Synthesis of fused pyranocarbazolones with biological interest

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Dedicated to Prof. Michael Orphanopoulos

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Abstract
Fused pyranocarbazolones were synthesized from the reactions of hydroxycarbazoles with ethyl propiolate in the presence of catalytic amount of $InCl_3$. Carbalkoxypyranocarbazolones and methylenefurocarbazolones were obtained from the reactions of hydroxycarbazoles with dialkyl acetylene dicarboxylates in the presence of Ph_3P in refluxing toluene. Furanone **10a** (IC_{50} = 10 μM) followed by pyranocarbazolones **9a** and **11b** are interesting lipoxygenase inhibitors. Pyranocarbazolones **9b**, **11a-b** are potent anti-lipid peroxidation agents and this is correlated to the presence of the coumarin ring.

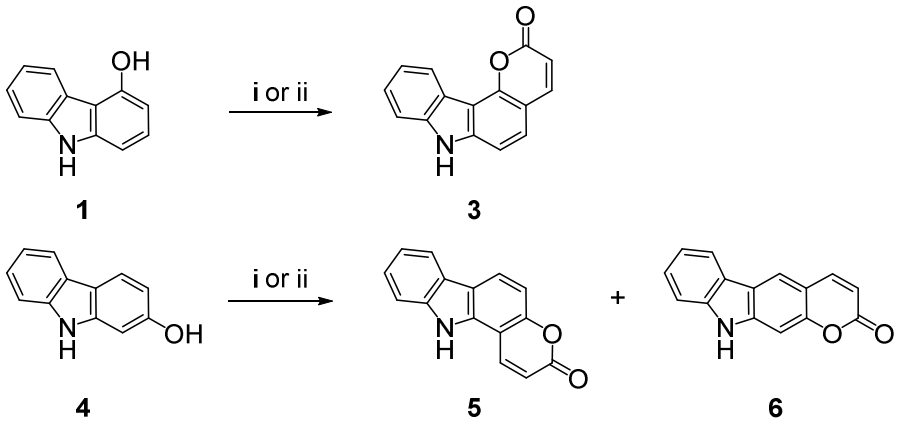
Keywords: Pyranocarbazolones; hydroxycarbazoles; coumarins; lipoxygenase inhibitors; anti-lipid peroxidation agents

Introduction

Coumarin derivatives are an interesting class of heterocycles, since coumarins are present in a variety of natural and synthetic biologically active compounds.[1-5] In particular, fused coumarins and among them pyrollocoumarins possess cytotoxic[6] and HIV-1 integrase inhibition[7] activity, act as fluorescent substrates for MAO enzymes,[8] and show antiinflammatory/antioxidant,[9] photobiological[10,11] and antitumor[10] properties. Carbazole derivatives, naturally occurring and synthetic, present interesting biological properties[12,13] like antibiotic, antioxidant, antibacterial, antifungal, cardiotonotic, antitumor, antilipid peroxidation.

There are many methods for the synthesis of coumarins including Pechmann, Perkin, Knoevenagel, Reformatsky, Wittig and Ring Closing Metathesis reactions.[3,14] One of the most attractive methods is the Pechmann condensation,[15] which starts from phenols and *β*-ketoesters or malic acid or alkynoates[16-18] in the presence mainly of concentrated H_2SO_4 or Lewis acids. The synthesis of carbazoles is achieved[12,13] mainly by multistep sequences involving coupling and cyclization reactions of anilines or iminoquinones, Fischer reactions of the corresponding phenylhydrazones, benzannulation reactions of indoles. The pyranone fragment is introduced starting from 1-hydroxycarbazoles by Friedel-Crafts reactions and subsequent cyclization,[19,20] by Pechmann cyclization,[21] by reactions[21] with DMAD and Ph_3P or from 2-formyl-1-hydroxy-carbazoles[22] by Wittig or Knoevenagel reactions.

Recently we have prepared coumarins by the reactions of phenols[23-26] and especially hydroxyindoles[9] with DMAD and Ph_3P and by Pechmann reactions of phenols with malic acid and H_2SO_4 under microwave (MW) irradiation or with propiolates in the presence of $ZnCl_2$.[27] In the course of our interest on the synthesis of coumarin hybrid derivatives, to study further their biological activity, we report herein the reactions of 2- and 4-hydroxycarbazoles with ethyl propiolate or with DMAD and Ph_3P and the biological study of new hybrid compounds as antioxidant agents. Free radicals are highly implicated in the induction of several significant pathophysiological disorders.[28] Inflammation, cancer, arthritis, myocardial and central nervous system (CNS) ischemia among them are under intensive study. Consequently, compounds with antioxidative character can be expected to offer protection in several diseases and to lead to potentially effective drugs.



Scheme 1. Reagents and conditions: (i) Ethyl propiolate (2), $InCl_3$ (11 mol%), 90 ^oC, 24 h; (ii) $Pd(OAc)_2$, (2), TFA, 0 ^oC, 5 min then r.t., 44 h.

