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ORIGINAL PAPER

Synthesis and biological evaluation of heterocyclic privileged medicinal structures containing (benz)imidazole unit

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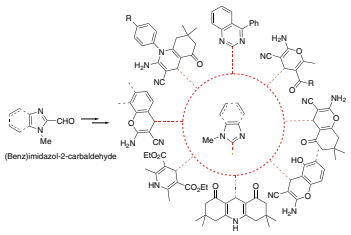
Abstract New heterocyclic privileged medicinal scaffolds involving 4*H*-pyran, 1,4-dihydropyridine, quinazoline, and 2-aminochromene bearing a (benz)imidazole unit were prepared in good to excellent yields, and evaluated for their in vitro hepatotoxicity on HepG2 cells and antioxidant activity using DPPH radical-scavenging assay. From these results, 2-amino-4-(1-methyl-1*H*-imidazol-2-yl)-4*H*-benzo[*h*]chromene-3-carbonitrile has been identified as a racemic, readily available imidazole derivative, significantly less hepatotoxic than tacrine at 100 μM concentration. One compound was found to be a potent antioxidant agent. The catalytic effect of the 1-methylimidazole unit, in the synthesis of some heterocycle, was also demonstrated. Crystal X-ray structures are reported for six compounds.

Graphical abstract



Keywords (Benz)imidazole · Polyheterocycle · Hepatotoxicity · DPPH · Crystal structure

Introduction

Heterocyclic compounds, and particularly those bearing nitrogen and oxygen atoms, such as 4*H*-pyran, quinazoline, 1,4-dihydropyridine (DHP), and chromene, continue to attract an increasing interest due to their wide range of pharmacological activities [1–9]. The imidazole and benzimidazole are common heterocyclic motifs present in some well-known components of human organisms, such as histidine, vitamin B12, purines, histamine, and biotin. These cores were also found in many natural products [10, 11], as lepidiline A and B [12] or synthetic molecules such as dacarbazine [13], zoledronic acid [14], tipifarnib [15], and azathioprine [16, 17], showing significant cytotoxicity against various types of human cancer cell lines. To improve the pharmacological profile associated with (benz)imidazoles, a number of investigations have been

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carried out by coupling them to other heterocyclic systems [18–22], or using them as bio-isostere [23–28].

In connection with our research on the preparation of new heterocyclic compounds with biological interest possessing an imidazole unit [29–31] and the synthesis and biological evaluation of tacrine analogs [32–34], we describe here the synthesis of new highly functionalized 2-substituted (benz)imidazole derivatives, and the evaluation of cytotoxicity and antioxidant properties. Furthermore, the in vitro hepatotoxicity test on HepG2 was carried out in the context of a project targeted to identify new suitable heterocyclic β-enaminonitrile precursors for the synthesis of non-toxic tacrine analogs for the potential treatment of Alzheimer's disease (AD).

Results and discussion

Chemistry

1-Methyl-1*H*-(benz)imidazole derivatives **1**–**4** have been used as starting materials to prepare the diverse heterocycle-(benz)imidazoles reported here. The synthetic pathways adopted for their preparations are outlined in Scheme 1. Known 1-methyl-1*H*-imidazole-2-carbaldehyde (**1**) was prepared from 1-methyl-1*H*-imidazole via the carbanion generated at C-2 with *n*-BuLi in the presence of DMF, according to a modified procedure [35]. 1-Methyl-1*H*-benzo[d]imidazole-2-carbaldehyde (**2**) was obtained via an addition–cyclization/methylation sequence [36, 37], followed by oxidation with SeO_2. The conversion of the (benz)imidazole-2-carbaldehyde to the corresponding α-cyanoacrylonitriles **3** and **4** proceeded cleanly with 94 and 67 % yield, respectively, by reaction with malononitrile (1–1.1 equiv), in EtOH at room temperature (in the case of compound **4**, a few drops of piperidine were added).

First of all, and based on our previous work [32–34], we explored the synthesis of 4*H*-pyran derivatives. Polyfunctionalized 4*H*-pyrans are common structural units in a number of natural products [38]. The 4*H*-pyran ring can be transformed into pyridine systems related to pharmacologically important DHP type of calcium antagonists [39]. The presence of a 4*H*-pyran moiety in organic molecules imparts them an extensive range of biological and pharmacological properties, such as spasmolytic, diuretic, anticoagulant, anti-cancer, and anti-anaphylactic activities [40–44]. Highly substituted pyran/chromenes tethered with $–NH_2$ and –CN functionalities possess diverse pharmacological properties [45–47]; they can also be used as precursors for designing new tacrine analogs for the potential treatment of Alzheimer's disease [32–34].

2-Amino-3-cyano-4*H*-pyrans **5a**–**5f** bearing an imidazole or benzimidazole moiety was prepared in a "one-pot" reaction. The treatment of (benz)imidazole 2-carbaldehydes **1** (or **2**) with malononitrile gave (benz)imidazole-α-cyanoacrylonitrile derivatives, which were immediately reacted in situ with acetylacetone, ethyl acetoacetate, or dimedone to afford the 2-(4*H*-pyran)-(benz)imidazole derivatives **5a**–**5f** (Table 1; Scheme 2). For the synthesis of compounds **5a**, **5c**, and **5d**, the reaction was generally over in 2 h, in good yields, and no catalyst was required. The molecular structures of compounds **5c**, **5d**, and **5f** (Fig. 1

