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Indolizines as Novel Potent Inhibitors of 15-Lipoxygenase

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Abstract—15-Lipoxygenase (15-LO) has been implicated in oxidation of low-density lipoproteins (LDL) and this enzyme may be involved in the development of atherosclerosis. We have examined 1-substituted indolizines as possible inhibitors of 15-LO from soy beans and from rabbit reticulocytes. Most compounds studied were significantly more active as inhibitors of 15-LO from soy beans than quercetin. The indolizines were slightly less potent inhibitors of the mammalian enzyme, but we found good correlation between inhibitory activity against both 15-LO enzymes studied. Several of the compounds were only weak DPPH scavengers and they may therefore be regarded as so-called non antioxidant inhibitors of 15-LO.

Introduction

15-Lipoxygenase (15-LO) has been implicated in oxidation of low-density lipoproteins (LDL). This process is believed to be important for the development of atherosclerosis.[1,2] The role of 15-lipoxygenase in the development of atherosclerosis is still not a clear-cut one,[3] but recent results in animals show a significant contribution of this enzyme towards atherogenesis.[4–7] In addition, 15-hydroperoxyeicosatetraenoic acid has been shown to decrease prostacyclin synthesis[8] and may thus have a pro-thrombotic effect. 15-Lipoxygenase has been implicated in prostate cancer[9,10] and in spontaneous abortion.[11] At present, it is not known whether these effects are clinically important in humans. Nevertheless, development of new and selective 15-lipoxygenase inhibitors appears to be an important task.

We have previously synthesized a number of indolizines and azaindolizines and screened their ability to act as scavengers of lipid peroxidation in vitro.[12–14] In the assay we used boiled rat liver microsomes, hence no enzyme activity was present and the active compounds must have acted as direct radical scavengers or as chelators of the iron used to initiate the peroxidation. We found that an oxygen atom connected to the indolizine C-1 was required for antioxidant activity, but that a wide variety of oxygen substituents (ethers, esters, sulfonates, carbonates and carbamates) were tolerated. We have now extended our study of indolizines as potential antioxidants, and we are currently reporting that a large number of indolizines are highly potent inhibitors of 15-lipoxygenase from soybeans as well as from rabbit reticulocytes.

Results and Discussion

1-Substituted-7-cyano-2,3-diphenylindolizines **3**–**7** were prepared by cycloaddition of 4-cyanopyridine to diphenylcyclopropenone followed by further functionalization in the position 1 of the indolizine ring employing reaction sequences previously described by us.[12,13] The syntheses of compounds **1**–**7** are summarized in Scheme 1.

Reaction of the bromide **2a** with butyllithium followed by trapping with DMF, acid chlorides, aldehydes or ketones gave the indolizinyl ketones **8** or alcohols **9** as depicted in Scheme 2, generally in high yields. Reaction of the aldehyde **8a** with sodium borohydride or methyllithium gave the alcohols **9a** and **9b**. The hydroxy group in compound **9c** was also alkylated giving the ether **10**.

In the initial screening of the indolizines **3**–**10** as potential inhibitors of 15-lipoxygenase, we used 15-LO from soybeans. However, it has been shown that there is a good correlation for inhibitory activity for the soybean