Results and Discussion

The reactions studied and the products obtained are depicted in Schemes 1-2. The treatment of the mixture of 4-hydroxycarbazole (**1**) and ethyl propiolate (**2**) with catalytic amount (11 mol%) of $InCl_3$ under stirring at 90 °C for 24 h gave pyrano[3,2-*c*]carbazol-2(7*H*)-one (**3**) (Scheme 1) in

77% yield (Method A, Table 1, entry 1). This reaction is a tandem Michael addition followed by cyclization for the formation of pyranone ring.[18] In the literature, it is referred also that this process with $InCl_3$ failed to give amino substituted coumarin.[18] The above procedure gave much better results than the attempts for the formation of derivative **3** from compounds **1** and **2** in the presence of catalytic amount of $Pd(OAc)_2$ and TFA[16] (Method B, Table 1, entry 2) or $ZnCl_2$ in dioxane[27] (Method C, Table 1, entry 3). The reaction also of **1** with malic acid in the presence of concentrated H_2SO_4 under microwave irradiation[27] led to 8% of product **3** (Method D, Table 1, entry 4).

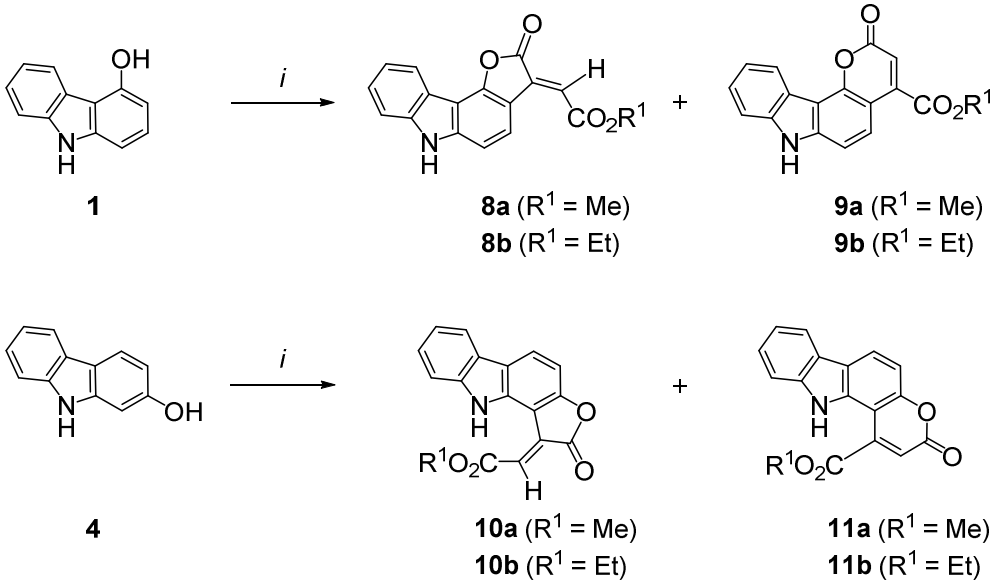
Table 1. Synthesis of pyranocarbazolones and furocarbazolones from hydroxycarbazoles

Entry	Starting hydroxycarbazole	Conditions	Products or unreacted hydroxycarbazole (yields %)
1	**1**	**Method A**: Ethyl propiolate (**2**), $InCl_3$ (11 mol%), 90 °C, 24 h	**3** (77), **1** (21)
2	**1**	**Method B**: **2**, $Pd(OAc)_2$ (1.1 mol%), TFA, 0 °C, 5 min then r.t. 44 h	**3** (15), **1** (62)
3	**1**	**Method C**: **2**, $ZnCl_2$, dioxane, reflux, 6 d	**3** (traces), **1** (mainly)
4	**1**	**Method D**: Malic acid, H_2SO_4, MW, 80 °C, 3 min	**3** (8), **1** (73)
5	**4**	**Method A**	**5** (20), **6** (25), **4** (44)
6	**4**	**Method B**	**5** (5), **6** (9), **4** (80)
7	**4**	**Method C**	**6** (4), **4** (75)
8	**4**	**Method D**	**4** (mainly)
9	**1**	**Method E**: Ph_3P, toluene, 0 °C, addition (45 min) DMAD (**7a**) in toluene, then reflux 4 d	**8a** (30), **9a** (25), **1** (30)
10	**1**	**Method F**: Ph_3P, toluene, 0 °C, addition (45 min) DMAD (**7a**) in toluene, then MW, 110 °C, 90 min	**8a** (29), **9a** (26), **1** (40)
11	**1**	**Method E** [diethyl acetylene dicarboxylate (**7b**)]	**8b** (34), **9b** (28), **1** (25)
12	**4**	**Method E**	**10a** (28), **11a** (25), **4** (29)
13	**4**	**Method F** (90 °C, 6 h)	**10a** (35), **11a** (28), **4** (17)
14	**4**	**Method G**: Ph_3P, DCM, 0 °C, addition (45 min) DMAD (**7a**) in DCM, then reflux 3 d	**10a** (13), **11a** (8), **4** (47)
15	**4**	**Method E** [diethyl acetylene dicarboxylate (**7b**)]	**10b** (35), **11b** (30), **4** (15)

The similar reaction of 2-hydroxycarbazole (**4**) with **2** (Scheme 1) under Method A (Table 1, entry 5) gave pyrano[3,2-*a*]carbazole-3(11*H*)-one (**5**) in 20% yield and pyrano[2,3-*b*]carbazole-2(10*H*)-one (**6**) in 25% yield. The same reaction at r.t. or under MW irradiation at different temperatures left unchanged the 2-hydroxycarbazole (**4**). This reaction under Method B led to compounds **5** (5%) and **6** (9%) (Table 1, entry 6), while under Method C only the linear compound **6** (4%) was obtained (Table 1, entry 7). The reaction of **4** with malic acid according to Method D at 100 °C for 1 min gave an intractable tar, while at 80 °C for 10 min the starting compound was left unchanged (Table 1, entry 8).