Scheme 1

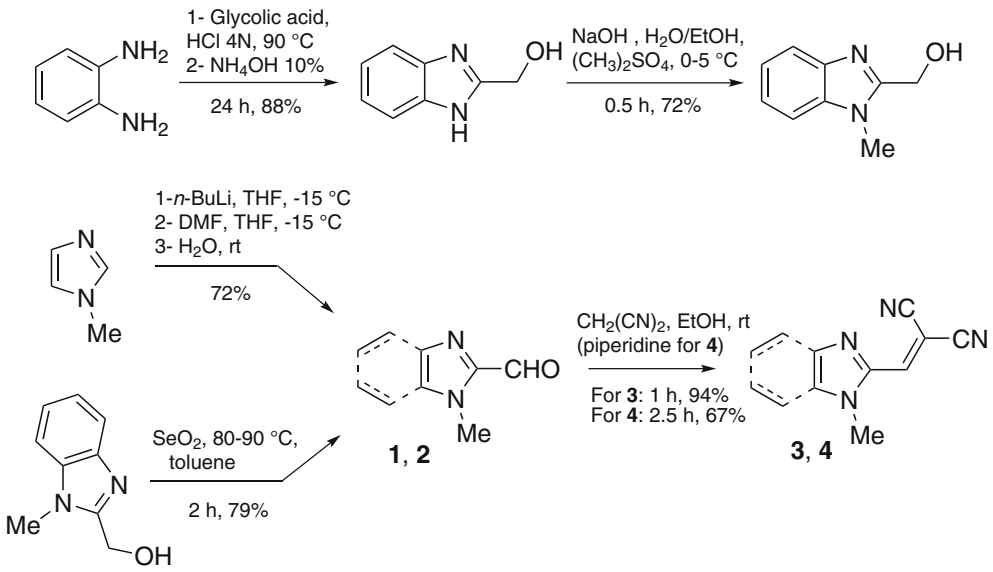
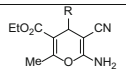
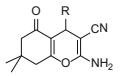
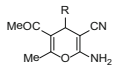
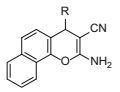
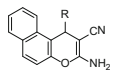
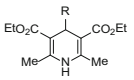
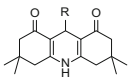
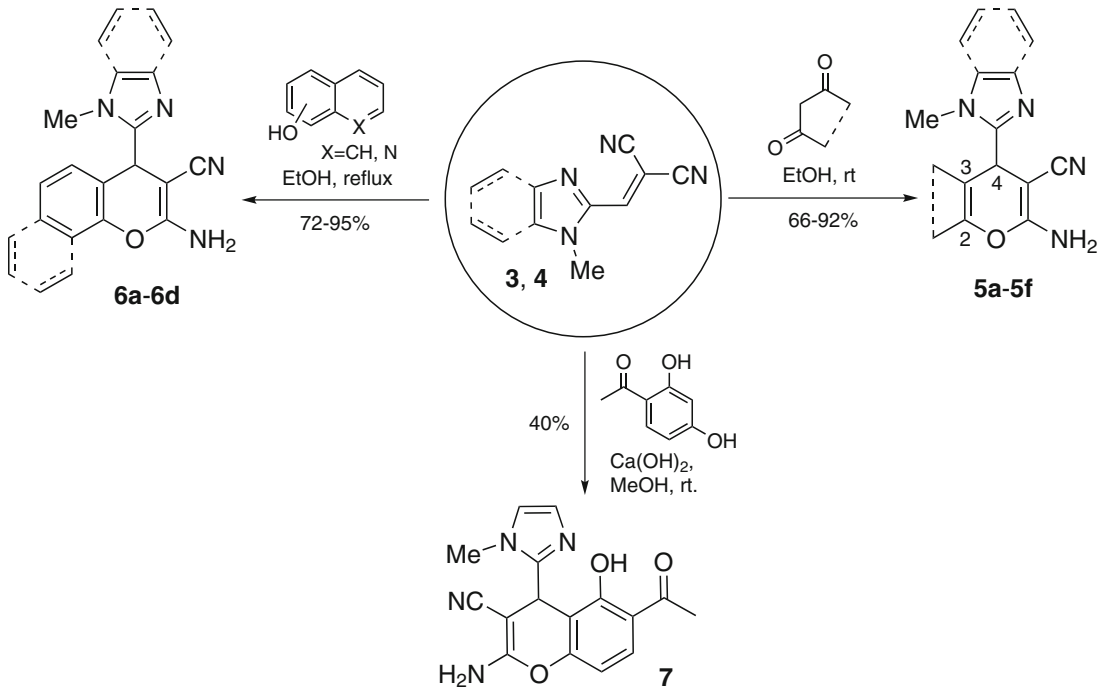


Table 1 Synthesis of (benz)imidazole-4*H*-pyran, -2-aminochromene, and -1,4-dihydropyridines

Structure	R = imidazole		R = benzimidazole	
	Comp.	Yield/% [a]	Comp.	Yield/% [a]
	**5a**	92	**5b**	85
	**5c**	90	**5d**	73
	**5e**	69	**5f**	66
	**6a**	75	**6b**	95
	**6c**	72	**6d**	90
	**8a**	73	**8b**	77
	**8c**	80		

[a] Yield of pure product

Scheme 2



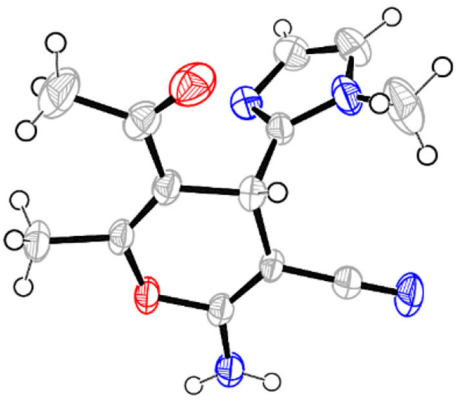


Fig. 1 ORTEP plot of the X-ray crystal structure of compound **5c**. Displacement ellipsoids are drawn at the 50 % probability level

and Fig. S1, S2 in Supplementary Material) were confirmed by crystal X-ray diffraction analysis.

2-Aminochromenes are important compounds in many naturally occurring products used as cosmetics, pigments, and potential biodegradable agrochemicals [48, 49]. These compounds are usually synthesized by reaction of benzylidenemalononitriles and 1-naphthol in organic solvents in the presence of organic bases such as piperidine or 4-methylmorpholine, which are frequently used in stoichiometric amounts [50]. In this context, 1-methyl(benz)imidazole-α-cyanoacrylonitrile **3** (or **4**) was treated with 1-naphthol (or 2-naphthol) to give the corresponding 2-aminochromene derivatives **6a**–**6d** (Table 1; Scheme 2).

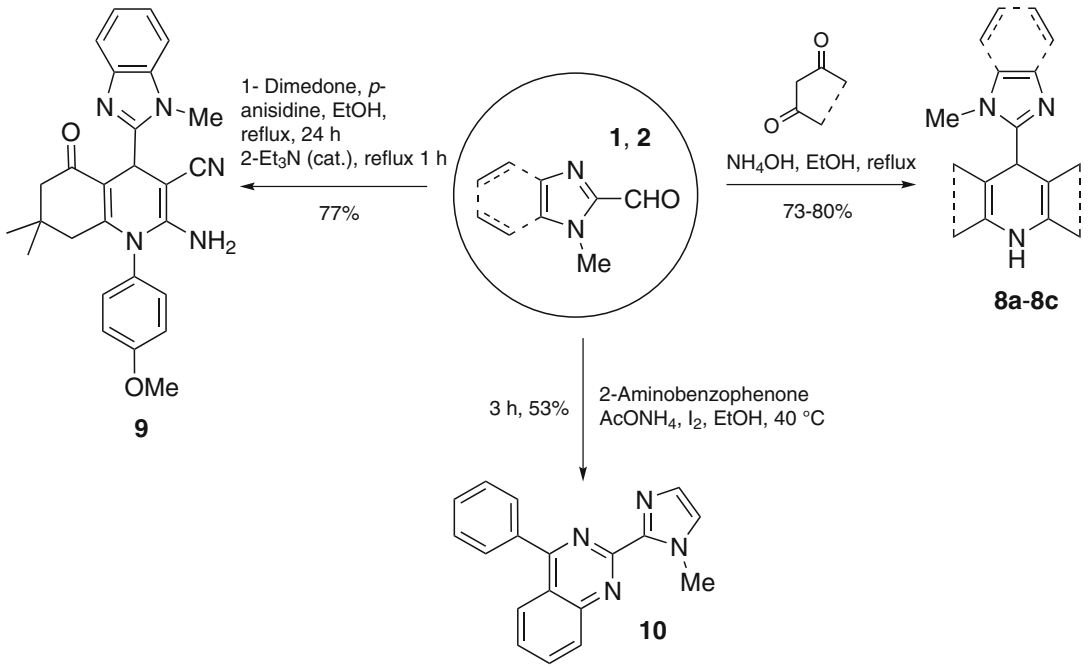
Similarly, the reaction of 1-methyl-1*H*-imidazole-α-cyanoacrylonitrile (**3**) with 2,4-dihydroxyacetophenone at rt in the presence of $Ca(OH)_2$, according to the procedure reported by Kolla and Lee [51], gives the corresponding 4*H*-pyran **7** (Scheme 2) in moderate yield (40 %). The molecular structure of **7** was confirmed by single crystal X-ray diffraction analysis (Fig. S4 in Supplementary Material).