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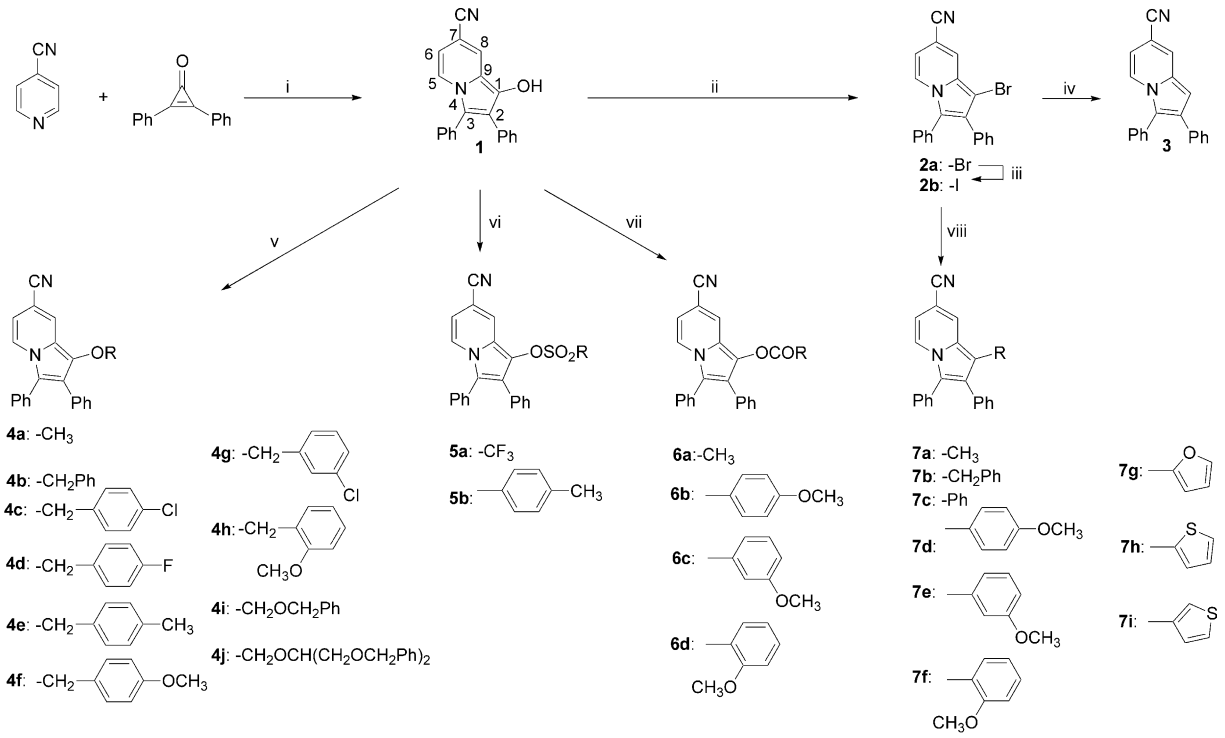
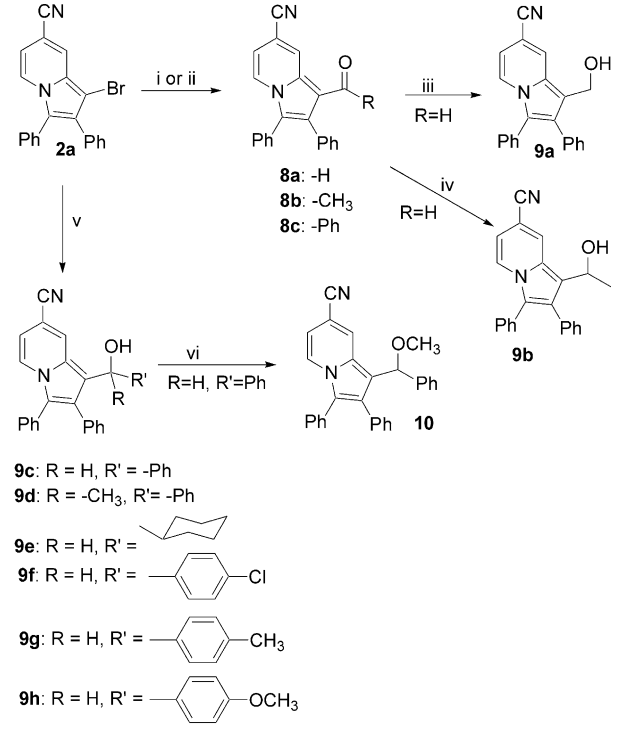


Scheme 1. Reagents and conditions: (i) DCE, Δ; (ii) Br_2, PPh_3, MeCN, 70 °C; (iii) (1) BuLi, THF, −78 °C; (2) I_2; (iv) (1) BuLi, THF, −78 °C; (2) NH_4Cl(aq); (v) NaH, alkyl halide, THF; (vi) TsCl/0 °C or Tf_2O/−78 °C, DMAP, CH_2Cl_2; (vii) $(RCO)_2O$ or RCOCl, DMAP, DCE; viii, $RSnBu_3$, RZnX or $RB(OH)_3$, cat Pd(0), 60–100 °C, dioxane or DMF.



Scheme 2. Reagents and conditions: (i) (1) BuLi, THF, −78 °C; (2) DMF or PhCOCl; (ii) $Bu_3SnC(Oet)=CH_2$, cat Pd(0), DMF, 100 °C; (iii) $NaBH_4$, EtOH; (iv), CH_3Li, −78 °C, THF; (v) BuLi, THF, −78 °C; (2) RCOR′; (vi) NaH, CH_3I, THF.

and mammalian 15-LO enzyme from rabbit or human reticulocytes[15–17] or human monocytes.[18] These enzymes are closely related, but not identical.[19] The results are given in Table 1.

The well known 15-LO inhibitor quercetin[20] was used as positive control (IC_{50} 51 μM) and the parent indolizine **3** as well as most of the derivatives **4**–**10**, were considerably more active. Introduction of alkoxy groups in the indolizine 1-position (compounds **4**) had essentially no effect on activity. There appears to be little restrictions as to the size of the indolizine 1-substituent in active compounds; the bulky acetal **4j** exhibits essentially the same activity as the parent compound **3**. The inhibitory activity of the esters **6** was in the same range as the corresponding ethers **4**. Introduction of sulfonates in the 1-position resulted in marked alteration of activity. The triflate **5a** was noticeably less active than the parent indolizine **3**, but the tosylate **5b**, on the other hand, was one of the three most potent compounds examined. Among the 1-alkyl and 1-arylindolizines **7** high inhibitory activities were found for all compounds studied. The same was true for the aldehyde and the ketones **8**. In compounds **9**–**10**, a hydroxy, methoxy or acyloxy group is present in the alkyl side chain connected to the C-1 in the heterocycle. In this group compounds more active as well as slightly less active than the parent indolizine **3** are found, but all compounds are substantially more potent 15-LO inhibitors than quercetin.