A solution of DMAD **7a** in toluene was added dropwise in 45 min to the solution of Ph_3P and **1** in toluene at 0 °C (Scheme 2). The mixture was heated at reflux for 4 d to give after chromatography methyl (2*E*)-(2-oxo-2*H*-furo[3,2-*c*]carbazol-3(6*H*)-ylidene)acetate (**8a**) (30%), followed by methyl 2-oxo-2,7-dihydropyrano[3,2-*c*]carbazole-4-carboxylate (**9a**) (25%) (Method E, Table 1, entry 9). The IR spectrum of **8a** exhibits a peak at 1798 cm^{-1} characteristic for the formation of furanone ring.[29,30] In the ^1H NMR spectrum there is a doublet at 8.71 ppm revealing the *E*-stereochemistry of this derivative.[29,30] The formation of **8a** can be explained by *γ*-lactonization[29,30] of the intermediate fumarate,[9,21,23-26] which by *δ*-lactonization leads to the pyranone **9a**. When the above reaction was performed under MW irradiation (Method F, Table 1, entry 10) the products **8a** and **9a** were isolated again.

The similar reaction of **1** with diethyl acetylene dicarboxylate (**7b**) under Method E (Table 1, entry 11) resulted to the furanone **8b** (34%) followed by the pyranone **9b** (28%).



Scheme 2. Reagents and conditions: (i) Dimethyl- or diethyl acetylenedicarboxylate **7a** or **7b**, Ph_3P, PhMe, 0 ^oC, 45 min, then 110 ^oC, 4 d.

The reaction of 2-hydroxycarbazole (**4**) with DMAD **7a** according to Method E (Table 1, entry 12) (Scheme 2) led to the furanone **10a** (28%) and the pyranone **11a** (25%). The above reaction following Method F (Table 1, entry 13) gave the derivatives **10a** (35%) and **11a** (28%). When the reaction of the hydroxycarbazole **4** with DMAD **7a** and Ph_3P was performed in DCM heated at reflux (Method G, Table 1, entry 14) the yields for the products **10a** and **11a** were only 13 and 8%, respectively. The last effort with DCM made to compare with the literature,[21] where the reaction of 1-hydroxycarbazole with DMAD and Ph_3P in DCM at r.t. for 24 h afforded methyl 2-oxo-2,11-dihydropyrano[2,3-*a*]carbazole-4-carboxylate (95%).

From the analogous reaction of compound **4** with **7b** under Method E (Table 1, entry 15) the furanone **10b** (35%) and the pyranone **11b** (30%) were obtained.

Taking into account the multifactorial character of oxidative stress and inflammation, and the role of indoles and coumarins in them, we decided to evaluate in the present investigation the new pyranocarbazolones (Table 2) as antioxidative agents. Nordihydroguaiaretic acid (NDGA) and Trolox, well known antioxidant agents, are used as reference compounds. Several assays should be used to assess *in vitro* antioxidant activity because the antioxidant ability of a compound must be evaluated in a variety of milieus. Most of them require a spectrophotometric measurement and a certain reaction time to obtain reproducible results.

Table 2. Biological evaluation of pyranocarbazolones and furocarbazolones as antioxidants

Compd No.	R^1	RA%[a] @ 100 µM 20/60 min	%LOX Inh.[b] @ 100 µM / IC_{50} µM	AAPH%[c] @ 100 µM
3		58/60	14	34
5		50/50	na[d]	27
6		>3	5	na[d]
8a	Me	16/na[d]	na[d]	24
8b	Et	11/na[d]	2	19
9a	Me	4/5	100 µM	39
9b	Et	3/12	33	81
10a	Me	26/37	10 µM	na[d]
10b	Et	26/40	32	na[d]
11a	Me	10/na[d]	43	78
11b	Et	>1	100 µM	54
NDGA		87/93	5.5 µM	
Trolox				71

[a] Interaction with DPPH; [b] *In vitro* % inhibition of soybean lipoxygenase (LOX); [c] % Inhibition of lipid peroxidation (AAPH); [d] na = no activity under the experimental conditions.

The interaction/reducing activity (RA) of the examined compounds with the stable free radical DPPH is shown in Table 2. This interaction, which indicates their radical scavenging ability in an iron-free system, was measured at 100 μM for 20/60 min. In the DPPH assay, the dominant chemical reaction involved is the reduction of the DPPH radical by an electron transfer from the antioxidant. Particularly effective such antioxidants are the phenoxide anions from phenolic compounds like catechol and derivatives, such as NDGA. Under our experimental conditions[25] very low interaction values (11-43%) were observed with the exception of compounds **3** and **5** which both contain an indolyl and a coumarin group. The results are similar, although compound **3** is a pyrano[3,2-*c*]carbazol-2(7*H*)-one and **5** is a pyrano[3,2-*a*]carbazole-3(11*H*)-one. An increase in reducing abilities is observed after 60 min of interaction for: **3**, **5**, **10**a and **10b**.