Next, we expanded this work to the preparation of azoles such as DHPs coupled to the (benz)imidazole unit. 4-Aryl-DHPs are a class of calcium channel blockers and, to improve their pharmacological profile, a number of investigations have been carried out by replacing the 4-aryl substituent by various heterocycles [52–56]. Consequently, we considered the synthesis of compounds containing a DHP entity linked to (benz)imidazole unit.

Symmetrical DHP analogs **8a**–**8c** (Table 1, Scheme 3) were prepared by the classical Hantzsch reaction of the (benz)imidazole-2-carbaldehyde **1** (or **2**) with ethyl acetoacetate or dimedone in ethanol, and in the presence of a large excess of ammonia. Single crystals of **8a** were grown by slow evaporation of a $CHCl_3$ solution and X-ray crystallographic analysis confirmed the structural assignment (Fig. 2).

In a parallel approach, we explored the synthesis of heterocycle containing two nitrogen atoms, such as quinazoline possessing an imidazole subunit. It is well known that modified quinazoline derivatives have shown

Scheme 3



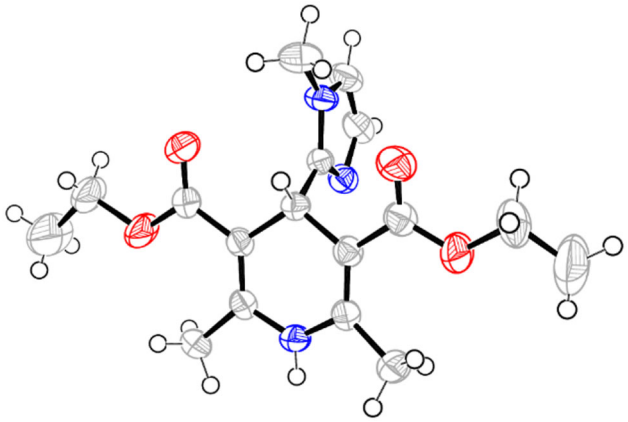


Fig. 2 ORTEP plot of the X-ray crystal structure of compound **8a**. Displacement ellipsoids are drawn at the 50 % probability level

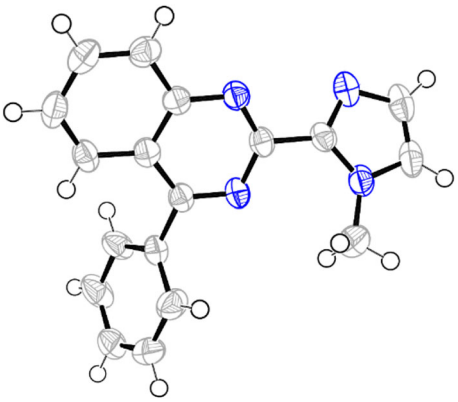


Fig. 3 ORTEP plot of the X-ray crystal structure of compound **10**. Displacement ellipsoids are drawn at the 50 % probability level

remarkable anti-cancer, anti-tubercular, and antibacterial activities [57–62]. In this context and in accordance with literature [63], 2-(1-methyl-1*H*-imidazol-2-yl)-4-phenylquinazoline (**10**) was prepared in a "one-pot" three-component reaction of 1-methyl-1*H*-imidazole-2-carbaldehyde (**1**), *o*-aminobenzophenone, ammonium acetate, and iodine as catalyst (Scheme 3). It is important to note that no purification was needed and compound **10** was isolated in pure form in 53 % of yield by simple filtration and washing with ethanol. The molecular structure of **10** was confirmed by single crystal X-ray diffraction analyses (Fig. 3).

Note that the preparation of compound **3** and 1-methyl-1*H*-imidazol-2-yl derivatives **5a**, **5c**, **5e**, **6a**, **6c**, **8a**, and **8c** can be achieved well in a short time reaction, without catalyst, the 2-substituted-1-methyl-1*H*-imidazole playing itself this role [64–66]. In contrast, the synthesis of heterocyclic compounds bearing a 1-methyl-1*H*-benzimidazole unit required piperidine as catalyst and a longer time. A plausible reaction mechanism has been detailed in Supplementary Material (Scheme S1).

In vitro hepatotoxicity evaluation

Hepatotoxicity of drug candidates represents one of the crucial factors for their possible clinical development, and the in vitro screening is a valuable tool for preselection of the best drug candidates in preclinical research reducing the high costs in drug development [67]. This is particularly of interest in projects targeted to design new tacrine analogs for AD, due to the fact that tacrine was withdrawn from the clinics for its secondary toxic effects on the liver and hepatotoxicity. The in vitro screening of the hepatotoxic activity of the newly synthesized compounds was performed on human hepatoma cell line HepG2 [68], the best-characterized and most frequently used cell line with respect to hepatotoxic end points to test the metabolism and liver toxicity of several drugs. For comparative purposes, tacrine was used as a standard control. The results of the cytotoxicity screening of the prepared compounds are summarized in Table 2.

The cytotoxicity of (benz)imidazole-4*H*-pyrans **5a–5f**, **6a–6d**, **7**, DHPs **8a–8c**, **9**, and quinazoline **10** was determined at micromolar concentrations ranging from 1, 3, 10, 30, 100, to 300 μM, using the MTT assay [69]. These compounds were well tolerated after a 24 h incubation period in human HepG2 cells compared with tacrine.

As shown in Table 2, and not surprisingly, tacrine significantly decreased the number of living cells, being more hepatotoxic gradually at higher doses, particularly at 100 and 300 μM concentrations. In contrast, all evaluated compounds showed a reduced hepatotoxicity compared with tacrine, especially at the highest concentrations used, 100 μM being the concentration that we are going to use next to discuss the observed structure–activity relationships (SAR) on this series of compounds.

At the 100 μM concentration, and according to the percentage of cell viability test, the four less toxic compounds were, in decreasing order: **6a** (85.7 %), **5c** (83.3 %), **5f** (82.6 %), and **5e** (82.2 %), with tacrine showing a significant lower (64.3 %) value at the same concentration. The least hepatotoxic compound, **6a**, was 1.3-fold less toxic than tacrine. This means that the pyrans, regardless of the type of the substituents at C2 and C3 in the central heterocyclic core, are less toxic than DHPs.

Among the analyzed pyrans, 1-methyl(benz)imidazole-4*H*-pyrans **5a–5f** (Table 2, entries 1–6) are clearly less hepatotoxic than tacrine at the different concentrations tested. Compounds **5c** (entry 3) and **5f** (entry 6) have the best non-hepatotoxic profile at 100 μM concentration with 83.3 and 82.6 % cell viability, respectively. For the same type of substituent at C2 and C3, pyrans bearing the imidazole ring are less (compare pyrans bearing C3 = CO_2Et; C2 = Me: **5a** (76.8 %) and **5b** (71.7 %); C3 = COMe; C2 = Me: **5c** (83.3 %) and **5d** (72.2 %)) or similarly

Table 2 In vitro toxicity of 1-methyl-1*H*-(benz)imidazole-heterocycles **5–10** and tacrine in HepG2 cells