Some of the indolizines in Table 1 were also tested as inhibitors of mammalian enzyme; 15-LO from rabbit reticulocytes. All compounds examined were slightly less active towards this enzyme, but we found a good correlation between inhibitory activity against both 15-LO enzymes studied. Linear regression yields an R value for the correlation between IC_{50} values for the two enzymes of 0.693, and the correlation is highly significant ($p<0.001$; Student's *t*-test). This is in good

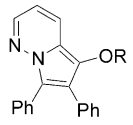
Table 1. Inhibitory activity of the indolizines **3**–**10** against 15-lipoxygenase from soybeans and rabbit reticulocytes and scavenging of DPPH

Compd	Substituent in the indolizine 1-position	IC_{50} (μM)[a,b] 15-LO from soybeans	IC_{50} (μM)[a,c] 15-LO from rabbit reticulocytes	% DPPH scavenging after 5 min, 667 μM concn[a]
3	–H	30±2	51±4	−0.2±0.1
4a	$-OCH_3$	33±2	77±3	−0.6±0.2
4b	$-OCH_2Ph$	31±2	—[d]	0.8±0.2
4c	$-OCH_2-C_6H_4-p$—Cl	31±3	n.d.[e]	1.3±0.1
4d	$-OCH_2-C_6H_4-p$–F	35±3	n.d.	1.8±0.4
4e	$-OCH_2-C_6H_4-p-CH_3$	37±4	n.d.	−0.8±0.3
4f	$-OCH_2-C_6H_4-p-OCH_3$	32±3	n.d.	33.3±4.6
4g	$-OCH_2-C_6H_4-m$–Cl	31±2	n.d.	−0.1±0.3
4h	$-OCH_2-C_6H_4-o-OCH_3$	29±3	n.d.	1.6±0.3
4i	$-OCH_2OCH_2Ph$	27±3	39±3	0.1±0.2
4j	$-CH_2OCH(CH_2OCH_2Ph)_2$	31±3	n.d.	4.2±2.4
5a	$-OSO_2CF_3$	46±4	59±4	79.5±1.9
5b	–OTs	17±1	25±3	n.d.
6a	$-OCOCH_3$	31±2	75±8	−0.5±0.7
6b	$-OCO-C_6H_4-p-OCH_3$	28±2	n.d.	5.4±0.4
6c	$-OCO-C_6H_4-m-OCH_3$	28±3	n.d.	1.2±0.4
6d	$-OCO-C_6H_4-o-OCH_3$	30±4	n.d.	4.7±0.9
7a	$-CH_3$	27±2	n.d.	−0.9±0.8
7b	$-CH_2Ph$	27±2	46±2	n.d.
7c	–Ph	28±1	n.d.	n.d.
7d	$-C_6H_4-p-OCH_3$	20±1	37±3	−0.9±0.1
7e	$-C_6H_4-m-OCH_3$	20±1	n.d.	−0.3±0.2
7f	$-C_6H_4-o-OCH_3$	20±1	n.d.	−0.2±0.3
7g	–(2-furyl)	24±1	n.d.	−0.6±0.2
7h	–(2-thienyl)	20±3	n.d.	−1.8±0.2
7i	–(3-thienyl)	19±2	n.d.	0.0±0.4
8a	–CHO	29±2	61±4	n.d.
8b	$-COCH_3$	23±1	44±3	n.d.
8c	–COPh	23±1	43±6	n.d.
9a	$-CH_2OH$	26±1	34±3	n.d.
9b	$-CH(OH)CH_3$	26±2	n.d.	n.d.
9c	–CH(OH)Ph	35±4	48±5	n.d.
9d	$-C(CH_3)(OH)Ph$	17±1	n.d.	n.d.
9e	–CH(OH)cyclohexyl	23±2	n.d.	n.d.
9f	$-CH(OH)-C_6H_4-p$–Cl	23±2	n.d.	n.d.
9g	$-CH(OH)-C_6H_4-p-CH_3$	22±1	n.d.	n.d.
9h	$-CH(OH)-C_6H_4-p-OCH_3$	17±1	n.d.	n.d.
10	$-CH(OCH_3)Ph$	21±3	n.d.	n.d.

[a]Data are shown±SD.
[b]Quercetin was used as positive control: IC_{50} 51±3 μM.
[c]Quercetin was used as positive control: IC_{50} 37±4 μM.
[d]Determination of exact IC_{50} value was not possible due to high UV-absorbtion of the compound itself at higher concn.
[e]Not determined.

accordance with most previous comparisons between inhibition of soybean 15-LO and mammalian 15-LO were significant correlation generally have been observed.[15–18, 21] Recently, some experiments have failed to demonstrate this correlation.[22,23] In these experiments, however, epicatechin and caffeic acid were reported not to inhibit soybean 15-LO. This is in contrast to previously reported results.[24–28] Conceivably, differences in methodology may be the reason for this discrepancy.

The azaindolizines **11a**–**e**[14] (Fig. 1) were also tested as 15-LO inhibitors (Table 2) and the results show that the activity of the alkoxyazaindolizines **11a** and **11b** were comparable to that of the methoxyindolizine **4a** (Table 1), and the same was true when the sulfonates **11c** and **11d** and the acetate **11e** were compared with compounds **5a**, **5b** and **6a** (Table 1). Also in the azaindolizine series the most potent compound tested was the tosylate, compound **11d**, which exhibited the same activity as the tosylate **5b** (Table 1) against the enzyme from soybeans as well as rabbit reticulocytes.



11a: $-CH_3$
11b: $-CH_2CH_3$
11c: $-SO_2CF_3$
11d: -Ts
11e: $-COCH_3$

Figure 1.

