (bs, 1H, H_{benz}), 10.78 (s, 2H, $H_{amidine}$), 8.29 (s, 1H, H_{arom}), 7.96 (s, 2H, H_{arom}), 7.82 (d, 1H, J = 1.47 Hz, H_{arom}), 7.54 (d, 1H, J = 1.48 Hz, H_{arom}), 7.27 (d, 1H, J = 8.27 Hz, H_{arom}), 4.06–4.03 (m, 4H, CH_2), 2.40 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6) (δ/ppm): 152.9, 151.9, 148.0, 147.5, 145.3, 134.9, 133.0, 132.4, 128.2, 126.9, 119.8, 119.5, 112.0 (2C), 92.8, 50.3 (2C), 39.9, 28.8, 20.2; anal. calcd. for $C_{21}H_{20}Cl_2N_4O_2$: C, 58.48; H, 4.67; Cl, 16.44; N, 12.99; O, 7.42. Found: C, 58.43; H, 4.71; Cl, 16.40; N, 12.94; O, 7.46%.

2-(2-(4-chloro-6-methyl-2-oxo-2H-chromen-3-yl)-1H-benzo[d] imidazol-5-yl)-3,4,5,6-tetrahydropyrimidin-1-ium chloride ***12***. The compound **12** was prepared following the general method from 4-chloro-6-methyl-2-oxo-2*H*-chromene-3-carbaldehyde **3** (0.10 g, 0.4 mmol), 2-(3,4-diaminophenyl)-3,4,5,6-tetrahydropyrimidin-1-ium chloride **6** (0.11 g, 0.4 mmol) and *p*-benzoquinone (0.05 g, 0.4 mmol) in apsolute ethanol (2.5 mL) to obtain 0.10 g (57%) of green powder. m.p. > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) (δ/ppm): 13.66 (bs, 1H, H_{benz}), 13.60 (bs, 1H, H_{benz}), 10.18 (s, 2H, $H_{amidine}$), 8.11 (s, 1H, H_{arom}), 7.94 (d, 1H, J = 8.47 Hz, H_{arom}), 7.84 (s, 1H, H_{arom}), 7.70 (dd, 1H, J_1 = 8.49, J_2 = 1.67 Hz, H_{arom}), 7.49 (dd, 1H, J_1 = 8.37, J_2 = 2.00 Hz, H_{arom}), 7.27 (d, 1H, J = 8.28 Hz, H_{arom}), 3.53 (s, 4H, CH_2), 2.41 (s, 3H, CH_3), 2.04–1.99 (m, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) (δ/ppm): 176.7, 166.9, 162.9, 160.0, 151.9, 151.5, 150.2, 134.9, 133.5, 130.3, 125.3, 124.7, 124.2, 120.7, 117.0, 116.10, 114.0, 113.5, 85.9, 29.5, 20.9, 18.5; anal. calcd. for $C_{22}H_{22}Cl_2N_4O_2$: C, 59.33; H, 4.98; Cl, 15.92; N, 12.58; O, 7.19. Found: C, 59.36; H, 4.92; Cl, 15.87; N, 12.53; O, 7.09%.

2-(2-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-1H-benzo[d] imidazol-5-yl)-4,5-dihydro-1H-imidazol-3-ium chloride ***13***. The compound **13** was prepared following the general method from 7-(*N*,*N*-diethylamino)-2-oxo-2*H*-chromene-3-carbaldehyde **4** (0.10 g, 0.4 mmol), 2-(3,4-diaminophenyl)-4,5-dihydro-1*H*-imidazol-3-ium chloride **5** (0.09 g, 0.4 mmol) and *p*-benzoquinone (0.04 g, 0.4 mmol) in apsolute ethanol (2.5 mL) to obtain 0.16 g (90%) of orange powder. m.p. > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) (δ/ppm): 12.90 (bs, 1H, H_{benz}), 12.77 (bs, 1H, H_{benz}), 10.64 (s, 2H, $H_{amidine}$), 8.64 (s, 1H, H_{arom}), 8.40–8.19 (m, 1H, H_{arom}), 8.88–7.78 (m, 2H, H_{arom}), 7.75 (d, 1H, J = 8.97 Hz, H_{arom}), 6.84 (dd, 1H, J_1 = 9.09, J_2 = 2.27 Hz, H_{arom}), 6.68 (d, 1H, J = 2.06 Hz, H_{arom}), 4.02 (s, 4H, CH_2), 3.53–3.49 (m, 4H, CH_2), 1.17 (d, 6H, J = 6.96 Hz, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6) (δ/ppm): 166.0, 160.6, 157.3, 152.6, 150.2 (2C), 131.7, 122.6, 119.7, 116.1 (2C), 110.6, 108.6 (2C), 107.1 (2C), 96.7, 44.9 (2C), 44.7 (2C), 12.8 (2C); anal. calcd. for $C_{24}H_{28}ClN_5O_2$: C, 63.50; H, 6.22; Cl, 7.81; N, 15.43; O, 7.05. Found: C, 63.45; H, 6.16; Cl, 7.74; N, 15.39; O, 7.12%.