Entry	Compound	Viability (%) HepG2 cells[a]					
		1 μM	3 μM	10 μM	30 μM	100 μM	300 μM
1	**5a**	99.9 ± 1.47^{ns}	92.5 ± 0.95**	81.8 ± 1.40***	76.4 ± 1.32***	76.8 ± 1.75***	72.6 ± 0.33***
2	**5b**	97.1 ± 0.79^{ns}	91.2 ± 0.4*	84.9 ± 2.50***	73.4 ± 1.73***	71.7 ± 1.81***	72.3 ± 2.92***
3	**5c**	95.4 ± 1.51^{ns}	91.5 ± 2.00*	85.0 ± 0.62***	83.7 ± 0.49***	**83.3 ± 0.72*** **	80.8 ± 1.41***
4	**5d**	97.3 ± 1.58^{ns}	90.9 ± 1.44*	89.0 ± 2.39**	85.8 ± 1.83***	72.2 ± 0.58***	74.9 ± 2.19***
5	**5e**	99.3 ± 1.77^{ns}	94.3 ± 1.06^{ns}	89.8 ± 0.54*	87.0 ± 1.23**	82.2 ± 0.91***	71.7 ± 3.71***
6	**5f**	98.9 ± 0.22^{ns}	89.4 ± 0.95***	85.1 ± 1.00***	84.3 ± 0.57***	**82.6 ± 1.79*** **	77.6 ± 1.38***
7	**6a**	97.1 ± 0.70^{ns}	95 ± 0.83^{ns}	93.2 ± 0.54^{ns}	86.6 ± 0.32*	**85.7 ± 1.86*** **	78.0 ± 0.62***
8	**6b**	99.2 ± 1.33^{ns}	96.2 ± 1.55^{ns}	97 ± 1.08^{ns}	83.4 ± 1.81*	81.6 ± 1.64***	78.2 ± 1.42***
9	**6c**	98.6 ± 0.97^{ns}	93.5 ± 1.47^{ns}	91.7 ± 2.08^{ns}	78.8 ± 3.66*	77 ± 3.36***	70.5 ± 2.97***
10	**6d**	100 ± 0.73^{ns}	97.8 ± 1.04^{ns}	96.6 ± 1.95^{ns}	81.4 ± 0.85*	81.5 ± 0.39***	80.8 ± 1.83***
11	**7**	96.6 ± 1.33^{ns}	88 ± 1.55^{ns}	84.8 ± 1.08^{ns}	82.3 ± 1.81*	71.1 ± 1.64***	67.4 ± 1.42***
12	**8a**	95.1 ± 2.89^{ns}	87.7 ± 0.99**	81.5 ± 1.90***	82.1 ± 2.12***	76.1 ± 1.55***	73.0 ± 0.51***
13	**8b**	94.1 ± 1.28^{ns}	85.8 ± 4.38^{ns}	77.5 ± 3.69**	75.6 ± 4.05***	69.1 ± 4.15***	63.4 ± 0.34***
14	**8c**	97.5 ± 0.88^{ns}	91.0 ± 2.30**	87.7 ± 1.34***	81.4 ± 1.22***	80.7 ± 0.68***	78.2 ± 1.23***
15	**9**	95.4 ± 0.45^{ns}	94.2 ± 0.46^{ns}	90.1 ± 2.03*	84.0 ± 1.69***	75.2 ± 2.91***	71.4 ± 3.54***
16	**10**	95.6 ± 1.19^{ns}	92.8 ± 1.40*	89.3 ± 1.38***	85.2 ± 1.46***	77.1 ± 1.47***	61.5 ± 2.11***
17	Tacrine	93.4 ± 4.69^{ns}	90 ± 2.95^{ns}	88.7 ± 3.42^{ns}	81.6 ± 4.88*	**64.3 ± 4.54*** **	40 ± 2.20***

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ and *ns* not significant, with respect to the control group. Comparisons between drugs and control group were per-formed by one-way ANOVA followed by the Newman-Keuls post hoc test

[a] Cell viability was measured as MTT reduction and data were normalized as % control. Data are expressed as the mean ± SEM of triplicate of at least four different cultures. All compounds were assayed at increasing concentrations (1–300 μM)

(compare pyrans bearing C2/C3: 3,3-dimethylcyclohexan-1-one: **5e** (82.2 %) and **5f** (82.6 %)) toxic than those bearing the benzimidazole nucleus. In this group, we observed also that for the pyrans bearing an imidazole ring at C4, **5c** bearing a C3 = COMe; C2 = Me substitution was less toxic, and that for the pyrans bearing a benzimidazole ring at C4, **5f** bearing a C2/C3; 3,3-dimethylcyclohexan-1-one substitution was less toxic.

Among the analyzed pyrans, 1-methyl(benz)imidazole-4*H*-pyrans **6a–6d** (Table 2, entries 7–10) are clearly less hepatotoxic than tacrine at 100 μM concentration. Compound **6a** (entry 7) has the best non-hepatotoxic profile, with 85.7 % cell viability. For the same type of naphthalene-fused ring, pyrans bearing the imidazole ring are less (compare pyrans bearing a C1′, C2′-naphthalene; **6a** (85.7 %) and **6b** (81.6 %)) or more [compare pyrans bearing a C2′, C1′-naphthalene; **6c** (77 %) and **6d** (81.5 %)] toxic than those bearing the benzimidazole nucleus. Finally, note that among the pyran derivatives, compound **7** (Table 2, entry 11) was the most toxic.

Among the analyzed 1-methyl(benz)imidazole-1,4-dihydropyridines **8a–8c**, **9** (Table 2, entries 12–15), DHP **8c** bearing a double 3,3-dimethylcyclohexan-1-one substitution at C2/C3 and C5/C6 showed lower hepatotoxicity than tacrine (80.7 % at 100 μM). It is also interesting to highlight that, in good agreement with what we have observed in pyrans of type **5**, for the same arrangement (C3 = CO_2Et; C2 = Me), the pyran bearing the imidazole ring at C4 (**8a** 76.1 %) was less hepatotoxic than the one bearing a benzimidazole ring at C4 (**8b** 69.1 %).

Compound **10** (Table 2, entry 16) is the least efficient of all compounds tested at higher concentration. Nevertheless, it is much less toxic than tacrine with 77.1 vs. 64.3 % cell viability at 100 μM.

DPPH radical scavenging

The in vitro antioxidant activity of all prepared compounds was carried out by the DPPH assay [70] which measures the hydrogen-donating ability of antioxidants to convert stable DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical to 2,2-diphenyl-1-picrylhydrazine. Ascorbic acid (Asc) was used as a standard antioxidant. The results are expressed as mean ± standard deviation (SD) and summarized in Table 3.

As shown in Table 3, all the tested compounds show a relatively poor radical-scavenging capability at $t = 0$ (~20 %), and no significant change was observed over time (0–24 h) for most of them. The replacement of the imidazole ring with benzimidazole in analogs does not have an influence on the DPPH activity. However, a noticeable increase in the antioxidant activity was detected

Table 3 DPPH radical-scavenging activities of 1-methyl-1*H*-(benz)imidazole-heterocycles **5–10**