In order to further evaluate the importance of the indolizine/azaindolizine ring for enzyme inhibitory activity, we also examined the simple ether **12** and the esters **13a**–**c** (Fig. 2, Table 3). These compounds may be regarded as phenyl analogues of the indolizines **4f**, and **6b**–**d**. Compounds **12** and **13** were actually able to inhibit the 15-LO from soybeans but they were much less active than the corresponding indolizines.

Table 2. Inhibitory activity of the azaindolizines **11** against 15-lipoxygenase from soybeans and rabbit reticulocytes and scavenging of DPPH

Compd	Substituent in the indolizine 1-position	IC_{50} (μM)[a,b] 15-LO from soybeans	IC_{50} (μM)[a,c] 15-LO from rabbit reticulocytes	% DPPH Scavenging after 5 min, 667 μM concn[a]
11a	$-OCH_3$	29±3	59±4	−0.5±0.2
11b	$-OCH_2CH_3$	28±2	n.d.[d]	−0.1±0.2
11c	$-OSO_2CF_3$	43±2	n.d.	31.9±1.9
11d	–OTs	17±1	28±3	0.8±0.0
11e	$-OCOCH_3$	32±3	59±4	n.d.

[a]Data are shown±SD.
[b]Quercetin was used as positive control: IC_{50} 51±3 μM.
[c]Quercetin was used as positive control: IC_{50} 37±4 μM.
[d]Not determined.

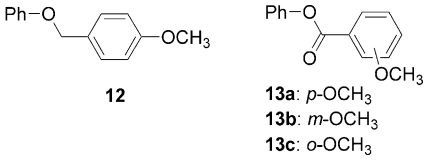


Figure 2.

The indolizines **4a**, **5**, **6a**, **7** and **9c**, as well as the azaindolizines **11**, have previously been examined as potential scavengers of lipid peroxidation in vitro.[12–14] In this assay, generally only indolizines with an oxygen atom bound directly to the indolizine C-1 exhibited antioxidant activity, and there is no correlation between the activity in the two test systems. Furthermore, many compounds described herein were also screened as potential scavengers of diphenylpicrylhydrazyl (DPPH; Tables 1–3),[29] but in most instances they were essentially inactive as radical scavengers and no correlation between enzyme inhibition and DPPH scavenging activity was found, indicating that most compounds examined in some way bind to the enzyme and thereby perform their inhibitory activity, thus the compounds may be regarded as so-called non-antioxidant inhibitors of 15-LO. DPPH scavenging activity was found for the indolizine-triflates **5a** and **11c**. However, these compounds are readily cleaved to the corresponding indolizin-1-ols[13] which may very well be the actual scavengers.

Experimental

Compounds **2a**,[13] **2b**,[22] **3**,[13] **4a**,[12] **5a**,[13] **5b**,[13] **6a**,[12] **7a**,[13] **7b**,[22] **7c–i**,[13] **8a**,[22] **8b**,[13] **8c**,[30] **9a–9b**,[30] **9c**,[12] **9d–9h**,[30] **10**,[30] and **11a–e**[14] are previously described. 1,3-Dibenzyloxy-2-chloromethoxy propane,[31] compounds **12**,[32] **13a**,[33] **13b**,[34] and **13c**,[35] were synthesized according to the literature. THF and 1,4-dioxane were distilled from Na/benzophenone, DMF from BaO, and DCE and dichloromethane was distilled from CaH_2. 15-Lipoxygenase from soybeans (Type 1) was purchased from Sigma (St Louis, MO, USA), and 15-lipoxygenase from rabbit reticulocytes from Oxford Biomedical Research (Oxford, MI, USA). Silica gel for flash chromatography was available from Merck (Darmstadt, Germany) (Merck No. 9385) or Fluka (Fluka No. 60752). The ^1H NMR spectra were recorded at 500 MHz with a Bruker Avance DRX 500 instrument, at 300 MHz with a Bruker Avance DPX 300 instrument or at 200 MHz with a Bruker Avance DPX 200 instrument. The ^{13}C NMR spectra were recorded at 125, 75 or 50 MHz using instruments mentioned above. Unless otherwise stated, the spectra are recorded at ambient temperature. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane. Mass spectra (EI) were recorded with a VG Prospec instrument at 70 eV ionizing voltage unless otherwise stated, and are presented as *m*/*z* (% rel. int.). Electrospray MS spectra (ESI) were recorded with a Bruker Apex 47e FT-ICR mass spectrometer. Melting points are uncorrected. All measurements of 15-lipoxygenase activity were carried out in a Shimadzu UV-160A spectrophotometer equipped with a Shimadzu CPS-240A thermostatted cell changer.

Table 3. Inhibitory activity of the compounds **12** and **13** against 15-lipoxygenase from soybeans and scavenging of DPPH

Compd	Substituent on benzene	IC_{50} (μM)[a,b] 15-LO from soybeans	% DPPH Scavenging after 5 min, 667 μM concn[a]
12	$-OCH_2-C_6H_4-p-OCH_3$	129±9	3.4±2.0
13a	$-OCO-C_6H_4-p-OCH_3$	>167	−1.1±0.7
13b	$-OCO-C_6H_4-m-OCH_3$	91±7	−0.4±0.3
13c	$-OCO-C_6H_4-o-OCH_3$	85±4	−0.6±0.1

[a]Data are shown±SD.
[b]Quercetin was used as positive control: IC_{50} 51±3 μM.

