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Efficient synthesis of 2-substituted 1-phenylchromen[3,4-*d*]imidazol-4(1*H*)-ones with possible anti-inflammatory activity

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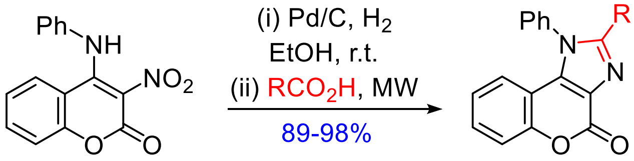
Abstract

Rare 2-substituted 1-phenylchromeno[3,4-*d*]imidazol-4(3*H*)-ones have been synthesized in excellent yields via a two-step reaction mediated by microwave irradiation that involves the reduction of 3-nitro-4-phenylaminocoumarin using H_2 and Pd/C to give *in situ* an intermediate diamine, that then reacts with aliphatic acids. A one-pot two-step reaction can also be performed in the presence of tin(II) chloride to give the same products in lower yields, accompanied by a small amount of 6*H*-chromeno[3,4-*b*]quinoxaline-6-one as side product. Preliminary *in vitro* studies support the possibility of anti-inflammatory activity and point to a new lead compound 1-phenyl-2-propylchromeno[3,4-*d*]imidazol-4(1*H*)-one.



Keywords: Pd/C Reduction, 3-nitro-4-phenylaminocoumarin, imidazolo[3,4-*d*]coumarins, microwave irradiation.

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Introduction

Coumarins are widely distributed in Nature presenting, along with their synthetic derivatives, interesting biological activities such as anticoagulant, antibiotic, anti-inflammatory, anti-HIV, and anticancer properties.[1-10] Fused coumarins are also biologically active. In particular, fused imidazolocoumarins have biological actions as antagonists of toll-like receptor 7 (TLR7) for the treatment of immune-mediated inflammatory disorders,[11] inhibitors of phosphodiesterase (PDE) VII,[12,13] for the treatment of allergic disorders,[12] psoriasis,[12] autoimmune diseases,[12] and movement disorders,[13] and growth inhibitors for mammalian cancer.[14] They also have neurotropic activity with a definite influence in CNS,[15] to bind to benzodiazepine receptors (BZR) with weak potency,[16] to present antibacterial activity[17] and to inhibit the hepatitis C virus NS5B polymerase.[18]

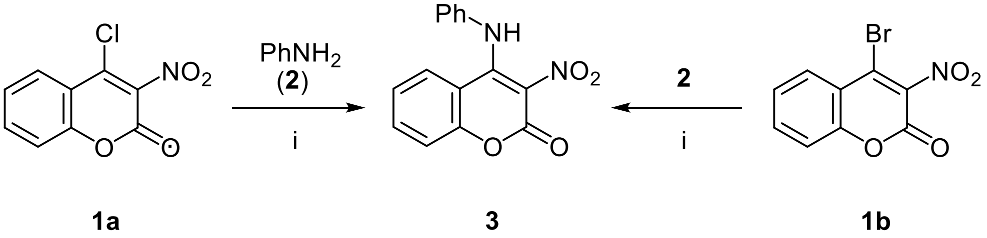
To the best of our knowledge, there is only one reference in the literature for 2-substituted 1-phenylimidazolo[3,4-*d*]coumarins, a 2-methyl derivative (acquired from ChemBridge database), with activity as inhibitor of the hepatitis C virus NS5B polymerase.[18] There are also a few known 2,3-disubstituted imidazolo[3,4-*d*]coumarins. The 3-methyl-2-propylimidazolo[3,4-*d*]coumarin has been prepared by the methylation of 2-propylimidazolo[3,4-*d*]coumarin,[19] while the 3-chloroethyl-2-propylimidazolo[3,4-*d*]coumarin was obtained by us during the chloromethylation of the same starting compound.[20] 3-Alkyl-2-(morpholin-4-yl)imidazolo[3,4-*d*]coumarins were synthesized from 4-[(1-morpholin-4-yl)amino]-3-nitrocoumarins by heating at reflux in excess of triethyl phosphite.[19]

There are only two routes to mono-1-*N*-substituted fused imidazolocoumarins: (i) Construction of the pyranone ring, for 1-substituted chromeno[3,4-*d*]imidazol-4(1*H*)-ones, by cyclization of alkyl 5-(2-hydroxyphenyl)-1*H*-imidazole-4-carboxylates in aqueous sulfuric acid under heating[11] or in the presence of base under MW irradiation;[21] and, (ii) construction of the imidazole ring, for 1-phenyl or 1-alkyl substituted chromeno[3,4-*d*]imidazol-4(1*H*)-ones, by the reaction of 3-amino-4-phenylamino- or 4-alkylaminocoumarin in boiling formic acid.[15]

Previously, our group developed an efficient synthesis of 2-substituted imidazolocoumarins starting from 4-amino-3-nitrocoumarin.[20] We envisioned that this sequence could also work for 3-nitro-4-phenylaminocoumarin which would give access to valuable 2-substituted 1-phenylimidazolo[3,4-*d*]coumarins. Herein, we present our investigations towards this goal and the biological evaluation of the formed products.

Results and Discussion

In continuation of our, above mentioned, previous work,[20] and due to our interest in the synthesis of coumarins fused with five-membered heterocycles,[19,22-25] we focused on the synthesis of the title compounds. Furthermore, a number of new structures were designed and subjected to *in silico* docking studies on soybean lipoxygenase (SLOX-1). The *in silico* results led us to choose and synthesize the most promising structures (Scheme 2), which were then tested *in vitro* to verify their LOX inhibitory activity.