Entry	Compound	Percentage inhibition of DPPH free radical (*I* %)[a]			
		0 min	30 min	3 h	24 h
1	**5a**	19.65 ± 0.94	21.07 ± 3.02	24.51 ± 0.79	21.29 ± 3.43
2	**5b**	22.84 ± 2.46	19.44 ± 1.60	23.60 ± 2.30	19.16 ± 0.94
3	**5c**	21.66 ± 0.55	21.57 ± 1.41	23.91 ± 4.45	24.59 ± 1.71
4	**5d**	21.97 ± 2.93	21.99 ± 1.95	23.85 ± 1.68	22.39 ± 2.47
5	**5e**	20.19 ± 0.95	23.27 ± 1.05	25.12 ± 1.03	42.84 ± 0.50
6	**5f**	18.16 ± 0.20	18.14 ± 1.34	25.14 ± 0.23	36.06 ± 5.17
7	**6a**	20.09 ± 2.91	20.27 ± 1.00	21.88 ± 4.30	18.15 ± 1.88
8	**6b**	19.91 ± 3.34	18.42 ± 2.93	18.57 ± 3.78	23.71 ± 4.29
9	**6c**	21.35 ± 3.01	21.35 ± 1.30	21.74 ± 0.17	19.88 ± 3.33
10	**6d**	18.99 ± 0.56	17.88 ± 1.10	21.60 ± 0.31	27.39 ± 0.46
11	**7**	23.63 ± 1.34	19.50 ± 0.88	23.96 ± 2.09	21.50 ± 0.83
12	**8a**	21.19 ± 3.56	22.08 ± 3.05	23.51 ± 1.33	22.13 ± 1.09
13	**8b**	19.56 ± 0.78	19.39 ± 0.34	22.53 ± 0.97	25.28 ± 4.25
14	**8c**	20.56 ± 0.79	18.89 ± 2.16	21.80 ± 3.04	18.34 ± 3.31
15	**9**	26.13 ± 3.02	31.56 ± 2.33	46.10 ± 0.34	67.82 ± 0.70
16	**10**	22.14 ± 0.61	19.30 ± 1.62	24.35 ± 1.26	20.86 ± 1.91
Standard	**Asc**	86.22 ± 1.62	84.11 ± 1.55	83.83 ± 1.38	78.13 ± 1.19

[a] Data are an average for triplicate determinations

after 24 h incubation for 4*H*-pyran compounds **5e** (Table 3, entry 5) and **5f** (Table 3, entry 6) tethered with $–NH_2$ and –CN functionalities bearing an acetyl group at C3, a methyl at C2, and an imidazole ring or benzimidazole at C4 (42.84 ± 0.50 vs. 20.19 ± 0.95 % for **5e**, and 36.06 ± 5.17 vs. 18.16 ± 0.20 % for **5f**). Among the tested compounds tethered with $–NH_2$ and –CN functionalities (**5a–5f**, **6a–6d**, **7**, and **9**), compound **9** (Table 3, entry 15) which contains a DHP entity, bearing at C2/C3: 3,3-dimethylcyclohexan-1-one, a 4-methoxyphenyl group on the nitrogen atom and a benzimidazole unit at C4, shows higher DPPH radical-scavenging activity (67.82 ± 0.70 %). This result is in good agreement with the values observed for ascorbic acid (78.13 ± 1.19 %) at 100 μM for 24 h. This can be explained by the presence of high electron-releasing methoxy group, which facilitates free radical inhibition after long reaction times [71]. The remaining compounds (Table 3, entries 12–14, 16) exhibited non-significant antioxidant activity compared to the standard reference (<25 %).

Conclusion

In conclusion, we have prepared and characterized by analytical spectroscopic data, in some cases, by X-ray diffraction analyses, some novel heterocyclic compounds bearing a 4*H*-pyran, quinazoline, DHP, and 2-aminochromene as central heterocyclic core and possessing additional (benz)imidazole motifs. Our synthetic approach allowed us to prepare 16 new compounds of biological importance in good to excellent yields, without the need of chromatographic separation. The free radical-scavenging activity for all prepared compounds was measured by DPPH. The data reported indicates that compound **9** exhibited significant antioxidant activity (>67 %) after 24 h of incubation. The results obtained in the in vitro hepatotoxicity test on human hepatoma cells HepG2 demonstrated that all prepared compounds are significantly less toxic than tacrine in the micromolar range, especially at higher concentration (100–300 μM), and racemic 2-amino-4-(1-methyl-1*H*-imidazol-2-yl)-4*H*-benzo[*h*]chromene-3-carbonitrile (**6a**) is a readily available imidazole derivative. This result is important because it paves the way to select and develop further the 2-amino-4*H*-benzo[*h*]chromene-3-carbonitrile core as the appropriate heterocyclic core to design new tacrine analogs for the potential treatment of Alzheimer's disease, and also confirms the suitability of the coupled imidazole ring for the same purpose. All these endeavors are now in progress in our laboratory and will be reported in due course.

Experimental

IR spectra were recorded on a Shimadzu FT IR-8201 PC spectrophotometer and only significant absorption band frequencies are cited. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance DPX250 or VARIAN Mercury-300 spectrometers. Chemical shifts (δ) are given in ppm and

J values in hertz (Hz). Elemental analyses were recorded on a Carlo-Erba CHNS apparatus/O. EA 1108. The measurements of the diffracted intensities were recorded on an APEX II diffractometer equipped with a two-dimensional detector kappa CCD (λ K$\alpha = 0.71073$ Å). Melting points were determined on an Electrothermal Digital Melting Points Apparatus IA 9200 or on a Kofler melting point apparatus. Thin layer chromatography (TLC) was carried out on precoated Merck silica gel aluminum sheets 60 F_{254} and detection by UV light at 254 nm. Column chromatography was performed on silica gel 60 (230 mesh). Solvents were freshly distilled before use: MeOH from magnesium in the presence of I_2, DMF was kept for few hours over $CaCl_2$ and distilled from CaO, and THF from Na/benzophenone. Commercial-grade reagents were used as supplied.

General procedure for the synthesis of 1-methyl-1H-(benz)imidazole-α-cyanoacrylonitrile (3) and (4)

To a solution of 1-methyl-1*H*-imidazole-2-carbaldehyde (**1**) or 1-methyl-1*H*-benzo[*d*]imidazole-2-carbaldehyde (**2**) in ethanol (e.g., 1.0 mmol in 5 cm^3), 1–1.1 equivalent of malononitrile and for compound **4** few drops of piperidine were added, and the mixture was stirred at room temperature for 1 h. The resultant residue was filtered off, washed with cold ethanol, and dried on air to afford the pure product.

2-[(1-Methyl-1H-imidazol-2-yl)methylene]malononitrile (**3**, $C_8H_6N_4$)
Yield 94 %; m.p.: 200–202 °C; IR (KBr): $\bar{\nu} = 2210$ (CN) cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6): $\delta = 8.15$ (s, 1H, H-imid.), 7.58 (s, 1H, H-imid.), 7.40 (s, 1H, C*H* = C(CN)$_2$), 3.79 (s, 3H, NCH_3) ppm; ^{13}C NMR (62.9 MHz, CD_3CN): $\delta = 142.7$, 141.1, 134.0, 129.2, 115.8, 114.1, 79.3, 34.1 ppm.

2-[(1-Methyl-1H-benzo[d]imidazol-2-yl)methylene]-malononitrile (**4**, $C_{12}H_8N_4$)
Yield 67 %; m.p.: >260 °C; IR (KBr): $\bar{\nu} = 2152$ (CN) cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 7.99$–7.95 (m, 1H, H-benzimid.), 7.76 (s, 1H, C*H* = C(CN)$_2$), 7.55–7.41 (m, 3H, benzimidazole), 3.98 (s, 3H, NCH_3) ppm; ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 144.8$, 144.3, 143.0, 136.3, 126.2, 124.5, 120.8, 114.5, 112.9, 111.8, 83.8, 30.3 ppm.

General procedure for the synthesis of 4H-pyran derivatives 5

To a solution of the 2-[(1-methyl-1*H*-imidazol-2-yl)methylene]malononitrile (**3**) or 2-[(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)methylene]malononitrile (**4**) in ethanol (1 mmol/10 cm^3), 1.3-dicarbonylic compound (1.1–1.2 equiv) and some drops of piperidine were added. After complete reaction (TLC), the reaction mixture was concentrated, and the solid residue was isolated by filtration, washed with cold EtOH, and dried on air.