In our studies, AAPH was used as a free radical initiator to follow oxidative changes of linoleic acid to conjugated diene hydroperoxide. The water-soluble azo compound AAPH has been extensively used as a clean and controllable source of thermally produced alkylperoxyl free radicals.[27] In the AAPH assay the highly reactive alkylperoxyl radicals are intercepted mainly by hydrogen atom transfer (HAT) from the antioxidant. Particularly effective HAT agents[31] are compounds with high hydrogen atom donating ability, that is compounds with low heteroatom-H bond dissociation energies and/or compounds from which hydrogen abstraction leads to sterically hindered radicals as well as compounds from which abstraction of hydrogen leads to C-centered radicals stabilized by resonance. Only pyranocarbazolones **9b**, **11a-b,** shown is Table 2 exhibit interesting anti-lipid peroxidation activity compared to the reference compound Trolox. Furanone **8b** exhibits lower activity compared to the corresponding pyranon**e 9b**. The same results are taken from furanones **10a-b** and pyranones **9a-b** and **11a-b**. It seems that pyranocarbazolones are more potent and this is correlated to the presence of the coumarin ring in **11a-b**. The rest carbazolones present low or no activity.

We evaluated compounds from both series for their ability to inhibit soybean lipoxygenase (LOX) by the UV absorbance based enzyme assay.[32] The lipoxygenase (LOX) catalyzes the first two steps in the metabolism of arachidonic acid to leukotrienes. LTB4 generation is considered to be important in the pathogenesis of neutrophil-mediated inflammatory diseases with a marked relation to the severity of cardiovascular diseases, asthma and cancer. The most potent inhibitor is furanone **10a** with an IC_{50} value of 10 μM followed by pyranocarbazolones **9a** and **11b** (100 μM). Judging the special structural characteristics within the two groups of derivatives, compounds of the furanone group seem to be more potent.

Conclusions

Pyranocarbazolones were synthesized from ethyl propiolate and hydroxycarbazoles in the presence of catalytic amount of $InCl_3$. Carbalkoxypyranocarbazolones and methylene-furocarbazolones were obtained from the reactions of hydroxycarbazoles with dialkyl acetylene

dicarboxylates in the presence of Ph_3P in toluene heated at reflux. Furanone **10a** (IC_{50} = 10 μM) followed by pyranocarbazolones **9a** and **11b** are interesting lipoxygenase inhibitors. Pyranocarbazolones **9b**, **11a-b** are potent anti-lipid peroxidation agents and this is correlated to the presence of the coumarin ring. Thus, the combination of carbazole and coumarin or furanone leads to interesting hybrid compounds in terms of biological activity.

Experimental Section

General. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were obtained with a Perkin-Elmer 1310 spectrophotometer as KBr disks. ^1H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively on a Bruker AM 300 with $CDCl_3$ as solvent and TMS as internal standard. Mass spectra were determined on a LCMS-2010 EV Instrument (Shimadzu) under Electrospray Ionization (ESI) conditions. Elemental analyses for C, H and N were obtained using a Perkin-Elmer 2400-II Element analyzer. The MW experiments were performed in a Biotage (Initiator 2.0) scientific MW oven. Silica gel N^o 60, Merck A.G. was used for column chromatography. Ethyl propiolate, DMAD, diethyl acetylene dicarboxylate, $InCl_3$, Ph_3P were purchased from Fluka, (Buchs, Switzerland) and used without further purification. 1,1-Diphenyl-2-picrylhydrazyl free radical (DPPH), nordihydroguaiaretic acid (NDGA), trolox, 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH), soybean lipoxygenase, linoleic acid sodium salt were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA).

General synthetic procedure, exemplified by pyrano[3,2-*c*]carbazol-2(7*H*)-one (3). **Method A.** A mixture of 4-hydroxycarbazole (**1**) (0.1 g, 0.55 mmol), ethyl propiolate (**2**) (0.07 mL, 73.5 mg, 0.75 mmol) and $InCl_3$ (13 mg, 0.059 mmol was heated under stirring at 90 °C for 24 h. After cooling, H_2O (5 mL) was added and the mixture was extracted with EtOAc (6 × 10 mL). The organic extracts were dried (Na_2SO_4), evaporated and the residue chromatographed (hexane/EtOAc 8:1) to give the compounds **3** (99 mg, 77%) and then **1** (21 mg, 21%).
Method B. $Pd(OAc)_2$ (6 mg, 0.025 mmol) and ethyl propiolate (**2**) (0.62 mL, 65.0 mg, 6.57 mmol) were added to the solution of 4-hydroxycarbazole (**1**) (0.40 g, 2.18 mmol) in TFA (6 mL) at 0 °C and stirred for 5 min at this temperature and for 44 h at r.t. The mixture was then poured into 10% $NaHCO_3$ (30 mL) and extracted with CH_2Cl_2 (5 × 40 mL). The organic layer was dried (Na_2SO_4), evaporated and chromatographed (hexane/EtOAc 8:1) to give the compounds **3** (77 mg, 15%) and then **1** (0.248 g, 62%).
Method C. Ethyl propiolate (**2**) 0.16 mL, 0.147 g, 1.5 mmol) and $ZnCl_2$ (0.153 g, 1.64 mmol) were added to the solution of 4-hydroxycarbazole (**1**) (0.30 g, 1.64 mmol) in dry dioxane (10 mL) and the mixture was heated at reflux for 6 d. After cooling to r.t., the mixture was poured into 5% HCl (10 mL) and extracted with CH_2Cl_2 (5 × 40 mL). The organic layer was dried (Na_2SO_4), evaporated and chromatographed (hexane/EtOAc 8:1) to give traces of the compound **3** and quantitatively **1**.