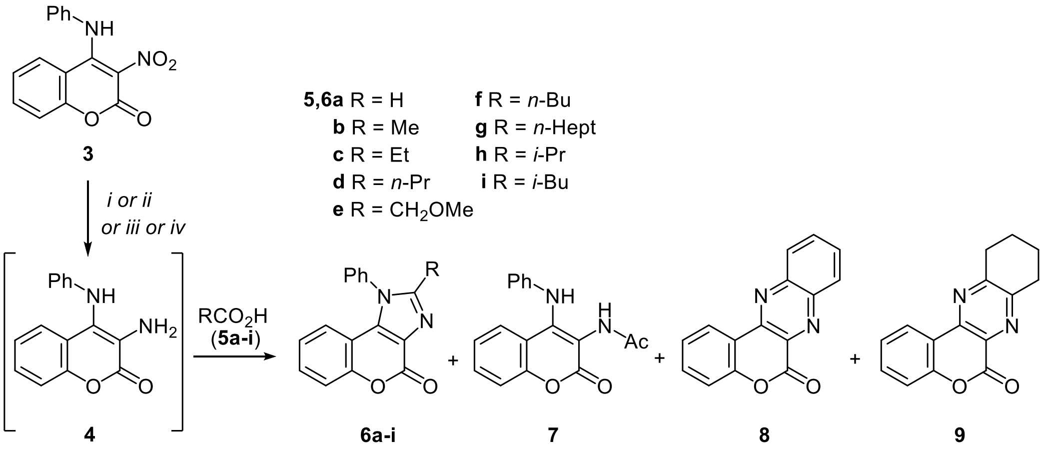
Scheme 1. Reaction conditions: (i) H_2O, MW (35-40 W, 3 bar), 100 °C, 15 min.

The starting 4-phenylamino-3-nitrocoumarin[26] (**3**) was obtained from 4-chloro-3-nitrocoumarin (**1a**)[27,28] or from 4-bromo-3-nitrocoumarin (**1b**)[29] by treatment with aniline (**2**) in water under microwave irradiation at 100 °C for 15 min (Scheme 1). Suitable conditions for the one-pot transformation of nitrocoumarin **3** to the fused 1-phenyl-2-alkylimidazolocoumarins **6** were investigated by performing the reactions with formic acid (**5a**) in the presence of tin(II) chloride under microwave irradiation at 100 °C for 5 min (Method A, Table 1). This reaction was expected to give the derivative **6a** in analogy to the synthesis of benzimidazoles from *o*-nitroanilines.[30] Indeed, the imidazolocoumarin **6a** was isolated in 62% yield followed by the quinoxaline derivative **8** (7%), as a side product (Table 1, entry 1). The use of acetic acid (**5b**) led to the formation of imidazolocoumarin **6b** (47%), the amide **7** (11%) and the side product **8** (2%) (Table 1, entry 2). The presence of the amide **7** reveals that the *in situ* formed 3-amino-4-phenylaminocoumarin (**4**)[31-33] reacted with acid **5b**. Then, amide **7** cyclized and dehydrated, under the reaction conditions, to give the expected product **6b**. Similarly, the reaction of acids **5c-e** at 120 °C under microwave irradiation gave the expected imidazolocoumarins **6c-e**, respectively, in moderate yields (Table 1, entries 3-5).

Next, we developed a one-pot protocol using 5% Pd/C under hydrogen as reducing agent (Method B). The reaction of formic acid (**5a**) with nitrocoumarin **3** in the presence of Pd/C under H_2 atmosphere in room temperature for 1 h (consumption of starting compound) followed by removing of the H_2 balloon and heating the resulting mixture at reflux, led to compound **6a** (43%) and the quinoxaline **8** (32%) (Table 1, entry 6). The analogous reaction of acetic acid (**5b**) gave the imidazolocoumarin **6b** and products **7**-**9**. Compound **9** was also prepared by reduction of the benzene ring of quinoxaline **8**. The yield of compound **9** was increased when 10% Pd/C as a catalyst was used (Table 1, entry 7 and note b). The reaction of nitrocoumarin **3** with propionic acid (**5c**) gave imidazolocoumarin **6c**, and an increased amount of side product **8**, tentatively, attributed to the longer heating time under the air atmosphere (Table 1, entry 8).

Owing to the increased quantities of side products with Pd/C as catalyst, we then investigated the use of microwave irradiation for the second step of the one-pot reaction (Method C). After finishing the first step of the above procedure with Pd/C under H_2 atmosphere and removing the balloon of H_2, the mixture of the reaction of **5a** with nitrocoumarin **3** was transferred in a vial, suitable for microwaves, and irradiated at 110 °C for 25 min to give the imidazolocoumarin **6a** in 92% yield, while the side product **8** was formed in only 3% yield (Table 1, entry 9). The similar process with acetic acid (**5b**) led to the products **6b** and **8** (Table 1, entry 10). The analogous reactions of propanoic acid (**5c**), butanoic acid (**5d**) and pentanoic acid (**5f**) needed more time for the initial reduction, and also for the microwave irradiation performed at 140 °C to give the imidazolocoumarins **6c**, **6d**, and **6f**, respectively (Table 1, entries 11, 12 & 14). The second step of the reaction of methoxyacetic acid (**5e**) at 130 °C completed in 45 min (Table 1, entry 13). More time for both steps and higher temperatures for the second step were needed for the similar reactions of octanoic acid (**5g**), 2-methylpropanoic acid (**5h**) and 3-methylbutanoic acid (**5i**) to give the imidazolocoumarins **6g**, **6h** and **6i**, respectively (Table 1, entries 15-17).