Ethyl 6-amino-5-cyano-2-methyl-4-(1-methyl-1H-imidazol-2-yl)-4H-pyran-3-carboxylate (**5a**, $C_{14}H_{16}N_4O_3$)
Yield 92 %; m.p.: 230–232 °C; IR (KBr): $\bar{\nu} = 3390$ (NH_2), 2318 (CN), 1689 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 6.96$–6.94 (m, 3H, NH_2, H-imid.), 6.69 (s, 1H, H-imid.), 4.59 (s, 1H, H-pyran), 3.99–3.87 (m, 2H, O*CH*$_2$CH$_3$), 3.65 (s, 3H, NCH_3), 2.30 (s, 3H, CH_3), 0.97 (t, $J = 7.1$ Hz, 3H, OCH$_2$*CH*$_3$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 165.2$, 158.7, 157.6, 149.6, 126.6, 120.7, 119.8, 105.0, 60.0, 54.5, 32.1, 30.1, 18.2, 13.8 ppm.

Ethyl 6-amino-5-cyano-2-methyl-4-(1-methyl-1H-benzo[d]imidazol-2-yl)-4H-pyran-3-carboxylate (**5b**, $C_{18}H_{18}N_4O_3$)
Yield 85 %; m.p.: 240–241 °C; IR (KBr): $\bar{\nu} = 3355$ (NH_2), 2198 (CN), 1720 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 7.54$ (d, $J = 7.5$ Hz, 2H, H-benzimid.), 7.26–7.14 (m, 2H, H-benzimid.), 7.08 (br s, 2H, NH_2), 4.92 (s, 1H, H-pyran), 3.92–3.88 (m, 5H, O*CH*$_2$CH$_3$, NCH_3), 2.39 (s, 3H, CH_3), 0.88 (t, $J = 7.1$ Hz, 3H, OCH$_2$*CH*$_3$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 165.5$, 159.3, 158.6, 157.4, 142.5, 135.7, 122.2, 121.9, 120.0, 119.0, 110.5, 104.9, 60.5, 54.2, 31.2, 30.0, 18.8, 14.0 ppm.

5-Acetyl-2-amino-6-methyl-4-(1-methyl-1H-imidazol-2-yl)-4H-pyran-3-carbonitrile (**5c**, $C_{13}H_{14}N_4O_2$)
Yield 90 %; m.p.: 220–222 °C; IR (KBr): $\bar{\nu} = 3394$ (NH_2), 2218 (CN), 1616 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 7.00$–6.96 (s, 3H, NH_2, H-imid.), 6.71 (s, 1H, H-imid.), 4.76 (s, 1H, H-pyran), 3.66 (s, 3H, NCH_3), 2.32 (s, 3H, OCH_3), 2.01 (s, 3H, CH_3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 197.5$, 159.0, 155.9, 148.8, 126.7, 121.6, 119.9, 113.2, 54.2, 32.3, 30.9, 29.6, 18.8 ppm.

5-Acetyl-2-amino-6-methyl-4-(1-methyl-1H-benzo[d]imidazol-2-yl)-4H-pyran-3-carbonitrile (**5d**, $C_{17}H_{16}N_4O_2$)
Yield 73 %; m.p.: 230 °C; IR (KBr): $\bar{\nu} = 3301$ (NH_2), 2187 (CN), 1661 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 7.54$ (d, $J = 7.0$ Hz, 2H, H-benzimid.), 7.26–7.14 (m, 2H, H-benzimid.), 7.10 (br s, 2H, NH_2), 5.02 (s, 1H, H-pyran), 3.91 (s, 3H, NCH_3), 2.36 (s, 3H, OCH_3), 2.13 (s, 3H, CH_3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 197.5$, 159.6, 157.6, 156.8, 142.4, 136.0, 122.3, 122.0, 120.1, 119.1, 113.8, 110.6, 54.1, 31.6, 30.5, 30.2, 19.6 ppm.

2-Amino-7,7-dimethyl-4-(1-methyl-1H-benzo[d]imidazol-2-yl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**5e**, $C_{16}H_{18}N_4O_2$)
Yield 69 %; m.p.: 192–194 °C; IR (KBr): $\bar{\nu} = 3367$ (NH_2), 2183 (CN), 1689 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 7.01$ (s, 2H, NH_2), 6.92 (s, 1H, H-imid.), 6.66 (s,

1H, H-imid.), 4.44 (s, 1H, H-pyran), 3.70 (s, 3H, NCH_3), 2.94–2.84 (m, 2H, CH_2), 2.69 and 2.47 (ABq, $J = 16.0$ Hz, 2H, CH_2), 1.03 (s, 3H, CH_3), 0.99 (s, 3H, CH_3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 196.0$, 162.6, 158.9, 149.3, 126.5, 120.6, 119.9, 111.3, 55.5, 49.8, 32.3, 32.1, 25.5, 26.9, 26.5 ppm.

2-Amino-7,7-dimethyl-4-(1-methyl-1H-benzo[d]imidazol-2-yl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**5f**, $C_{20}H_{20}N_4O_2$)
Yield 66 %; m.p.: 250–251 °C; IR (KBr): $\bar{\nu} = 3429$ (NH_2), 2360 (CN), 1724 (CO) cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6): $\delta = 7.56$–7.48 (m, 2H, H-benzimid.), 7.26–7.11 (m, 2H, H-benzimid.), 6.98 (br s, 2H, NH_2), 4.79 (s, H, H-pyran), 3.94 (s, 3H, NCH_3), 2.64–2.46 (m, 2H, CH_2), 2.29 and 2.10 (ABq, $J = 16.0$ Hz, 2H, CH_2), 1.07 (s, 3H, CH_3), 1.06 (s, 3H, CH_3) ppm; ^{13}C NMR (62.9 MHz, C_5D_5N): $\delta = 196.8$, 163.9, 161.0, 158.1, 143.9, 136.7, 122.7, 122.4, 121.2, 120.0, 112.6, 110.7, 56.8, 50.8, 40.8, 32.7, 30.5, 29.3, 29.0. 27.6 ppm.

General procedure for the synthesis of 2-aminochromene derivatives 6

To 2-[(1-methyl-1*H*-(benz)imidazol-2yl)methylene]-malononitrile (**3**) (or **4**) (1.0 mmol) dissolved in 10 cm^3 ethanol, 1 mmol of 1- or 2-naphtol and two to three drops of piperidine were added under stirring, and then the reaction mixture was heated at reflux overnight. After completion (TLC), the reaction mixture was cooled, concentrated in a vacuum, and the resultant precipitate was filtered off and washed with cold ethanol.

2-Amino-4-(1-methyl-1H-imidazol-2-yl)-4H-benzo[h]chromene-3-carbonitrile (**6a**, $C_{18}H_{14}N_4O$)
Yield 75 %; m.p.: >260 °C; IR (KBr): $\bar{\nu} = 3433$ (NH_2), 2376 (CN) cm^{-1}; ^1H NMR (250 MHz, CD_3CN): $\delta = 7.95$–7.90 (m, 2H, H-naph.), 7.78–7.75 (m, 1H, H-naph.), 7.53–7.43 (m, 2H, H-naph.), 7.30 (d, $J = 8.9$ Hz, 1H, H-naph), 7.15 (br s, 2H, NH_2), 7.69 (s, 1H, H-imid.), 6.67 (s, 1H, H-imid.), 5.56 (s, 1H, H-pyran), 3.65 (s, 3H, NCH_3) ppm; ^{13}C NMR (62.9 MHz, CD_3CN): $\delta = 160.4$, 148.9, 146.8, 130.7, 130.6, 129.8, 128.5, 127.3, 126.6, 125.1, 123.2, 121.9, 120.5, 116.8, 113.1, 53.7, 32.4, 31.5 ppm.