1-Phenylmethoxy-2,3-diphenyl-7-indolizinecarbonitrile (4b). A mixture of diphenylcyclopropenone (206 mg, 1.0 mmol) and 4-cyanopyridine (104 mg, 1.0 mmol) in dry DCE (20 mL) was refluxed under N_2 for 16 h and evaporated under N_2. The residue was dissolved in dry THF (30 mL), and sodium hydride (53 mg, 2.2 mmol) was added. The resulting mixture was stirred under N_2 at ambient temperature for 1 h, before benzyl bromide (264 μL, 2.2 mmol) was added. After stirring at ambient temperature for 24 h, the reaction mixture was evaporated in vacuo, and purified by flash chromatography eluting with EtOAc–hexane (1:19); yield 288 mg (72%), yellow crystals, mp 172–174 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 4.78 (s, 2H, CH_2), 6.36 (dd, J=7.5 and 1.8 Hz, 1H, H-6), 7.27–7.31 (m, 9H, Ph), 7.31–7.39 (m, 6H, Ph), 7.81 (br d, J=1.8 Hz, 1H, H-8), 7.82 (dd, J=7.5 and 0.6 Hz, 1H, H-5). ^{13}C NMR (75 MHz, $CDCl_3$) δ 77.4, 97.6, 110.4, 119.9, 121.9, 122.1, 122.2, 123.9, 124.7, 127.5, 128.7, 128.8 (2 C), 128.9, 129.0, 129.6, 130.2,

130.4, 130.9, 132.6, 137.3, 138.5. MS (EI): 400 (M^+, 3), 309 (100), 281 (3), 220 (4), 178 (5), 131 (9), 103 (24), 91 (5), 65 (2). Anal: Found: C, 83.79; H, 5.08; N, 7.04. $C_{28}H_{20}N_2O$ requires C, 83.98; H, 5.03; N, 6.99.

1-(4-Chlorophenyl)methoxy-2,3-diphenyl-7-indolizinecarbonitrile (4c). The compound was prepared as described for compound **4b** above. After addition of the alkyl halide (4-chlorobenzyl chloride) the reaction mixture was stirred for 24 h at ambient temperature and at 50 °C for 72 h. EtOAc–hexane (1:29) followed by (1:19) were used as eluents for flash chromatography; yield 223 mg (51%), yellow crystals, mp 163–166 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.80 (s, 2H, CH_2), 6.45 (dd, J = 7.4 and 1.7 Hz, 1H, H-6), 7.14–7.17 (m, 2H, Ph), 7.17–7.29 (m, 10H, Ph), 7.29–7.41 (m, 2H, Ph), 7.75 (dd, J = 1.7 and 0.8 Hz, 1H, H-8), 7.88 (dd, J = 7.4 and 0.8 Hz, 1H, H-5). ^{13}C NMR (75 MHz, $CDCl_3$) δ 76.5, 97.8, 110.4, 119.8, 122.0, 122.0, 122.1, 123.9, 124.5, 127.6, 128.8, 129.0, 129.1, 129.6, 130.1 (2 C), 130.4, 130.8, 132.5, 134.6, 135.7, 138.2. MS (EI): 436/434 (M^+, 0.4/2), 309 (100), 178 (4), 131 (8), 103 (22). Anal: Found: C, 77.23; H, 4.55; N, 6.14. $C_{28}H_{19}ClN_2O$ requires C, 77.33; H, 4.40; N, 6.44%.

1-(4-Fluorophenyl)methoxy-2,3-diphenyl-7-indolizinecarbonitrile (4d). The compound was prepared as described for compound **4b** above and purified by flash chromatography, eluting with EtOAc–hexane (1:19); yield 382 mg (91%), yellow crystals, mp 152–154 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.74 (s, 2H, CH_2), 6.38 (dd, J = 7.4 and 1.8 Hz, 1H, H-6), 6.94–7.00 (m, 4H, Ph), 7.00–7.41 (m, 10H, Ph), 7.72 (1H, br d, J = 1.8 Hz, 1H, H-8), 7.83 (dd, J = 7.4 and 0.8 Hz, 1H, H-5). ^{13}C NMR (75 MHz, $CDCl_3$) δ 76.6, 97.7, 110.4, 115.6, 115.9, 119.8, 122.0 (d, J_{CF} = 4.8 Hz, CH in Ar), 122.1, 122.2, 123.9, 124.5, 127.5, 128.7, 129.1, 129.6, 130.1, 130.4, 130.6, 130.7, 130.8, 132.6, 133.0 (d, J_{CF} = 3.3 Hz, CH in Ar), 138.2, 163.1 (d, J_{CF} = 247 Hz). MS (EI): 418 (M^+, 4), 309 (100), 178 (4), 131 (8), 109 (6), 103 (21). Anal. Found: C, 80.17; H, 4.79; N, 6.30. $C_{28}H_{19}FN_2O$ requires C, 80.37; H, 4.58; N, 6.69%.

