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N-Aminoindoline Derivatives as Inhibitors of 5-Lipoxygenase

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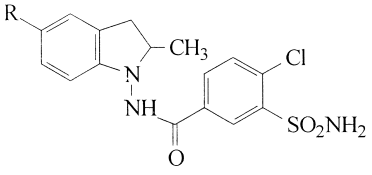
Abstract—*N*-Aminoindoline derivatives were prepared and their 5-lipoxygenase inhibitory activities were evaluated in vitro and compared with those of phenidone and NDGA. Compound **4** presents the most effective 5-LO inhibition.

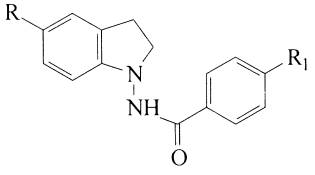
The peptidoleukotrienes LTC_4, LTD_4 and LTE_4 are potent constrictors of human airways, and it has been widely assumed that they play an important role in asthma.[1] They are responsible for potent pro-inflammatory and spasmogenic manifestations, and are present in the airways of asthmatic patients at rest and during an acute crisis. The 5-LO enzyme catalyses two key steps in the LT biosynthetic pathway: the arachidonate-5-HPETE oxidation step followed by the dehydrogenation phase resulting in the formation of LTA_4.[2] Inhibition of 5-LO could provide a novel therapy for human inflammatory diseases such as asthma.

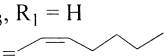
At least four strategies can be considered for 5-LO inhibition: (a) the antioxidant and/or free radical scavenger mechanism; (b) the iron-chelation process; (c) the inhibition of the 5-LO translocation reaction; and (d) the substrate mimic procedure.[3] The aim of this work was to synthesise new antioxidants as inhibitors of 5-LO.

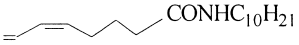
Indapamide, a diuretic drug used in cardiovascular disease treatment, has antioxidant properties: this drug is effective in the inhibition of LDL oxidation and its major metabolite, 5-hydroxyindapamide, is even more active.[4] This could be due to capto-dative stabilisation of the radical[5] resulting from the abstraction of a hydrogen radical. Molecule **1**, like indapamide, presents an *N*-aminoindoline skeleton substituted by a carbonyl group. The non-cyclized analogues of this molecule possessing a carbonyl (**2**) or a sulfonyl group (**3**) should help evaluate the influence of the indoline cycle opening and may be compared with indapamide and also with phenidone, another antioxidant inhibitor of 5-LO.[6]

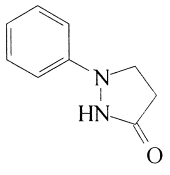


Indapamide : R = H
5-Hydroxyindapamide : R = OH

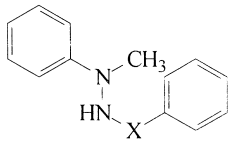


1 : R= H, R_1 = H
4 : R = OH, R_1 = H
5 : R = OCH_3, R_1 = H
6 : R = H, R_1 = 

7 : R = H, R_1 = 



Phenidone



2 : X = CO
3 : X = SO_2

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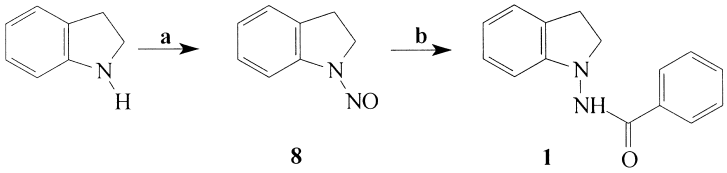
In order to test the influence of an oxygen moiety on antioxidant properties, different pharmacomodulations were performed at C_5 and led to **4** (R = OH), that could be considered as an analogue of 5-hydroxyindapamide, and to **5** ($R = OCH_3$). Compounds **6** and **7**,[7] with a chain containing a double bond as does arachidonic acid, were also tested.