2-Amino-4-(1-methyl-1H-benzo[d]imidazol-2-yl)-4H-benzo[h]chromene-3-carbonitrile (**6b**, $C_{22}H_{16}N_4O$)
Yield 95 %; m.p.: >260 °C; IR (KBr): $\bar{\nu} = 3471$ (NH_2), 2179 (CN) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 7.96$ (d, $J = 9.0$ Hz, 1H, H-Ar), 7.92–7.90 (m, 1H, H-Ar), 7.52–7.50 (m, 1H, H-Ar), 7.43–7.41 (m, 1H, H-Ar), 7.43–7.33 (m, 4H, H-Ar), 7.23 (br s, 2H, NH_2), 7.20–7.08 (m, 2H, H-Ar), 6.00 (s, 1H, H-pyran), 3.97 (s, 3H, NCH_3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 161.3$, 156.8, 147.7, 142.6, 136.4, 131.3, 131.0. 130.5, 129.2, 128.1, 125.7, 123.6, 122.7, 122.4, 121.0, 119.4, 117.5, 113.5, 111.0, 53.7, 32.1, 30.7 ppm.

3-Amino-1-(1-methyl-1H-imidazol-2-yl)-4H-benzo[f]chromene-2-carbonitrile (**6c**, $C_{18}H_{14}N_4O$)
Yield 72 %; m.p.: >260 °C; IR (KBr): $\bar{\nu} = 3440$ (NH_2), 2183 (CN) cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6): $\delta = 7.96$–7.92 (m, 2H, H-naph.), 7.78–7.75 (m, 1H, H-naph.), 7.51–7.43 (m, 2H, H-naph.), 7.30 (d, $J = 8.9$ Hz, 1H, H-naph.), 7.15 (br s, 2H, NH_2), 6.99 (d, $J = 0.9$ Hz, 1H, H-imid.), 6.67 (d, $J = 0.9$ Hz, 1H, H-imid.), 5.67 (s, 1H, H-pyran), 3.66 (s, 3H, NCH_3) ppm; ^{13}C NMR (62.9 MHz, DMSO-d_6): $\delta = 160.4$, 148.9, 146.8, 130.7, 130.4, 129.7, 128.5, 127.3, 126.6, 125.0, 123.2, 121.9, 120.5, 116.8, 113.1, 53.7, 32.4, 31.4 ppm.

3-Amino-1-(1-methyl-1H-benzo[d]imidazol-2-yl)-4H-benzo[f]chromene-2-carbonitrile (**6d**, $C_{22}H_{16}N_4O$)
Yield 90 %; m.p.: >260 °C; IR (KBr): $\bar{\nu} = 3474$ (NH_2), 2180 (CN) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 7.96$ (d, $J = 9.0$ Hz, 1H, H-Ar), 7.92–7.90 (m, 1H, H-Ar), 7.52–7.50 (m, 1H, H-Ar), 7.43–7.41 (m, 1H, H-Ar), 7.43–7.33 (m, 4H, H-Ar), 7.23 (br s, 2H, NH_2), 7.20–7.09 (s, 2H, H-Ar), 5.96 (s, 1H, H-pyran), 3.97 (s, 3H, NCH_3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 161.3$, 156.8, 147.7, 142.6, 136.4, 131.3, 131.0. 130.5, 129.2, 128.1, 125.7, 123.6, 122.7, 122.4, 121.0, 119.4, 117.5, 113.5, 111.0, 53.7, 32.1, 30.7 ppm.

6-Acetyl-2-amino-5-hydroxy-4-(1-methyl-1H-imidazol-2-yl)-4H-chromene-3-carbonitrile (**7**, $C_{16}H_{14}N_4O_3$)
To 158 mg 2-[(1-methyl-1*H*-imidazol-2-yl)methylene]-malononitrile (**3**, 80 mmol, 1.0 equiv) dissolved in 5 cm^3 methanol, 152 mg 2,4-dihydroxyacetophenone (1.0 equiv.) and 75 mg $Ca(OH)_2$ (1.0 equiv.) were added under magnetic stirring. The reaction mixture was kept under stirring at rt until the disappearance of the starting product (TLC). The residue was filtered and washed with ethyl acetate (2×5 cm^3) to remove residual **3**. The solid was then dissolved in 15 cm^3 THF and the mixture were filtered off to remove the remaining $Ca(OH)_2$. The organic layer was dried over anhydrous $MgSO_4$, filtered, and the solvent removed under reduced pressure. The resultant residue was purified by crystallization in dioxane/MeOH solution to give pure compound **7** as a white solid. Yield 40 %; m.p.: >260 °C; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 7.88$ (d, $J = 8.9$ Hz, H-Ar), 7.13 (s, 2H, NH_2), 6.99 (s, 1H, H-imid.), 6.69–6.66 (m, 2H, H-imid., H-Ar), 4.98 (s, 1H, H-pyran), 3.76 (s, 3H, NCH_3), 3.50 (br s, 1H, OH) 2.58 (s, 3H, CH_3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 204.6$, 159.9, 159.8, 154.1, 149.4, 131.9, 126.6, 120.7, 120.2, 112.7, 110.1, 107.5, 54.3, 32.5, 27.6, 26.6 ppm.

General procedure for the synthesis of 1,4-dihydropyridine-1-methyl-1H-(benz)imidazoles 8

To 1.0 mmol of 1-methyl-1*H*-imidazole-2-carbaldehyde (**1**) or 1-methyl-1*H*-benzimidazole-2-carbaldehyde (**2**) dissolved in a minimum of EtOH, the activated methylene compound (2.2 equiv) and NH_4OH (4.0 equiv) were added; then the reaction mixture was refluxed until disappearance of the starting product (TLC). After cooling to rt, the residue was poured into cold-ice water, filtered off, and the solid was washed with cold ethanol.

Diethyl 2,6-dimethyl-4-(1-methyl-1H-imidazol-2-yl)-1,4-dihydropyridine-3,5-dicarboxylate (**8a**, $C_{17}H_{23}N_3O_4$)
Yield 73 %; m.p.: 230–231 °C; IR (KBr): $\bar{\nu} = 1670$ (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 9.19$ (br s, 1H, NH), 6.84 (s, 1H, H-imid.), 6.64 (s, 1H, H-imid.), 4.87 (s, 1H, H-pyrid.), 4.00 (q, $J = 7.1$ Hz, 4H, OCH_2CH$_3$), 3.72 (s, 3H, NCH_3), 2.18 (s, 6H, CH_3), 1.12 (t, $J = 7.1$ Hz, 6H, OCH$_2CH_3$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 166.9$, 152.6, 146.2, 125.9, 120.0, 99.6, 59.1, 32.5, 30.9, 18.3, 14.3 ppm.