Table 1. Synthesis of fused 1-phenyl-2-alkylimidazolocoumarins **6a-i** from 3-nitro-4-phenylaminocoumarin (**3**) and acids **5a-i** *via* one-pot procedures using Methods A-D

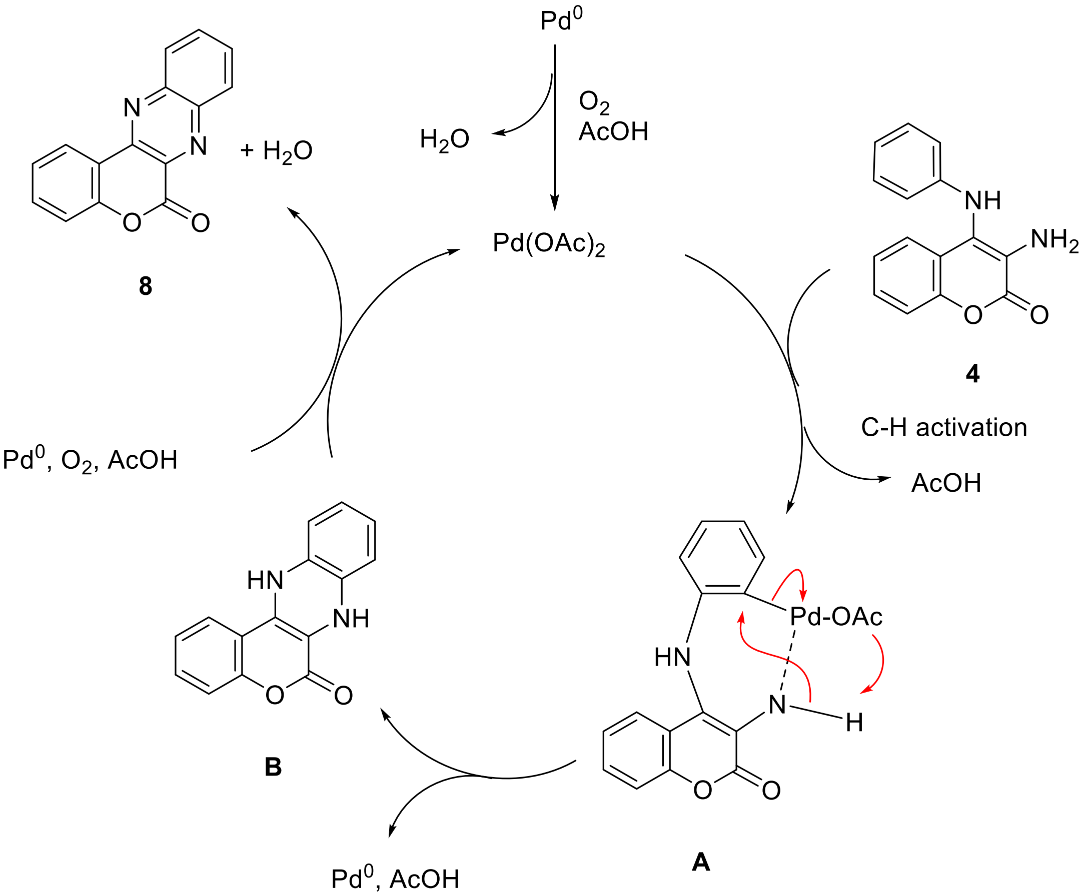


Entry	Acid **5**	R	Method[a]	Temp. (°C); Power[b] (W); Pressure[b] (bar)	Time	Products (Yields %)
1	**5a**	H	A	100; 25-30; 5	5 min	**6a** (62), **8** (7)
2	**5b**	Me	A	120; 35-40; 3	1 h	**6b** (47), **7** (11), **8** (2)
3	**5c**	Et	A	120; 25-30; 1	0.75 h	**6c** (66), **8** (11)
4	**5d**	*n*-Pr	A	120; 30-35; 1	0.75 h	**6d** (56), **8** (10)
5	**5e**	CH_2OMe	A	120; 20-25; 2	0.25 h	**6e** (44), **8** (8)
6	**5a**	H	B	r.t., then reflux	1 h, then 6 h	**6a** (43), **8** (32)
7	**5b**	Me	B	r.t., then reflux	1 h, then 8 h	**6b** (70), **7** (12), **8** (7), **9**[c] (4)
8	**5c**	Et	B	r.t., then reflux	3 h, then 14 h	**6c** (55), **8** (40)
9	**5a**	H	C	r.t., then 110; 20-25; 5	1 h, then 25 min	**6a** (92), **8** (3)
10	**5b**	Me	C	r.t., then 110; 30-35; 2	1 h, then 1.5 h	**6b** (88), **8** (8)
11	**5c**	Et	C	r.t., then 140; 40-45; 4	2 h, then 1.25 h	**6c** (85), **8** (10)
12	**5d**	*n*-Pr	C	r.t., then 140; 35-40; 3	3 h, then 2 h	**6d** (82), **8** (8)
13	**5e**	CH_2OMe	C	r.t., then 130; 25-30; 5	3 h, then 0.75 h	**6e** (82), **8** (8)
14	**5f**	*n*-Bu	C	r.t., then 140; 40-45; 3	3 h, then 2 h	**6f** (76), **8** (10)
15	**5g**	*n*-Hept	C	r.t., then 150; 70-75; 3	14 h, then 2.25 h	**6g** (72), **8** (17)
16	**5h**	*i*-Pr	C	r.t., then 150; 45-50; 3	4 h, then 2 h	**6h** (77), **8** (8)
17	**5i**	*i*-Bu	C	r.t., then 150; 45-50; 3	4 h, then 3 h	**6i** (74), **8** (10)
18	**5a**	H	D	r.t., then 110; 25-30; 5	0.5 h, then 0.25 h	**6a** (97)
19	**5b**	Me	D	r.t., then 110; 25-30;3	0.5 h, then 1 h	**6b** (98)
20	**5c**	Et	D	r.t., then 140; 35-40; 4	0.5 h, then 1 h	**6c** (96)
21	**5d**	*n*-Pr	D	r.t., then 140; 45-50; 1	0.5 h, then 1 h	**6d** (95)
22	**5e**	CH_2OMe	D	r.t., then 110; 25-30; 4	0.5 h, then 0.75 h	**6e** (97)
23	**5f**	*n*-Bu	D	r.t., then 140; 45-50; 1	0.5 h, then 1 h	**6f** (94)
24	**5g**	*n*-Hept	D	r.t., then 150; 75-80; 1	0.5 h, then 1.5 h	**6g** (89)
25	**5h**	*i*-Pr	D	r.t., then 150; 35-40; 2	0.5 h, then 1.5 h	**6h** (92)
26	**5i**	*i*-Bu	D	r.t., then 150; 35-40; 2	0.5 h, then 1.5 h	**6i** (91)