Compound **1** was prepared as outlined in Scheme 1. Nitrosoindoline **8** was prepared by nitrosation of indoline.[8] Reduction with $LiAlH_4$ and acylation of crude 1-aminoindoline with benzoyl chloride[9] produced **1**.

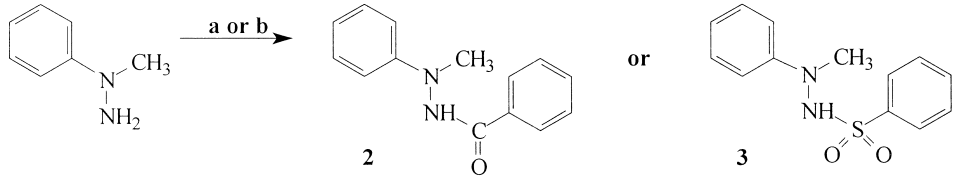
The reaction of *N*-methyl-*N*-phenylhydrazine with benzoyl chloride or benzenesulfonyl chloride led to compounds **2** and **3**, respectively (Scheme 2).

The substituted derivatives **4** and **5** were prepared from the corresponding indoles (Scheme 3).

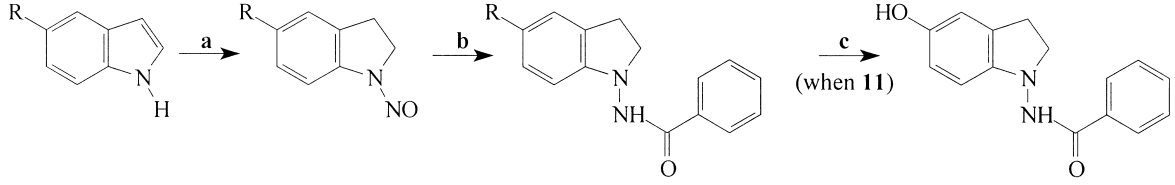
After reduction with sodium cyanoborohydride into the corresponding indolines,[10] nitrosation resulted in **9** and **10**. These compounds were reduced with $LiAlH_4$ to give 1-aminoindolines which were acylated in situ with benzoyl chloride. Compound **4** was obtained after hydrogenolysis of **11** in the presence of Raney nickel. Carboxylic acid **6** was synthesised as shown in Scheme 4. Ethyl hexynoate was coupled under Castro–Stephens conditions with *p*-iodobenzoic acid by palladium catalysis in the presence of catalytic amounts of copper(I) iodide to give the aryne **12**.[11] Reduction of the triple bond with hydrogen over Lindlar catalyst[12] did not lead to pure *cis* olefin **14**, but to a mixture also containing



Scheme 1. (a) $NaNO_2$, HCl, H_2O, 83%; (b) (i) $LiAlH_4$, anhydrous ether, 0 °C; (ii) C_6H_5COCl, AcOEt, NEt_3, 0 °C, 45%.