1-(4-Methylphenyl)methoxy-2,3-diphenyl-7-indolizinecarbonitrile (4e). The compound was prepared as described for compound **4b** above and purified by flash chromatography, eluting with EtOAc–hexane (1:39) followed by (1:29) and (1:19); yield 311 mg (75%), yellow crystals, mp 176–178 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.35 (s, 3H, CH_3), 4.74 (s, 2H, CH_2), 6.36 (dd, J = 7.4 and 1.8 Hz, 1H, H-6), 7.14–7.24 (m, 4H, Ph), 7.24–7.42 (m, 10H, Ph), 7.65 (d, J = 1.8 Hz, 1H, H-8), 7.83 (dd, J = 7.4 and 0.8 Hz, 1H, H-5). ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.7, 97.5, 110.3, 119.9, 121.9, 122.1, 122.3, 123.8, 124.8, 127.5, 128.7, 129.0, 130.4, 130.9, 132.7, 134.3, 138.5, 138.6. MS (EI): 414 (M^+, 4), 309 (100), 279 (4), 178 (6), 131 (10), 105 (12), 103 (30). Anal: Found: C, 84.15; H, 5.38; N, 6.38. $C_{29}H_{22}N_2O$ requires C, 84.03; H, 5.35; N, 6.76%.

1-(4-Methoxyphenyl)methoxy-2,3-diphenyl-7-indolizinecarbonitrile (4f). The compound was prepared as described for compound **4b** above and purified by flash chromatography, eluting with EtOAc–hexane (1:19), yield 382 mg (89%), yellow crystals, mp 156–158 °C, R_f 0.36 (SiO_2, EtOAc–hexane; 1:4). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.79 (s, 3H, CH_3), 4.69 (s, 2H, CH_2), 6.35 (dd, J = 7.4 and 1.8 Hz, 1H, H-6), 6.80–7.24 (m, 4H, Ph), 7.24–7.34 (m, 10H, Ph), 7.64 (dd, J = 1.8 and 0.9 Hz, 1H, H-8), 7.82 (dd, J = 7.4 and 0.9 Hz, 1H, H-5). ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.7, 97.5, 110.3, 114.3, 119.5, 121.5, 121.8, 121.9, 123.4, 124.3, 127.0, 128.3, 128.6, 129.0, 129.4, 129.6, 130.2, 130.4, 130.6, 130.9, 132.7, 138.8, 160.1. MS (EI): 430 (M^+, 1), 414 (12), 311 (21), 310 (100), 309 (48), 281 (13), 178 (10), 170 (28), 121 (34), 115 (8), 103 (18), 99 (11). HRMS: calcd for $C_{29}H_{22}N_2O_2$: 430.1681; found 430.1684. Anal: Found: C, 80.58; H, 5.15; N, 6.02. $C_{29}H_{22}N_2O_2$ requires C, 80.91; H, 5.15; N, 6.51%.

1-(3-Chlorophenyl)methoxy-2,3-diphenyl-7-indolizinecarbonitrile (4g). The compound was prepared as described for compound **4b** above and purified by flash chromatography, eluting with EtOAc–hexane (1:29) followed by (1:19); yield 396 mg (91%), yellow crystals, mp 156–158 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.74 (s, 2H, CH_2), 6.39 (dd, J = 7.5 and 1.8 Hz, 1H, H-6), 7.13–7.16 (m, 2H, Ph), 7.16–7.30 (m, 10H, Ph), 7.30–7.44 (m, 2H, Ph), 7.75 (d, J = 1.8, 1H, H-8), 7.86 (br d, J = 7.5, 1H, H-5). ^{13}C NMR (75 MHz, $CDCl_3$) δ 76.5, 97.8, 110.5, 119.8, 122.0, 122.1, 122.1, 123.9, 124.5, 126.7, 127.6, 128.8 (2 C), 128.8, 129.1, 129.6, 130.1, 130.2, 130.4, 130.8, 132.4, 134.7, 138.2, 139.2. MS (EI): 434/434 (M^+, 1/3), 309 (100), 281 (6), 178 (7), 131 (9), 103 (25). Anal: Found: C, 77.30; H, 4.54; N, 6.02. $C_{28}H_{19}ClN_2O$ requires C, 77.23; H, 4.40; N, 6.44%.

1-(2-Methoxyphenyl)methoxy-2,3-diphenyl-7-indolizinecarbonitrile (4h). The compound was prepared as described for compound **4b** above. After addition of the alkyl halide (2-methoxybenzyl chloride) the reaction mixture was stirred at 60 °C for 24 h. EtOAc–hexane (1:19) was used as eluent for flash chromatography; yield 258 mg (60%), yellow crystals, mp 152–154 °C, R_f 0.38 (SiO_2, EtOAc–hexane; 1:4). ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.89 (s, 3H, CH_3), 4.76 (s, 2H, CH_2), 6.42 (dd, J = 7.4 and 1.7 Hz, 1H, H-6), 6.92–7.04 (m, 2H, Ph), 7.04–7.48 (m, 12H, Ph), 7.84 (dd, J = 1.7 and 0.9 Hz, 1H, H-8), 7.88 (dd, J = 7.4 and 0.9 Hz, 1H, H-5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 55.7, 72.9, 97.2, 110.3, 110.8, 120.1, 120.8, 121.9, 122.3, 122.4, 123.7, 125.1, 125.5, 127.3, 128.6, 129.0, 129.6, 130.4, 130.4 (2 C), 130.9, 130.9, 132.7, 138.8, 158.1. MS (EI): 430 (M^+, 9), 309 (100), 178 (4), 131 (8), 121 (8), 103 (21), 91 (8). HRMS: calcd for $C_{29}H_{22}N_2O_2$: 430.1681; found 430.1693. Anal: Found: C, 80.57; H, 5.44; N, 6.59. $C_{29}H_{22}N_2O_2$ requires C, 80.91; H, 5.15; N, 6.51%.