[a] Method A: $SnCl_2{\cdot}2H_2O$, MW; Method B: 5% Pd/C, H_2, then remove H_2, reflux; Method C: 5% Pd/C, H_2, then remove H_2, MW; Method D: EtOH, 5% Pd/C, H_2, then remove H_2, addition of acids **5a-i**, MW. [b] MW experimental conditions. [c] With 10% Pd/C: **6b** (68), **7** (3), **8** (8), **9** (20).

The reduction of the first step was tried next in ethanol, in an effort to reduce reduction time in all procedures (Method D). The 4-phenylamino-3-nitrocoumarin (**3**) was reduced with 5% Pd/C in ethanol under hydrogen in 30 min. After the removing of the catalyst by filtration through celite and evaporation of the ethanol, the light yellow residue was directly transferred to a vial, and formic acid (**5a**) was added. The mixture was then heated under microwave irradiation at 110 °C for 25 min to give the imidazolocoumarin **6a** in 97% yield, and, notably, no side product **8** was detected in the reaction mixture (Table 1, entry 18). Similar reactions of the reduction product of nitrocoumarin **3** with acetic acid (**5b**) or methoxyacetic acid (**5e**) at 110 °C for 1 h or 45 min gave the imidazolocoumarins **6b** or **6e** in excellent yields, respectively (Table 1, entries 19 & 22). Furthermore, propanoic acid (**5c**), butanoic acid (**5d**), and pentanoic acid (**5f**) also reacted at 140 °C for 1 h to give the imidazolocoumarins **6c**, **6d**, and **6f**, respectively, in excellent yields (Table 1, entries 20, 21 & 23). Higher temperature (150 °C for 1.5 h) was needed for the analogous reactions of the more hindered acids **5g**, **5h**, and **5i** to give the imidazolocoumarins **6g**, **6h**, and **6i**, respectively, in slightly lower yields (Table 1, entries 24-26).

Tentatively, the formation of side product, quinoxaline **8**, could be attributed to palladation of the intermediate diamine **4** from *in situ* formed palladium acetate to give intermediate **A**, followed by reductive elimination to give the fused piperazine derivative **B**. Oxidation of the latter led to the formation of quinoxaline **8** (Scheme 3).



Scheme 3. Tentative reaction mechanism for the formation of quinoxaline **8.**

The tested compounds showed medium inhibitory activity on soybean lipoxygenase. For all the compounds the IC_{50} values were determined with the exception of **6b.** Derivative **6d** was the most active followed by **6i**, **6c**, **6a**, **6f**, **6g**, **6h**, **6e**, whereas **6b** was the most inactive (38% at 100 μM). Perusal of these activities in comparison to the earlier synthesized compounds[20] showed that the presence of the phenyl group led to more potent compounds, e.g., **6a** (IC_{50} = 52 μM) is more potent than the unsubstituted (98 μM). Furthermore, the insertion of the phenyl led to bioactivity in the cases where no activity was recorded (**6h**, 65 μM- **6g**, 60 μM- **6e**, 100 μM). No improvement was observed when a phenyl group was inserted next to the methyl group (**6b**). It seems that the increase of lipophilicity is the main reason, since this physicochemical property is cited[34] to influence the anti-LOX activity. NDGA was used as a reference compound for the anti-LOX activity. The compounds, with the exception of **6a**, **6b** and **6d**, inhibit lipid peroxidation within the range 69-100% at 100 μM (Table 2). Lipophilicity does not fully explain the results, since **6i** (clog P = 5.23) presents LP% = 69%, whereas **6f** with the higher anti-lipid peroxidation ability (LP% = 100%) presents lower clog P value (4.96), following by **6e** (clog P = 2.90, LP% 93%), and **6g** (clog P = 6.55, LP% = 89%). Trolox was used as a reference compound for the anti-lipid peroxidation activity.

Table 2. *In vitro* antioxidant activity: Inhibition of lipid peroxidation, LP%; Inhibitory activity of compounds on soybean lipoxygenase (IC_{50} μM / LOX%)

Compounds	Clog P[35]	LP % @ 100 μM	LOX% @ 100 μM or IC_{50} μM
6a	3.11	37	52 μM
6b	3.38	59	38% @ 100 μM
6c	3.90	70	50 μM
6d	4.43	32	22 μM
6e	2.90	93	100 μM
6f	4.96	100	55 μM
6g	6.55	89	60 μM
6h	4.30	84	65 μM
6i	5.23	69	47 μM
NDGA			0.5 μM
Trolox		88	

The *in silico* docking study was helpful in interpreting the *in vitro* experimental results. The novel synthesized derivatives present allosteric interactions with the enzyme. The preferred docking orientation for the most potent derivative **6d** is shown in Figure 1. The binding of **6d** to soybean LOX (PDB code: 3PZW) has an AutoDock Vina score of -7.5. Compound **6d** presents two H-bonds between the carbonyl group of the coumarin scaffold and TRP618 and the nitrogen of the imidazolyl ring with LYS622. Docking studies on the most potent compound **6d** showed that, this is able to accommodate the extensively hydrophobic cavity close to the active site with possible hydrophobic interactions (π-π stacking) between its propyl-group and VAL676, GLU677 and LEU680.