Method D. Concd. H_2SO_4 (0.8 mL) was added to the mixture of 4-hydroxycarbazole (**1**) (0.31 g, 1.7 mmol) and malic acid (228 mg, 1.7 mmol) and irradiated in the MW oven at 80 °C for 3 min. After cooling the mixture was poured in ice (5 g), neutralized by 10% Na_2CO_3 and extracted with CH_2Cl_2 (3 × 20 mL) and EtOAc (5 × 20 mL). The combined organic layers were dried (Na_2SO_4), evaporated and chromatographed (hexane/EtOAc 8:1) to give the compound **3** (32.0 mg, 8%) followed by **1** (226 mg, 73%).

3. Yellow prisms, mp 111-113 °C (hexane/EtOAc); IR (ν_{max}, cm^{-1}): 3406 (N-H), 1725 (C=O). ^1H NMR (300 MHz, $CDCl_3$): 6.34 (1H, d, $^3J_{HH}$ 9.4 Hz, O=C-CH=CH), 7.29-7.39 (2H, m), 7.42-7.52 (3H, m), 7.81 (1H, d, $^3J_{HH}$ 9.4 Hz, O=C-CH=CH), 8.41 (1H, brs, NH), 8.60 (1H, d, $^3J_{HH}$ 8.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 101.0, 107.8, 110.8, 111.4, 112.2, 115.1, 121.2, 123.8, 125.3, 126.4, 135.8, 142.3, 144.3, 153.8, 161.2 (C=O). MS (ESI) *m/z*: 236 $[M+H]^+$, 258 $[M+Na]^+$; Anal. Calcd for $C_{15}H_9NO_2$ (235.27): C, 76.59; H, 3.86; N, 5.95%. Found: C, 76.41; H, 4.15; N, 5.92%.

Pyrano[3,2-*a*]carbazole-3(11*H*)-one (5) and pyrano[2,3-*b*]carbazole-2(10*H*)-one (6). The reaction by method A of 2-hydroxycarbazole (**4**) gave after chromatography the compounds **5** (26 mg, 20%), **6** (32 mg, 25%) followed by **4** (44 mg, 44%). Method B led to **5** (5%) and **6** (9%) and **4** (80%). Method C gave **6** (4%) and **4** (75%).

5. Light yellow prisms, mp 101-103 °C (hexane/EtOAc); IR (ν_{max}, cm^{-1}): 3405 (N-H), 1716 (C=O). ^1H NMR (300 MHz, $CDCl_3$): 6.74 (1H, d, $^3J_{HH}$ 8.3 Hz, O=C-CH=CH), 6.87 (1H, brs, NH), 7.16-7.39 (5H, m), 7.88 (1H, d, $^3J_{HH}$ 8.3 Hz, O=C-CH=CH), 7.95 (1H, d, $^3J_{HH}$ 7.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 95.6, 107.7, 109.6, 115.5, 117.5, 117.8, 119.6, 121.7, 122.3, 127.2, 139.1, 140.0, 144.8, 156.4. 159.6 (C=O). MS (ESI) *m/z*: 236 $[M+H]^+$; Anal. Calcd for $C_{15}H_9NO_2$ (235.27): C, 76.59; H, 3.86; N, 5.95%. Found: C, 76.74; H, 4.03; N, 5.74%.

6. Colorless prisms, mp 107-109 °C (hexane/EtOAc); IR (ν_{max}, cm^{-1}): 3407 (N-H), 1705 (C=O). ^1H NMR (300 MHz, $CDCl_3$): 6.34 (1H, d, $^3J_{HH}$ 9.5 Hz, O=C-CH=CH), 7.31 (1H, t, $^3J_{HH}$ 6.8 Hz), 7.35 (1H, s), 7.45-7.49 (2H, m), 7.89 (1H, d, $^3J_{HH}$ 9.5 Hz, O=C-CH=CH), 8.08 (1H, d, $^3J_{HH}$ 7.4 Hz), 8.14 (1H, s), 8.31 (1H, brs, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 97.6, 111.0, 111.7, 119.1, 119.8, 120.1, 121.0, 126.3, 130.6, 138.4, 140.8, 142.5, 144.8, 152.9, 161.6 (C=O). MS (ESI) *m/z*: 236 $[M+H]^+$, 258 $[M+Na]^+$; Anal. Calcd for $C_{15}H_9NO_2$ (235.27): C, 76.59; H, 3.86; N, 5.95%. Found: C, 76.68; H, 3.72; N, 5.81%.