2-(2-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-1H-benzo[d] imidazol-5-yl)-3,4,5,6-tetrahydropyrimidin-1-ium chloride ***14***.

The compound **14** was prepared following the general method from 7-(*N*,*N*-diethylamino)-2-oxo-2*H*-chromene-3-carbaldehyde **4** (0.10 g, 0.4 mmol), 2-(3,4-diaminophenyl)-3,4,5,6-tetrahydropyrimidin-1-ium chloride **6** (0.09 g, 0.4 mmol) and *p*-benzoquinone (0.04 g, 0.4 mmol) in apsolute ethanol (2.5 mL) to obtain 0.04 g (21%) of orange powder. m. p. > 300 °C; ^{1}H NMR (400 MHz, DMSO-d$_6$) (δ/ppm): 12.80 (bs, 1H, H$_{benz}$), 12.70 (bs, 1H, H$_{benz}$), 9.98 (s, 2H, H$_{amidine}$), 8.10–8.00 (m, 2H, H$_{arom}$), 7.79 (d, 2H, J = 8.45 Hz, H$_{arom}$), 7.76 (d, J = 8.96 Hz, H$_{arom}$), 7.55 (d, 1H, J = 7.08 Hz, H$_{arom}$), 6.85 (dd, 1H, J_1 = 9.00, J_2 = 2.10 Hz, H$_{arom}$), 6.69 (d, J = 1.79 Hz, H$_{arom}$), 3.54–3.60 (m, 8H, CH$_2$), 2.04–1.99 (m, 2H, CH$_2$), 1.18 (t, 6H, J = 6.91 Hz, CH$_3$); ^{13}C NMR (101 MHz, DMSO-d$_6$) (δ/ppm): 160.6, 157.3, 152.5, 150.2, 131.7 (2C), 122.4 (2C), 121.6, 116.1, 110.6 (2C), 108.6 (2C), 107.3 (2C), 96.7, 44.8 (2C), 40.6, 40.4, 18.41, 12.8 (2C); anal. calcd. for $C_{25}H_{30}ClN_5O_2$: C, 64.16; H, 6.46; Cl, 7.57; N, 14.96; O, 6.84. Found: C, 64.22; H, 6.38; Cl, 7.63; N, 14.88; O, 6.77%.

General method for the synthesis of amidino-substituted benzothiazole derivatives 17–18. A mixture of 7-(*N*,*N*-diethylamino)-2-oxo-2*H*-chromene-3-carbaldehyde **4** (0.3 mmol) and 2-amino-5-(4,5-dihydro-1*H*-imidazol-3-ium-2-yl)-benzenethiolate hydrate (0.3 mmol) **15** or 2-amino-5-(3,4,5,6-tetrahydropyrimidin-1-ium-2-yl)-benzenethiolate **16** (0.3 mmol) in glacial acetic acid (5 ml) was stirred at reflux under nitrogen for 2 h, followed by the addition of diethyl-ether and the crude product was filtered off. Crude product was suspended in water and concentrated hydrochloric acid (0.02 mL, 0.2 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 3 h, followed by addition of acetone and the resulting precipitate was filtered off and washed with diethyl-ether.