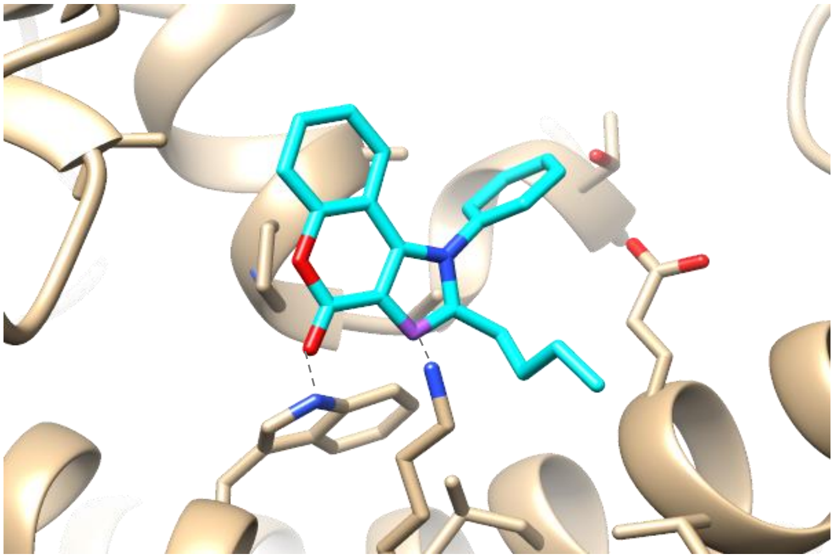


Figure 1. Docking pose of **6d** (depicted in cyan) bound to soybean lipoxygenase.

Conclusions

2-Substituted 1-phenylchromeno[3,4-*d*]imidazol-4(3*H*)-ones were synthesized in excellent yields by the two step reaction of 3-nitro-4-phenylaminocoumarin with aliphatic acids in the presence of hydrogen and Pd/C under microwave irradiation. The combination of an imidazolyl ring condensed to a coumarin moiety leads to a new hybrid structure. The preliminary *in vitro* results support the possibility of the anti-inflammatory activity and point to a new lead compound, 1-phenyl-2-propylchromeno[3,4-*d*]imidazol-4(1*H*)-one **6d**. Research is underway to design, synthesize and biologically evaluate new analogues.

Experimental Section

General. All the chemicals were procured from either Sigma-Aldrich Co. or Merck & Co., Inc. Melting points were determined on a Kofler hot-stage apparatus. IR spectra were obtained with a Perkin-Elmer 1310 spectrophotometer as Nujol mulls. NMR spectra were recorded on Agilent 500/54 (DD2) (500 and 125 MHz for ^{1}H and ^{13}C, respectively) using $CDCl_3$ as solvent and TMS as an internal standard. *J* values are reported in Hz. Mass spectra were determined on a LCMS-2010 EV Instrument (Shimadzu) under electrospray ionization (ESI) conditions. HRMS (ESI-MS) were received on ThermoFisher Scientific model LTQ Orbitrap Discovery MS. Silica gel N^o 60, Merck A.G. was used for column chromatography. The MW experiments were performed in a scientific focused microwave reactor (Biotage Initiator 2.0) using the appropriate sealed vials; power and pressure readings are shown in Table 1.

3-Nitro-4-phenylamino-2*H*-chromen-2-one (3) (Modification of literature methods[26,36])

A. 4-Chloro-3-nitrocoumarin (**1a**) (0.5 g, 2.2 mmol), aniline (**2**) (0.81 mL, 0.827 g, 8.87 mmol) and water (4 mL) were added to a flask for MW oven. The mixture was irradiated at 100 °C for 15 min. After cooling, HCl 1 N (1 mL) was added and the mixture was filtered. The solid was washed with water (2 × 5 mL) and ethyl ether (5 mL) and dried under vacuum to give a yellow microcrystalline powder (0.587 g, 95%), m.p. 234-236 °C (EtOH), (lit.[26] m.p. 235-236 °C).

B. 4-Bromo-3-nitrocoumarin (**1b**) (2.0 g, 7.4 mmol), aniline (**2**) (2.7 mL, 2.757 g, 29.62 mmol) and water (10 mL) were added to a flask for MW oven. The mixture was irradiated at 100 °C for 15 min. After work up like above, **4** was isolated (1.96 g, 94%).

1-Phenylchromeno[3,4-*d*]imidazol-4(1*H*)-one (6a): Typical procedures

Method A. 3-Nitro-4-phenylamino-2*H*-chromen-2-one (**3**) (0.282 g, 1 mmol), $SnCl_2 \cdot 2H_2O$ (0.677 g, 3 mmol) and formic acid (**5a**) (3 mL) were placed in a tube for MW oven. The mixture was irradiated at 100 °C for 5 min. After cooling, ethyl acetate (5 mL) was added and the mixture was filtered through celite. The solid was washed with boiling ethyl acetate (5 × 10 mL). The filtrate was extracted with water (3 × 20 mL) and brine (20 mL) and dried over anhydrous Na_2SO_4. After evaporation, the residue was separated by column chromatography [silica gel, hexane/ethyl acetate (2:1)] to give compound **6a** (0.162 g, 62%) as a colorless needles, m.p. 209-211 °C (EtOAc) (lit.[21] m.p. 211-213 °C), followed by compound **8** (18 mg, 7%).