General synthetic procedure, exemplified by methyl (2*E*)-(2-oxo-2*H*-furo[3,2-*c*]carbazol-3(6*H*)-ylidene)acetate (8a) and methyl 2-oxo-2,7-dihydropyrano[3,2-*c*]carbazole-4-carboxylate (9a). Method E. In a stirred solution of 4-hydroxycarbazole (**1**) (0.20 g, 1.09 mmol) and Ph_3P (285 mg, 1.09 mmol) in toluene (5 mL) at 0 °C was added dropwise over 45 min a solution of DMAD (**7a**) (0.13 mL, 156 mg, 1.1 mmol) in toluene (5 mL). The resulted orange mixture was refluxed for 4 d and after cooling to r.t. was evaporated and chromatographed (EtOAc/hexane 1:8) to give, after the elution of Ph_3P, the derivative **8a** (96 mg, 30%) followed by compound **9a** (80 mg, 25%) and the starting compound **1** (60 mg, 30%).

Method F. A mixture of DMAD (**7a**) (0.35 mL, 78 mg, 0.55 mmol) in toluene (1 mL) was added dropwise for 45 min at 0 °C in the stirred solution in the special vial for MW oven of Ph_3P (73

mg, 0.28 mmol) and 4-hydroxycarbazole (**1**) (50 mg, 0.28 mmol) in toluene (1 mL). After that the mixture was irradiated in the MW oven at 110 °C for 90 min. After cooling to r.t. the mixture was chromatographed (EtOAc/hexane 1:8) to give, after the elution of Ph_3P, derivative **8a** (24 mg, 29%), compound **9a** (21 mg, 26%) and compound **1** (20 mg, 40%).

8a. Red prisms, mp 161-163 °C (hexane/EtOAc); IR (ν_{max}, cm^{-1}): 3351 (N-H), 1798 (C=O, furanone), 1719 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 3.90 (3H, s, OCH_3), 6.80 (1H, s), 7.21 (1H, d, $^3J_{HH}$ 8.7 Hz), 7.32 (1H, t, $^3J_{HH}$ 7.3 Hz), 7.43-7.52 (2H, m), 8.21 (1H, d, $^3J_{HH}$ 7.9 Hz), 8.41 (1H, brs, NH), 8.71 (1H, d, $^3J_{HH}$ 8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 53.3 (OCH_3), 102.4, 106.7, 109.5, 120.9, 121.3, 123.0, 125.3, 126.8, 127.5, 128.5, 128.6, 132.0, 132.1, 152.1, 163.6 (C=O), 164.1 (C=O). MS (ESI) *m/z*: 294 $[M+H]^+$, 316 $[M+Na]^+$; Anal. Calcd for $C_{17}H_{11}NO_4$ (293.27): C, 69.62; H, 3.78; N, 4.78%. Found: C, 69.71; H, 3.59; N, 4.63%.

9a. Yellow prisms, mp 171-173 °C (hexane/EtOAc); IR (ν_{max}, cm^{-1}): 3367 (N-H), 1730 (C=O), 1714 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 4.03 (3H, s, OCH_3), 6.83 (1H, s O=C-CH=C), 7.35 (1H, d, $^3J_{HH}$ 8.8 Hz), 7.37 (1H, t, $^3J_{HH}$ 7.9 Hz), 7.45-7.52 (2H, m), 8.22 (1H, d, $^3J_{HH}$ 8.8 Hz), 8.43 (1H, brs, NH), 8.59 (1H, d, $^3J_{HH}$ 7.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 52.1 (OCH_3), 99.1, 107.9, 110.7, 114.5, 115.0, 119.0, 121.3, 123.7, 125.1, 126.6, 132.1, 140.6, 144.1, 154.3, 161.2 (C=O), 165.8 (C=O). MS (ESI) *m/z*: 294 $[M+H]^+$; Anal. Calcd for $C_{17}H_{11}NO_4$ (293.27): C, 69.62; H, 3.78; N, 4.78%. Found: C, 69.48; H, 4.06; N, 4.67%.

Ethyl (2*E*)-(2-oxo-2*H*-furo[3,2-*c*]carbazol-3(6*H*)-ylidene)acetate (8b) and ethyl 2-oxo-2,7-dihydropyrano[3,2-*c*]carbazole-4-carboxylate (9b). The reaction of 4-hydroxycarbazole (**1**) with diethyl acetylenedicarboxylate (**7b**) gave after chromatography the compounds **8b** (114 mg, 34%), **9b** (94 mg, 28%) followed by **1** (50 mg, 25%).

8b. Red prisms, mp 176-178 °C (hexane/EtOAc); IR (ν_{max}, cm^{-1}): 3429 (N-H), 1806 (C=O, furanone), 1712 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 0.78 (3H, d, $^3J_{HH}$ 7.1 Hz, OCH_2CH_3), 3.88 (1H, d, $^3J_{HH}$ 7.1 Hz, OCH_2CH_3), 6.83 (1H, s), 7.16 (1H, d, $^3J_{HH}$ 8.7 Hz), 7.36 (1H, t, $^3J_{HH}$ 7.3 Hz), 7.42-7.50 (2H, m), 8.22 (1H, d, $^3J_{HH}$ 7.2 Hz), 8.70 (1H, d, $^3J_{HH}$ 8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 14.2 (OCH_2CH_3), 62.9 (OCH_2CH_3),, 106.1, 108.4, 113.3, 120.9, 121.3, 122.2, 123.1, 127.0, 131.9, 133.6, 135.1, 140.9, 144.8, 151.7, 165.6 (C=O), 168.9 (C=O). MS (ESI) *m/z*: 308 $[M+H]^+$; Anal. Calcd for $C_{18}H_{13}NO_4$ (307.30): C, 70.35; H, 4.26; N, 4.56%. Found: C, 70.29; H, 4.17; N, 4.48%.