2-[2-(7-(N,N-Diethylamino)-2-oxo-2H-chromen-3-yl) benzothiazol-6-yl]-4,5-dihydro-1H-imidazol-3-ium chloride ***17.*** The compound **17** was prepared following the general method from 2-amino-5-(4,5-dihydro-1*H*-imidazol-3-ium-2-yl)-benzenethiolate hydrate **15** (0.06 g, 0.3 mmol) and 7-(*N*,*N*-diethylamino)-2-oxo-2*H*-chromene-3-carbaldehyde **4** (0.07 g, 0.3 mmol) to obtain of 0.03 g (47%) red powder. m.p. > 300 °C; ^{1}H NMR (300 MHz, DMSO-d_6) (δ/ppm): 10.77 (s, 2H, NH$_{amidine}$), 9.00 (s, 1H, H$_{arom}$), 8.80 (s, 1H, H$_{arom}$), 8.12 (s, 2H, H$_{arom}$), 7.78 (d, 1H, J = 8.97 Hz, H$_{arom}$), 6.86 (d, 1H, J = 8.88 Hz, H$_{arom}$), 6.66 (s, 1H, H$_{arom}$), 4.04 (s, 4H, CH$_2$), 3.52 (q, 4H, J = 6.92 Hz, CH$_2$), 1.17 (t, 6H, J = 6.86 Hz, CH$_3$); ^{13}C NMR (DMSO-d_6, 151 MHz): δ/ppm = 166.1, 164.6, 160.3, 157.1, 155.6, 152.8, 143.5, 135.6, 132.1, 126.2, 123.3, 122.0, 117.6, 110.6, 109.6, 108.1, 96.2, 44.5 (2C), 44.5 (2C), 12.35 (2C); Anal. Calcd. for $C_{24}H_{27}ClN_4O_2S$: C, 61.20; H, 5.78; Cl, 7.53; N, 11.90; O, 6.79; S, 6.81. Found: C, 61.28; H, 5.86; Cl, 7.58; N, 11.97; O, 6.83; S, 6.86%.

2-[2-(7-(N,N-Diethylamino)-2-oxo-2H-chromen-3-yl) benzothiazol-6-yl]-3,4,5,6-tetra-hydropyrimidin-1-ium chloride ***18.*** The compound **18** was prepared following the general method from 2-amino-5-(3,4,5,6-tetrahydropyrimidin-1-ium-2-yl)-benzenethiolate **16** (0.06 g, 0.3 mmol) and 7-(*N*,*N*-diethylamino)-2-oxo-2*H*-chromene-3-carbaldehyde **4** (0.07 g, 0.3 mmol) to obtain of 0.04 g (54%) red powder. m.p. > 300 °C; ^{1}H NMR (300 MHz, DMSO-d_6) (δ/ppm): 10.16 (s, 2H, NH$_{amidine}$), 9.06 (s, 1H, H$_{arom}$), 8.57 (s, 1H, H$_{arom}$), 8.14 (d, 1H, J = 8.58 Hz, H$_{arom}$), 7.85 (dd, 1H, J_1 = 8.77 Hz, J_2 = 1.69 Hz, H$_{arom}$), 7.81 (d, 1H, J = 9.08 Hz, H$_{arom}$), 6.88 (dd, 1H, J_1 = 8.97 Hz, J_2 = 2.00 Hz, H$_{arom}$), 6.70 (s, 1H, H$_{arom}$), 4.05 (s, 8H, CH$_2$), 2.05–1.96 (m, 2H, CH$_2$), 1.17 (t, 6H, J = 6.87 Hz, CH$_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ/ppm = 165.7, 160.9, 159.6, 157.5, 155.3, 153.2, 143.9, 135.9, 132.5, 126.1, 124.6, 122.8, 122.3, 111.1, 110.2, 108.6, 96.7, 45.0 (2C), 18.2 (3C), 12.8 (2C); anal. calcd. for $C_{25}H_{29}ClN_4O_2S$: C, 61.91; H, 6.03; Cl, 7.31; N, 11.55; O, 6.60; S, 6.61. Found: C, 61.96; H, 6.08; Cl, 7.26; N, 11.48; O, 6.57; S, 6.66%.

Conflicts of interest

The authors declare no conflict of interest.

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