Method B. A mixture of **3** (0.282 g, 1 mmol), 10% Pd/C (26.6 mg, 0.05 mmol) and **5a** (3 mL) was stirred under an atmosphere of H_2 (balloon) for 1 h [consumption of **4** (checked by TLC)]. The balloon of H_2 was removed and the mixture was heated at reflux for 6 h and then was filtered through celite. The resulting solid was washed with ethyl acetate (5 × 10 mL). The filtrate was washed with a saturated solution of $NaHCO_3$ (3 × 20 mL) and brine (20 mL) and dried over anhydrous Na_2SO_4. After evaporation, the separation of the residue as above gave compound **6a** (0.112 g, 43%) followed by compound **8** (82 mg, 32%).

Method C. A mixture of **3** (0.282 g, 1 mmol), 10% Pd/C (26.6 mg, 0.05 mmol) and **5a** (3 mL) was stirred in a round bottom flask under an atmosphere of H_2 (balloon) for 1 h. The balloon of H_2 was removed, the mixture was transferred in a vial for MW oven, while some remaining in the flask was washed with formic acid (**5a**) (0.5 mL) and poured in the vial. This vial was irradiated at 110 °C for 25 min. Then, the treatment, like above, gave compound **6a** (0.24 g, 92%) followed by compound **8** (8 mg, 3%).

Method D. 3-Nitro-4-phenylamino-2*H*-chromen-2-one (**3**) (0.282 g, 1 mmol), 10% Pd/C (26.6 mg, 0.05 mmol) and ethanol (20 mL) were added in a round bottom flask and stirred under an atmosphere of H_2 (balloon) at r.t. for 30 min [consumption of **3** (checked by TLC)]. The balloon of H_2 was removed, the mixture was filtered under celite, the filtrate was evaporated and the resulted light-yellow solid was transferred in a tube for MW oven. Then, formic acid (**5a**) (2 mL) was added and the mixture was irradiated at 110 °C for 25 min. After cooling, ethyl acetate (30 mL) was added to the mixture and washed with a saturated solution of $NaHCO_3$ (3 × 20 mL) and brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to give compound **6a** (0.253 g, 97%).

6*H*-Chromeno[3,4-*b*]quinoxaline-6-one (8). Light-yellow microcrystalline powder, m.p. 248-249 °C (xylene) (lit.[37] m.p. 249 °C), IR (KBr): 3037, 1747, 1610, 1558 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.75 (dd, *J* 7.9, 1.3, 1H), 8.42 (d, *J* 8.4, 1H), 8.27 (d, *J* 8.5, 1H), 8.02–7.98 (m, 1H), 7.92–7.89 (m, 1H), 7.68–7.64 (m, 1H), 7.48 (t, *J* 7.6, 1H), 7.44 (d, *J* 8.4, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.1, 152.4, 146.2, 145.1, 143.5, 134.8, 134.2, 133.4, 131.4, 131.2, 129.5, 125.6, 125.5, 118.9, 117.7; MS (ESI): 249 $[M+H]^{+\cdot}$.

2-Methyl-1-phenylchromeno[3,4-*d*]imidazol-4(1*H*)-one (6b). (Method A, 0.13 g, 47% yield; Method B, 0.193 g, 70%; Method C, 0.243 g, 88%, Method D, 0.27 g, 98%), colorless needles, m.p. 254-255 °C (EtOAc), IR (KBr): 3055, 2854, 1741, 1589, 1534, 1500 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72–7.66 (m, 3H), 7.50-7.40 (m, 2H), 7.39–7.31 (m, 2H), 6.95 (t, *J* 7.5, 1H), 6.63 (d, *J* 8.0, 1H), 2.37 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.9, 152.4, 151.7, 136.7, 135.7, 130.9, 130.8, 129.5, 127.7, 125.3, 124.0, 120.6, 118.0, 112.9, 13.7; MS (ESI): 277 $[M+H]^{+\cdot}$; HRMS (ESI, MS/MS): Calcd for $C_{17}H_{13}N_2O_2$ $[M+H]^+$ 277.0977. Found: 277.0977.

2-Ethyl-1-phenylchromeno[3,4-*d*]imidazol-4(1*H*)-one (6c). (Method A, 0.191 g, 66% yield; Method B, 0.16 g, 55%; Method C, 0.247 g, 85%, Method D, 0.278 g, 96%), colorless needles, m.p. 240-242 °C (EtOAc), IR (KBr): 3061, 2921, 2917, 2875, 2851, 1735, 1587, 1535, 1498 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73–7.65 (m, 3H), 7.45-7.39 (m, 3H), 7.34 (dd, *J* 8.2, 7.3, 1H), 6.95 (t, *J* 7.6, 1H), 6.59 (d, *J* 8.0, 1H), 2.62 (q, *J* 7.5, 2H), 1.31 (t, *J* 7.5, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.3, 156.2, 152.4, 136.7, 135.7, 130.9, 130.7, 129.4, 127.9, 125.6, 123.9, 120.7, 118.0, 113.1, 21.0, 11.9; MS (ESI): 291 $[M+H]^{+\cdot}$, 313 $[M+Na]^{+\cdot}$; HRMS (ESI): Calcd for $C_{18}H_{15}N_2O_2$ $[M+H]^+$ 291.1133. Found: 291.1127.