9b. Yellow prisms, mp 183-185 °C (hexane/EtOAc); IR (ν_{max}, cm^{-1}): 3435 (N-H), 1727 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 1.44 (3H, t, $^3J_{HH}$ 7.1 Hz, OCH_2CH_3), 4.44 (2H, q, $^3J_{HH}$ 7.1 Hz, OCH_2CH_3), 6.84 (1H, s, O=C-CH=C), 6.92 (1H, d, $^3J_{HH}$ 8.2 Hz), 7.38-7.50 (2H, m), 7.53 (1H, d, $^3J_{HH}$ 8.1 Hz), 7.99 (1H, d, $^3J_{HH}$ 7.7 Hz), 8.13 (1H, d, $^3J_{HH}$ 8.2 Hz), 11.7 (1H, brs, NH). ^{13}C NMR (75 MHz, $CDCl_3$): 13.8 (OCH_2CH_3), 62.5 (OCH_2CH_3), 107.6, 110.4, 115.6, 119.5, 120.4, 121.9, 122.8, 123.8, 124.6, 127.2, 137.6, 139.6, 143.6, 150.8, 160.3 (C=O), 164.3 (C=O). MS (ESI) *m/z*: 308 $[M+H]^+$; Anal. Calcd for $C_{18}H_{13}NO_4$ (307.30): C, 70.35; H, 4.26; N, 4.56%. Found: C, 70.18; H, 4.41; N, 4.38%.

Methyl (2*E*)-(2-oxo-2*H*-furo[3,2-*a*]carbazol-1(10*H*)ylidene)acetate (10a) and methyl 3-oxo-3,11-dihydropyrano[3,2-*a*]carbazole-1-carboxylate (11a). The reaction of 2-hydroxycarbazole

(**4**) with DMAD (**7a**) by method E resulted to **10a** (89 mg, 28%) and **11a** (80 mg, 25%). Method F (90 °C, 6 h) gave **10a** (35%), **11a** (28%) and **4** (17%).

Method G. To a stirred solution of 2-hydroxycarbazole (**4**) (0.20 g, 1.09 mmol) and Ph_3P (285 mg, 1.09 mmol) in DCM (5 mL) at 0 °C was added dropwise over 45 min a solution of DMAD (**7a**) (0.13 mL, 0.156 g, 1.1 mmol) in DCM (5 mL). The resulted orange mixture was heated at reflux for 3 d and after cooling to r.t. was evaporated and chromatographed (EtOAc/hexane 1:8) to give, after the elution of Ph_3P, the derivative **10a** (41 mg, 13%) followed by the compound **11a** (26 mg, 8%) and the starting compound **4** (94 mg, 47%).

10a. Red needles, mp 158-160 °C (hexane/EtOAc); IR (ν_{max}, cm^{-1}): 3248 (N-H), 1794 (C=O, furanone), 1703 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 3.99 (1H, s, OCH_3), 6.85 (1H, s), 6.93 (1H, d, $^3J_{HH}$ 8.3 Hz, O-C_{ar}-CH=CH), 7.26 (1H, t, $^3J_{HH}$ 7.7 Hz), 7.43 (1H, t, $^3J_{HH}$ 7.7 Hz), 7.54 (1H, d, $^3J_{HH}$ 7.7 Hz), 7.99 (1H, d, $^3J_{HH}$ 7.7 Hz), 8.14 (1H, d, $^3J_{HH}$ 8.3 Hz, O-C_{ar}-CH=CH), 11.65 (1H, brs, NH). ^{13}C NMR (75 MHz, $CDCl_3$): 53.0 (OCH_3), 102.2, 104.0, 111.1, 119.7, 119.8, 120.6, 121.9, 122.8, 126.1, 127.0, 132.5, 136.5, 139.6, 156.2, 167.7 (C=O), 168.2 (C=O). MS (ESI) *m/z*: 294 $[M+H]^+$; Anal. Calcd for $C_{17}H_{11}NO_4$ (293.27): C, 69.62; H, 3.78; N, 4.78%. Found: C, 69.48; H, 4.01; N, 4.71%.

11a. Yellow prisms, mp 150-152 °C (hexane/EtOAc); IR (ν_{max}, cm^{-1}): 3991 (N-H), 1736 (C=O), 1716 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 4.14 (3H, s), 7.07 (1H, s, O=C-CH=C), 7.23-7.33 (2H, m), 7.46 (1H, t, $^3J_{HH}$ 7.1 Hz), 7.54 (1H, d, $^3J_{HH}$ 8.0 Hz), 8.06 (1H, d, $^3J_{HH}$ 7.1 Hz), 8.25 (1H, d, $^3J_{HH}$ 8.7 Hz), 10.6 (1H, brs, NH). ^{13}C NMR (75 MHz, $CDCl_3$): 54.1 (OCH_3), 101.3, 109.3, 111.4, 119.2, 119.7, 120.5, 120.9, 122.3, 124.8, 126.0, 134.6, 139.2, 141.2, 154.3, 159.8 (C=O), 167.6 (C=O). MS (ESI) *m/z*: 294 $[M+H]^{+\cdot}$; Anal. Calcd for $C_{17}H_{11}NO_4$ (293.27): C, 69.62; H, 3.78; N, 4.78%. Found: C, 69.50; H, 4.12; N, 4.62%.