1-Benzyloxymethoxy-2,3-diphenyl-7-indolizinecarbonitrile (4i). The compound was prepared as described for compound **4b** above and purified by flash chromatography, eluting with EtOAc–hexane (1:19); yield 333 mg (77%), yellow crystals, mp 132–134 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.65 (s, 2H, CH_2), 5.02 (s, 2H, CH_2), 6.39 (dd, J = 7.5 and 1.7 Hz, 1H, H-6), 7.17–7.32 (m, 10H, Ph), 7.38–7.40 (m, 5H, Ph), 7.86 (dd, J = 7.5 and 0.9 Hz, 1H, H-5), 7.88 (dd, J = 1.7 and 0.9 Hz, 1H,

H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δ 71.2, 77.2, 97.6, 110.0, 119.4, 121.7, 121.8, 121.9, 123.5, 124.5, 127.0, 127.9, 128.0, 128.3, 128.5, 128.6, 129.2, 129.7, 130.1, 130.4, 132.0, 136.2, 136.9. MS (EI): 430 (M^+, 4), 311 (7), 310 (41), 309 (100), 178 (4), 131 (7), 103 (19), 91 (11). Anal: Found: C, 80.74; H, 5.05; N, 6.20. $C_{29}H_{22}N_2O_2$ requires C, 80.91; H, 5.15; N, 6.51%.

1-(1,3-Dibenzyloxy-2-propyloxy)methoxy-2,3-diphenyl-7-indolizinecarbonitrile (4j). The compound was prepared as described for compound **4b** above and purified by flash chromatography, eluting with EtOAc–hexane (1:19) followed by (1:9) and (1:4); yield 446 mg (75%), yellow crystals, mp 108–110 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.50 (d, J = 5.0 Hz, 2H, CH_2), 4.84 (m, 1H, CH), 4.48 (s, 2H, CH_2), 5.15 (s, 2H, CH_2), 6.38 (dd, J = 7.5 and 1.7 Hz, 1H, H-6), 7.24–7.27 (m, 16H, Ph), 7.28–7.31 (m, 4H, Ph), 7.82 (dd, J = 7.5 and 0.6 Hz, 1H, H-5), 8.12 (br s, 1H, H-8). ^{13}C NMR (50 MHz, $CDCl_3$) δ 70.6, 72.2, 73.8, 97.8, 98.4, 110.3, 119.9, 121.8, 121.9, 123.8, 125.7, 127.4, 128.1 (2 C), 128.7, 128.8, 129.0, 129.6, 130.2, 130.5, 130.9, 132.5, 137.1, 138.4, 139.1. MS (EI): 311 (9), 310 (59), 309 (100), 281 (5), 181 (3), 178 (4), 131 (5), 103 (16), 92 (3), 91 (41), 65 (3). MS (ESI): 595 (M + 1). Anal: Found: C, 78.95; H, 5.79; N, 5.04. $C_{39}H_{34}N_2O_3$ requires C 78.77; H, 5.76; N, 4.71%.

7-Cyano-2,3-diphenylindolizin-1-yl 4-methoxybenzoate (6b). A mixture of diphenylcyclopropenone (206 mg, 1.0 mmol) and 4-cyanopyridine (104 mg, 1.0 mmol) in dry DCE (20 mL) was refluxed under N_2 for 16 h and evaporated under N_2. The residue was dissolved in dry dichloromethane (120 mL), and 4-(*N,N*-dimethylamino) pyridine (244 mg, 2.0 mmol) was added. The reaction mixture was stirred under N_2 at ambient temperature for 2 h, before 4-methoxybenzoyl chloride (597 μL, 3.5 mmol) was added, and stirred under N_2 at 40 °C for 48 h. The resulting mixture was evaporated in vacuo, and purified by flash chromatography, eluting with EtOAc–hexane (1:19) followed by (1:9); yield 282 mg (64%), yellow crystals, mp 240–242 °C, R_f 0.24 (SiO_2, EtOAc–hexane; 1:4). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.89 (s, 3H, CH_3), 6.49 (dd, J = 7.5 and 1.7 Hz, 1H, H-6), 6.97 (d, J = 7.0 Hz, 2H, Ar), 7.15–7.23 (m, 5H, Ph), 7.23–7.45 (m, 5H, Ph), 7.72 (br s, H-8, 1H,), 7.95 (dd, J = 7.5 and 0.5 Hz, 1H, H-5), 8.13 (d, J = 7.0 Hz, 2H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.0, 99.4, 110.8, 114.4, 119.4, 121.2, 122.1, 122.5, 122.9, 124.2, 124.6, 127.6, 128.7, 129.2, 129.7, 129.9 (2 C), 130.2 (2 C), 131.0, 131.7, 133.2, 164.6. MS (EI): 444 (M^+, 12), 310 (12), 135 (100), 103 (11), 77 (5). HRMS: calcd for $C_{29}H_{20}N_2O_3$: 444.1473; found 444.1474. Anal: Found: C, 77.94; H, 4.59; N, 6.03.