1-Phenyl-2-propylchromeno[3,4-*d*]imidazol-4(1*H*)-one (6d). (Method A, 0.17 g, 56% yield; Method C, 0.249 g, 82%; Method D, 0.289 g, 95%), colorless needles, m.p. 233-234 °C (EtOAc), IR (KBr): 3064, 2966, 2931, 2869, 1726, 1613, 1587, 1528, 1497 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73–7.65 (m, 3H), 7.44-7.38 (m, 3H), 7.33 (t, *J* 7.8, 1H), 6.94 (t, *J* 7.6, 1H), 6.57 (d, *J* 8.0, 1H), 2.57 (t, *J* 7.6, 2H), 1.81–1.74 (m, 2H), 0.91 (t, *J* 7.3, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.2, 155.1, 152.4, 136.6, 135.7, 130.9, 130.7, 129.4, 127.9, 125.65, 123.9, 120.6, 118.0, 113.1, 29.2, 21.1, 13.9; MS (ESI): 305 $[M+H]^{+\cdot}$; HRMS (ESI, MS/MS): Calcd for $C_{19}H_{17}N_2O_2$ $[M+H]^+$ 305.1290. Found: 305.1283.

2-(Methoxymethyl)-1-phenylchromeno[3,4-*d*]imidazol-4(1*H*)-one (6e). (Method A, 0.135 g, 44% yield; Method C, 0.251 g, 82%; Method D, 0.297 g, 97%), colorless needles, m.p. 213-214 °C (EtOAc), IR (KBr): 3074, 2930, 2820, 1736, 1587 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71–7.64 (m, 3H), 7.52-7.49 (m, 2H), 7.44–7.37 (m, 2H), 6.98 (t, *J* 7.6, 1H), 6.66 (d, *J* 8.0, 1H), 4.43 (s, 2H), 3.30 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.0, 152.7, 150.6, 137.5, 135.2, 130.9, 130.4, 130.0, 127.9, 125.6, 124.1, 121.1, 118.2, 112.9, 65.4, 58.4; MS (ESI): 307 $[M+H]^{+\cdot}$, 329 $[M+Na]^{+\cdot}$; HRMS (ESI, MS/MS): Calcd for $C_{19}H_{15}N_2O_2$ $[M+H]^+$ 307.1082. Found: 307.1073.

2-Butyl-1-phenylchromeno[3,4-*d*]imidazol-4(1*H*)-one (6f). (Method C, 0.242 g, 76% yield; Method D, 0.299 g, 94%), colorless microcrystalline powder, m.p. 167-169 °C (EtOAc), IR (KBr): 3076, 2969, 2874, 2857, 1743, 1536, 1502 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72–7.65 (m, 3H), 7.44-7.38 (m, 3H), 7.34 (t, *J* 7.8, 1H), 6.94 (t, *J* 7.6, 1H), 6.57 (d, *J* 8.0, 1H), 2.59 (t, *J* 7.8, 2H), 1.76–1.69 (m, 2H), 1.34–1.26 (m, 2H), 0.83 (t, *J* 7.4, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.3, 155.4, 152.4, 136.6, 135.7, 130.9, 130.7, 129.4, 127.9, 125.7, 123.9, 120.6, 118.0, 113.1, 29.7, 26.9, 22.4, 13.7; MS (ESI): 319 $[M+H]^{+\cdot}$, 341 $[M+Na]^{+\cdot}$; HRMS (ESI, MS/MS): Calcd for $C_{20}H_{19}N_2O_2$ $[M+H]^+$ 319.1446. Found: 319.1416.

2-Heptyl-1-phenylchromeno[3,4-*d*]imidazol-4(1*H*)-one (6g). (Method C, 0.259 g, 72% yield; Method D, 0.32 g, 89%), colorless microcrystalline powder, m.p. 136-138 °C (EtOAc), IR (KBr): 3064, 3049, 2924, 2857, 1754, 1558, 1536, 1497 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72–7.66 (m, 3H), 7.45–7.39 (m, 3H), 7.34 (t, *J* 7.8, 1H), 6.94 (t, *J* 7.6, 1H), 6.58 (d, *J* 8.0, 1H), 2.58 (t, *J* 7.8, 2H), 1.77–1.71 (m, 2H), 1.29–1.18 (m, 8H), 0.84 (t, *J* 6.8, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.3, 155.4, 152.4, 136.6, 135.8, 130.8, 130.7, 129.3, 128.0, 125.8, 123.9, 120.6, 118.1, 113.2, 31.7, 29.28, 28.9, 27.7, 27.3, 22.7, 14.2; MS (ESI): 361 $[M+H]^{+\cdot}$; HRMS (ESI, MS/MS): Calcd for $C_{23}H_{25}N_2O_2$ $[M+H]^+$ 361.1916. Found: 361.1894.

2-Isopropyl-1-phenylchromeno[3,4-*d*]imidazol-4(1*H*)-one (6h). (Method C, 0.234 g, 77% yield; Method D, 0.28 g, 92%), colorless microcrystalline powder, m.p. 293-294 °C (EtOAc), IR (KBr): 3053, 2969, 2930, 2857, 1734, 1592, 1524, 1494 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73–7.66 (m, 3H), 7.46-7.39 (m, 3H), 7.34 (t, *J* 7.6, 1H), 6.94 (t, *J* 7.6, 1H), 6.52 (d, *J* 8.0, 1H), 2.89–2.82 (m, 1H), 1.32 (d, *J* 6.8, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.0, 157.4, 152.4, 136.4, 135.7, 130.9, 130.7, 129.3, 128.1, 125.7, 123.9, 120.6, 118.1, 113.2, 26.6, 21.8; MS (ESI): 305 $[M+H]^{+\cdot}$, 327 $[M+Na]^{+\cdot}$; HRMS (ESI, MS/MS): Calcd for $C_{19}H_{17}N_2O_2$ $[M+H]^+$ 305.1290. Found: 305.1283.