Diethyl 2,6-dimethyl-4-(1-methyl-1H-benzo[d]imidazol-2-yl)-1,4-dihydropyridine-3,5-dicarboxylate
(**8b**, $C_{21}H_{25}N_3O_4$)
Yield 77 %; m.p.: >260 °C; IR (KBr): $\bar{\nu} = 3448$ (NH), 1631 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 9.00$ (br s, 1H, NH), 7.48–7.43 (m, 2H, H-benzimid.), 7.16–7.07 (m, 2H, H-benzimid.), 5.11 (s, 1H, H-pyrid.), 4.01–3.95 (m, 4H, OCH_2CH$_3$), 3.90 (s, 3H, NCH_3), 2.24 (s, 6H, CH_3), 1.13–1.10 (m, 6H, OCH$_2CH_3$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 193.9$, 167.5, 160.4, 147.2, 135.7, 122.2, 121.9, 119.0, 110.7, 100.0, 60.0, 31.2, 19.4, 14.9 ppm.

3,3,6,6-Tetramethyl-9-(1-methyl-1H-imidazol-2-yl)-3,4,6,7,9,10-hexahydroacridine-1,8(2H,5H)-dione
(**8c**, $C_{21}H_{27}N_3O_2$)
Yield 80 %; m.p.: 228–230 °C; IR (KBr): $\bar{\nu} = 1654$ (CO) cm^{-1}; ^{1}H NMR (250 MHz, C_6D_5N): $\delta = 7.30$ (s, 1H, H-imid.), 6.85 (s, 1H, H-imid.), 5.10 (br s, 1H, NH_2), 4.94 (s, 1H, H-pyrid.), 4.17 (s, 3H, NCH_3), 2.40 (s, 4H, CH_2), 2.22 and 2.08 (ABq, $J = 20$ Hz, 4H, CH_2), 0.94 (s, 12H, CH_3) ppm; ^{13}C NMR (62.9 MHz, C_6D_5N): $\delta = 197.4$, 163.6, 127.8, 120.7, 114.3, 50.9, 40.9, 33.4, 32.6, 29.2, 27.3, 24.9 ppm.

2-Amino-1-(4-methoxyphenyl)-7,7-dimethyl-4-(1-methyl-1H-benzo[d]imidazol-2-yl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (**9**, $C_{27}H_{27}N_5O_2$)
Dimedone (150 mg, 1.07 mmol, 1.0 equiv) and 131 mg *p*-anisidine (1.07 mmol, 1.0 equiv) were refluxed in 15 cm^3 ethanol for 24 h. The reaction mixture was cooled to rt, and 222 mg 2-[(1-methyl-1*H*-benzimidazol-2-yl)methylene]malononitrile (**4**, 1.0 equiv) and two to three drops of triethylamine were added. The mixture was refluxed again for 1 h. After cooling, the solid was filtered off, washed with cold ethanol, and dried on air to give compound **9** (375 mg) in a pure form as a yellow solid. Yield 77 %; m.p.: 214–216 °C; IR (KBr): $\bar{\nu} = 3452$ (NH_2), 2175 (CN), 1647 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 7.57$–7.52 (m, 2H, H-Ar), 7.43–7.41 (m, 2H, H-Ar), 7.23–7.13 (m, 4H, H-Ar), 5.51 (br s, 2H, NH_2), 4.95 (s, 1H, H-pyrid.), 3.97 (s, 3H, OCH_3), 3.84 (s, 3H, NCH_3), 2.19 (d, $J = 16.0$ Hz, 2H, CH_2), 2.00 and 1.85 (ABq, $J = 16.0$ Hz, 2H, CH_2), 0.97 (s, 3H, CH_3), 0.86 (s, 3H, CH_3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 195.5$, 160.0, 158.4, 152.9, 152.5, 142.8, 135.8, 135.8, 129.4, 121.9, 121.8, 121.6, 119.0, 115.6, 110.5, 110.4, 57.1, 56.4, 55.8, 49.6, 32.7, 30.1, 29.3, 29.1, 27.0, 18.9 ppm.

2-(1-Methyl-1H-imidazol-2-yl)-4-phenylquinazoline
(**10**, $C_{18}H_{14}N_4$)
1-Methyl-1*H*-imidazol-2-carbaldehyde (**1**, 315 mg, 2.0 mmol) dissolved in 5 cm^3 EtOH was placed in a 50 cm^3 round-bottomed flask, and under stirring 702 mg 2-aminobenzophenone (1.0 equiv.), 193 mg ammonium acetate (2.5 equiv.), and 13 mg iodine (5 % mmol) were added successively. The reaction mixture was heated at 40 °C for 3 h, and the reaction was then allowed to stand at rt overnight. The resultant solid was filtered off, washed with H_2O (3 × 10 cm^3) followed by ethanol (3 × 10 cm^3), and dried under reduced pressure to afford the compound **10** (303 mg) as a pale green solid, which was crystallized in an isopropanol/MeOH solution. Yield 53 %; m.p.: 112 °C; ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 8.18$–7.57 (m, 9H, H-Ar), 7.27 (s, 1H, H-imid.), 7.06 (s, 1H, H-imid.), 4.24 (s, 3H, NCH_3) ppm; ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 153.8$, 151.6, 144.2, 137.2, 133.9, 130.2, 130.0, 129.3, 128.5, 127.6, 127.0, 125.2, 121.5, 37.0 ppm.

Cell culture and treatment

The human hepatoma cell line HepG2 was cultured in Eaglés minimum essential medium (EMEM) supplemented with 15 non-essential amino acids, 1 mM sodium pyruvate, 10 % heat-inactivated fetal bovine serum (FBS), 100 units/cm^3 penicillin, and 100 μg/cm^3 streptomycin (reagents from Invitrogen, Madrid, Spain). Cultures were seeded into flasks containing supplemented medium and maintained at 37 °C in a humidified atmosphere of 5 % CO_2 and 95 % air. Culture media were changed every 2 days. Cells were sub-cultured after partial digestion with 0.25 % trypsin–EDTA. For assays, HepG2 cells were subcultured in 96-well plates at a seeding density of 1×10^5 cells per well. When the HepG2 cells reached 80 % confluence, the medium was replaced with fresh medium containing

1–300 μM compounds or 0.1 % DMSO as a vehicle control.

Measurement of cell viability

Cell viability, measured as the mitochondrial activity of living cells, was determined by quantitative colorimetric assay with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). Briefly, 50 mm^3 of the MTT labeling reagent, at a final concentration of 0.5 mg/cm^3, was added to each well at the end of the incubation period and the plate was placed in a humidified incubator at 37 °C with 5 % CO_2 and 95 % air (v/v) for an additional 2 h period. Metabolically active cells convert the yellow MTT tetrazolium compound to a purple formazan product. Then, the insoluble formazan was dissolved with dimethylsulfoxide; colorimetric determination of MTT reduction was measured in an ELISA microplate reader at 540 nm. Control cells treated with EMEM were taken as 100 % viability.

Statistical analysis

The data are shown as mean ± SEM of data obtained from two or three independent experiments from different cultures, each of which was performed in triplicate. Statistical analyses between two cortical neuronal cell conditions were made by a Student's test. A p value of 0.05 was considered to be statistically significant.

DPPH radical scavenging

To 3 cm^3 of a DPPH free radical solution in MeOH (0.1 mM), 1 cm^3 of each test sample and standard (Asc: ascorbic acid) solution in methanol were added at concentrations of 100 $\mu g/cm^3$. The absorbance was measured at 517 nm at $t = 0$ min with UV–visible spectrophotometer(Jenway 6300) and after 30 min, 3 h, and 24 h incubation in the dark at room temperature. The percentage inhibition of DPPH free radical (I %) was calculated as follows:

$$I\,\% = [(\text{Abs control}/\text{Abs sample})/\text{Abs control}] \times 100,$$

where Abs control is the absorbance of DPPH radical + methanol and Abs sample is the absorbance of DPPH radical + test sample/standard Asc.

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