Ethyl (2*E*)-(2-oxo-2*H*-furo[3,2-*a*]carbazol-1(10*H*)ylidene)acetate (10b) and ethyl 3-oxo-3,11-dihydropyrano[3,2-*a*]carbazole-1-carboxylate (11b). The reaction of 2-hydroxycarbazole (**4**) with diethyl acetylene dicarboxylate (**7b**) resulted to **10b** (117 mg, 35%) and **11b** (101 mg, 30%).

10b. Red needles, mp 182-184 °C (hexane/EtOAc); IR (ν_{max}, cm^{-1}): 3276 (N-H), 1795 (C=O, furanone), 1697 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 1.44 (3H, t, $^3J_{HH}$ 7.1 Hz, OCH_2CH_3), 4.44 (2H, q, $^3J_{HH}$ 7.1 Hz, OCH_2CH_3), 6.82 (1H, s), 6.91 (1H, d, $^3J_{HH}$ 8.2 Hz, O-C_{ar}-CH=CH), 7.25 (1H, t, $^3J_{HH}$ 7.7 Hz), 7.43 (1H, t, $^3J_{HH}$ 7.7 Hz), 7.53 (1H, d, $^3J_{HH}$ 7.7 Hz), 7.98 (1H, d, $^3J_{HH}$ 7.7 Hz), 8.12 (1H, d, $^3J_{HH}$ 8.2 Hz, O-C_{ar}-CH=CH), 11.67 (1H, brs, NH). ^{13}C NMR (75 MHz, $CDCl_3$): 14.2 (CH_3), 62.3 (OCH_2), 102.3, 104.2, 111.7, 119.7, 120.5, 120.6, 121.9, 123.0, 126.1, 126.9, 132.5, 136.6, 139.7, 156.3, 167.3 (C=O), 168.4 (C=O). MS (ESI) *m/z*: 308 $[M+H]^+$; Anal. Calcd for $C_{18}H_{13}NO_4$ (307.30): C, 70.35; H, 4.26; N, 4.56%. Found: C, 70.41; H, 4.41; N, 4.47%.

11b. Yellow prisms, mp 175-177 °C (hexane/EtOAc); IR (ν_{max}, cm^{-1}): 3350 (N-H), 1733 (C=O), 1712 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 1.51 (3H, t, $^3J_{HH}$ 7.1 Hz, OCH_2CH_3), 4.60 (2H, q, $^3J_{HH}$ 7.1 Hz, OCH_2CH_3), 7.07 (1H, s, O=C-CH=C), 7.23-7.35 (2H, m), 7.46 (1H, t, $^3J_{HH}$ 7.8 Hz), 7.54 (1H, d, $^3J_{HH}$ 8.0 Hz), 8.06 (1H, d, $^3J_{HH}$ 7.8 Hz), 8.24 (1H, d, $^3J_{HH}$ 8.5 Hz), 10.70 (1H, brs, NH). ^{13}C NMR (75 MHz, $CDCl_3$): 14.1 (CH_2), 63.7 (OCH_2), 101.5, 109.4, 111.5, 119.0, 119.7, 120.5,

121.0, 122.4, 124.9, 126.1, 134.8, 139.3, 141.7, 154.4, 160.0 (C=O), 167.3 (C=O). MS (ESI) *m/z*: 308 $[M+H]^+$; Anal. Calcd for $C_{18}H_{13}NO_4$ (307.30): C, 70.35; H, 4.26; N, 4.56%. Found: C, 70.15; H, 4.58; N, 4.32%.

Biological evaluation

Determination of the reducing activity of the stable radical 1,1-diphenyl-picrylhydrazyl (DPPH).[25] To a solution of DPPH (final concentration 50 μM) in absolute ethanol was added an equal volume of the compounds dissolved in DMSO. As a control solution ethanol was used. The final concentration of the test compounds was 100 μM. After 20 min/60 min at room temperature, the absorbance was recorded at 517 nm (Table 1).

Inhibition of linoleic acid peroxidation.[25,27] Production of conjugated diene hydroperoxide by oxidation of linoleic acid in an aqueous dispersion was monitored at 234 nm. Azobis(2-amidinopropane) dihydrochloride (AAPH) is used as a free radical initiator. Ten microliters of the 16 mM linoleic acid dispersion were added to the UV cuvette containing 0.93 mL of 0.05 M phosphate buffer, pH 7.4, thermostated at 37 ^oC under air by the addition of 50 μL of 40 Mm AAPH solution. Oxidation was carried out in the presence of aliquots (10 μL) of the tested compounds. In the assay without antioxidant, lipid oxidation was measured in the presence of the same level of DMSO. The rate of oxidation at 37 ^oC was monitored by recording the increase in absorption at 234 nm caused by conjugated diene hydroperoxide formation (Table 1).

Soybean lipoxygenase inhibition study *in vitro*.[25,27] The *in vitro* study was evaluated as reported previously. The test compounds dissolved in ethanol were incubated at room temperature with sodium linoleate (100 μL) and 0.2 mL of enzyme solution ($1/9 \times 10^{-4}$ w/v in saline). The conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was recorded and compared with the appropriate standard inhibitor (NDGA) (Table 1).

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