2-Isobutyl-1-phenylchromeno[3,4-*d*]imidazol-4(1*H*)-one (6i). (Method C, 0.235 g, 74% yield; Method D, 0.289 g, 91%), colorless microcrystalline powder, m.p. 202-204 °C (EtOAc), IR (KBr): 3064, 2958, 2924, 2868, 1726, 1589, 1624, 1494 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73–7.67 (m, 3H), 7.45–7.41 (m, 3H), 7.36 (t, *J* 7.7, 1H), 6.96 (t, *J* 7.5, 1H), 6.56 (d, *J* 8.0, 1H), 2.53 (d, *J* 7.2, 2H), 2.26–2.18 (m, 1H), 0.91 (d, *J* 6.5, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.8, 154.7, 152.5, 136.4, 135.6, 131.0, 130.7, 129.6, 128.1, 125.2, 124.0, 120.7, 118.1, 113.0, 35.8, 28.0, 22.6; MS (ESI): 319 $[M+H]^{+\cdot}$, 341 $[M+Na]^{+\cdot}$; HRMS (ESI, MS/MS): Calcd for $C_{20}H_{18}N_2O_2$ $[M+H]^+$ 319.1446. Found: 319.1434.

***N*-(2-Oxo-4-(phenylamino)-2*H*-chromen-3-yl)acetamide (7).**[38] (Method A, 32 mg, 11% yield; Method B, 35 mg, 12%; Method B (10% Pd/C, 9 mg, 3%) orange microcrystalline powder, m.p. 211-213 °C (EtOAc), IR (KBr): 3266, 3018, 2922, 2847, 1685, 1660, 1610, 1536 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.78 (bs, 1H), 7.47–7.42 (m, 2H), 7.34 (d, *J* 8.6, 1H), 7.23 (t, *J* 7.7, 2H), 7.07 (t, *J* 7.7, 1H), 7.01 (t, *J* 7.3, 1H), 6.86 (d, *J* 7.9, 2H), 2.23 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 161,1, 151.6, 142.0, 141.6, 131.2, 129.4, 126.8, 123.9, 122.8, 119.8, 117.3, 116.4, 109.5, 24.0; MS (ESI): 295 $[M+H]^{+\cdot}$.

8,9,10,11-Tetrahydro-6*H*-chromeno[3,4-*b*]quinoxalin-6-one (9). (Method B, 10 mg, 4%; Method B (10% Pd/C, 50 mg, 20%) light-yellow microcrystalline powder, m.p. 162-164 °C (xylene), IR (KBr): 3059, 2952, 2919, 2857, 1754, 1631, 1538 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.51 (d, *J* 8.3, 1H), 7.59 (t, *J* 7.7, 1H), 7.42–7.38 (m, 2H), 3.21–3.16 (m, 4H), 2.06–2.02 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.6, 159.9, 156.2, 152.2, 145.5, 132.4, 131.1, 125.1, 124.8, 118.7, 117.4, 33.4, 32.6, 22.5, 22.4; MS (ESI): 253 $[M+H]^{+\cdot}$, 275 $[M+Na]^{+\cdot}$.

3-Amino-4-phenylaminocoumarin (4). (Control experiment, modification of literature methods[32,33])
3-Nitro-4-phenylamino-2*H*-chromen-2-one (**3**) (0.282 g, 1 mmol), 10% Pd/C (26.6 mg, 0.05 mmol) and ethanol (20 mL) were added in a round bottom flask and stirred under an atmosphere of H_2 (balloon) at r.t. for 30 min [consumption of **3** (checked by TLC)]. The balloon of H_2 was removed, the mixture was filtered under celite, the filtrate was evaporated and the residue was washed by cold ethanol to give compound **4** (0.248 g, 98%) as light yellow microcrystalline powder, m.p. 198-200 °C (dec.) (EtOH), (lit.[33] m.p. 199-203 °C). ^1H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, *J* 7.8, 1H), 7.38–7.32 (m, 2H), 7.28–7.25 (m, 2H), 7.24–7.20 (m, 1H), 6.93 (t, *J* 7.4, 1H), 6.74 (d, *J* 7.8, 2H), 5.58 (bs, 1H), 4.09 (bs, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.22, 148.41, 141.64, 129.69, 127.68, 124.61, 123.98, 122.53, 121.42, 120.94, 119.68, 116.85, 116.04; MS (ESI): 253 $[M+H]^{+\cdot}$.

In vitro experiments

In the *in vitro* experiments assays of each experiment was performed at least in triplicate and the standard deviation of absorbance was less than 10% of the mean.

Inhibition of linoleic acid lipid peroxidation.[24] The *in vitro* assay is performed according to our previous published method. Production of conjugated diene hydroperoxide by oxidation of linoleic acid sodium salt in an aqueous solution was monitored at 234 nm. 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) was used as a free radical initiator in 0.05 M phosphate buffer, pH 7.4 prethermostated at 37 °C. Oxidation was carried out in the presence of 10 μL of the examined compounds (stock solution in DMSO). Trolox was used as a standard (Table 2).

Soybean lipoxygenase inhibition study *in vitro*.[24] *In vitro* study was evaluated as reported previously. The conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was recorded and compared with the appropriate standard inhibitor nordihydroguaretic acid (IC_{50} 0.5 μM). Several concentrations were used for the determination of IC_{50} values (Table 2).

In silico studies. See the Supplementary *Information* and references herein.

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Supplementary Material

Proton and carbon-13 NMR spectra are presented in the Supplementary file associated with this article.

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