7-Cyano-2,3-diphenylindolizin-1-yl 3-methoxybenzoate (6c). The compound was prepared as described for compound **6b** above and purified by flash chromatography, eluting with EtOAc–hexane (1:29) followed by (1:19); yield 244 mg (55%), yellow crystals, mp 222–225 °C, R_f 0.30 (SiO_2, EtOAc–hexane; 1:4). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.85 (s, 3H, CH_3), 6.50 (dd, J = 7.4 and 1.77 Hz, 1H, H-6), 7.03–7.24 (m, 6H, Ph), 7.24–7.43 (m, 6H, Ph), 7.66 (m, 1H, Ph), 7.72 (br s, 1H, H-8), 7.78 (d, J = 7.4 Hz, 1H, Ar), 7.95 (br d, J = 7.8 Hz, 1H, H-5). ^{13}C NMR (75 MHz, $CDCl_3$) δ 54.5, 98.1, 109.4, 113.7, 117.9, 119.6, 120.5, 121.1, 121.4, 121.7, 121.8, 122.8, 122.9, 126.2, 127.2, 127.3, 127.8, 128.2, 128.3, 128.7, 128.8, 129.5, 130.2, 158.7, 163.9. MS (EI): 444 (M^+, 53), 309 (59), 135 (100), 107 (10), 103 (24), 77 (6). HRMS: calcd for $C_{29}H_{20}N_2O_3$: 444.1473; found 444.1475. Anal: Found: C, 78.27; H, 4.52; N, 6.44. $C_{29}H_{20}N_2O_3$ requires C, 78.36; H, 4.54; N, 6.30%.

7-Cyano-2,3-diphenylindolizin-1-yl 2-methoxybenzoate (6d). The compound was prepared as described for compound **6b** above and purified by flash chromatography, eluting with EtOAc–hexane (1:19) followed by (1:9); yield 391 mg (88%), yellow crystals, mp 163–165 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.91 (s, 3H, CH_3), 6.49 (dd, J = 7.4 and 1.6 Hz, 1H, H-6), 6.98–7.04 (m, 2H, Ph), 7.04–7.43 (m, 10H, Ph), 7.51–7.56 (m, 1H, Ph), 7.79 (br s, 1H, H-8), 7.91–7.96 (m, 2H, Ph and H-5). ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.4, 99.2, 110.8, 112.6, 118.7, 119.5, 120.7, 122.1, 122.5, 123.0, 124.2, 124.7, 127.6, 128.7, 128.9, 129.2, 129.7, 129.9, 130.4, 131.0, 131.8, 132.7, 135.1, 160.3, 164.8. MS (EI): 444 (M^+, 12), 309 (11), 135 (100), 103 (12), 77 (87). Anal: Found: C, 77.98; H, 4.63; N, 6.37. $C_{29}H_{20}N_2O_3$ requires C, 78.36; H, 4.54; N, 6.30%.

Inhibition of 15-lipoxygenase from soybeans

Lipoxygenase activity was measured in borate buffer solutions (0.2M, pH 9.00) as previously described[29,36] by the increase in absorbance at 234 nm from 30 to 90 s after addition of the enzyme, using linoleic acid (134 μM) as substrate. The final enzyme concentration was 167 U/mL. Test substances were added as DMSO solutions (final DMSO concn 1.6%); DMSO alone was added in uninhibited control experiments. Six or more parallels of controls and three or more parallels for each test substance solution were measured. To ensure constant enzyme activity throughout the experiment, the enzyme solution was kept on ice, and controls were measured at regular intervals. Calculation of enzyme activity was carried out as previously described[36] and IC_{50} values were determined by linear interpolation between the measuring points closest to 50% activity. Values are expressed as means ±SD. Student's *t*-test was employed for determination of statistical significance.

Inhibition of 15-lipoxygenase from rabbit reticulocytes

The frozen rabbit reticulocyte enzyme (in phosphate buffer, used as received) was thawed immediately before experiment and subsequently kept on ice throughout the experimental period (maximum 1 day). A mixture of substrate solution [linoleic acid in abs. ethanol; 0.4% (w/v), 20 μL], 2-methoxyethanol (in uninhibited control experiments, 50 μL) or solution of test substance in 2-methoxyethanol (50 μL), and phosphate-buffered saline (0.1 M phosphate, pH 7.60, 2.80 mL) was stirred in a quartz cuvette. The oxidation reaction was started by addition of 8 μL enzyme solution. After a brief stirring, the increase in absorbance at 234 nm was measured from 30 to 90 s after enzyme addition. All measurements were carried

out in triplo, and controls without test substance were measured at intervals to ensure constant enzyme activity. Per cent inhibition and IC_{50} values were calculated as described for the soybean enzyme.

DPPH scavenging

Scavenging activity towards the diphenylpicrylhydrazyl (DPPH) was measured as the decrease in absorbance at 517 nm of a methanolic DPPH solution ($A_{517} = 1.0$, 2.95 mL) over a 5-min period after addition of 50 μL of a DMSO solution of the test substance.[29] Appropriate corrections were made for dilution and for absorbance of the reaction product, reduced DPPH. Calculation of radical scavenging activity was carried out as previously described.[37]

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