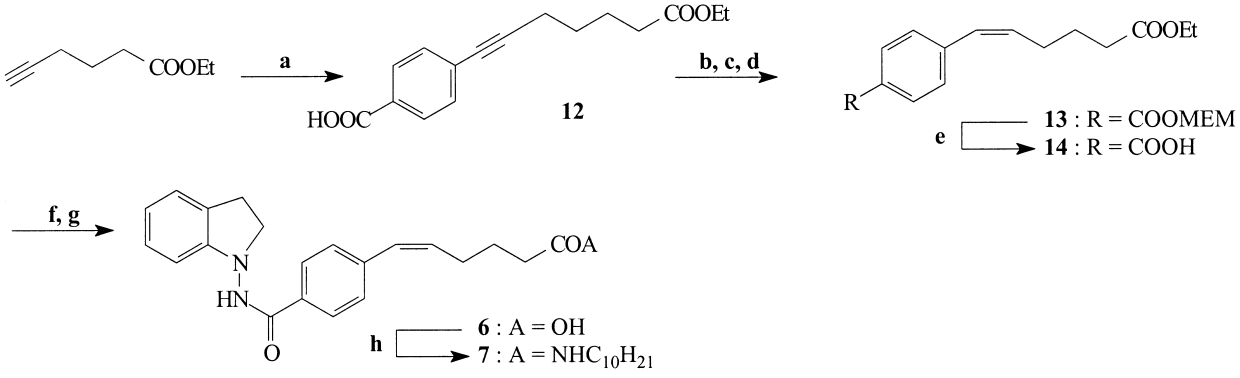


Scheme 2. (a) C_6H_5COCl, $CHCl_3$, NEt_3, 0 °C, 59%; (b) $C_6H_5SO_2Cl$, $CHCl_3$, NEt_3, 0 °C, 70%.



$R = C_6H_5CH_2O$ **9** : $R = C_6H_5CH_2O$ (85%) **11** : $R = C_6H_5CH_2O$ (37%) **4** : (32%)
$R = CH_3O$ **10** : $R = CH_3O$ (51%) **5** : $R = CH_3O$ (55%)

Scheme 3. (a) (i) $NaBH_3CN$, AcOH; (ii) $NaNO_2$, aq HCl; (b) (i) $LiAlH_4$, anhydrous ether, 0 °C; (ii) C_6H_5COCl, AcOEt, NEt_3, 0 °C; (c) H_2, Raney Ni, EtOH.



Scheme 4. (a) *p*-Iodobenzoic acid, NEt_3, CuI, $Pd(P(C_6H_5)_2)Cl_2$, 76%; (b) H_2, $Pd/BaSO_4$, pyridine, EtOH, 86%; (c) MEMCl, $(iPr)_2NEt$, CH_2Cl_2, 84%; (d) HPLC, 70%; (e) TFA, CH_2Cl_2, 98%; (f) (i) EDCI, HOBT, NEt_3, 0 °C; (ii) 1-aminoindoline, 0 °C, rt; (g) NaOH, N_2, dioxan/H_2O (33:67), 95%; (h) (i) EDCI, HOBT, NEt_3, 0 °C; (ii) $C_{10}H_{21}NH_2$, 0 °C, rt, 38%.

trans olefin and the corresponding alkane. In order to circumvent this difficulty, separation was performed by HPLC[13] after transforming the carboxylic function into MEM ester.[14] This strategy led to pure *cis* olefin **13**. The MEM ester was cleaved by TFA and the resulting carboxylic acid **14** was coupled with 1-aminoindoline in the presence of EDCI and HOBT.[15] The carboxylate ester was hydrolysed by NaOH to give compound **6** which was coupled with *N*-decylamine under peptidic synthesis conditions to give amide **7**.

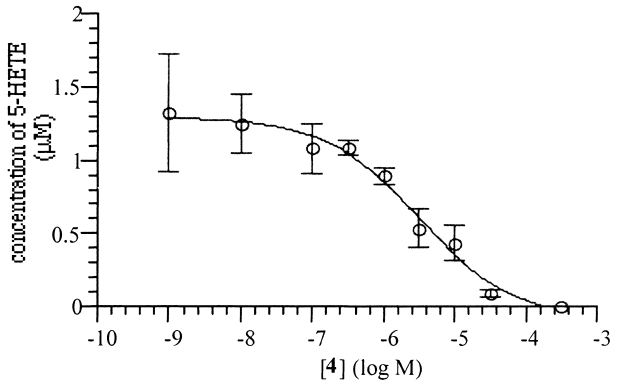
The activities of these different compounds were evaluated in vitro and compared with those of phenidone and NDGA (nordihydroguiaretic acid),[16] the latter being another antioxidant that inhibits 5-LO. Lipoxygenase products synthesised in RBL-1 cells—a rat basophilic leukemia cell line—were analysed by HPLC. The cells were harvested by centrifugation, washed with phosphate buffer saline (PBS), pH 7.4, and resuspended in PBS. Cells were stimulated and the reaction was stopped as previously described.[17] The 5-LO product 5-HETE was quantified by HPLC separation on a Chromspher C_{18} column. Detection of 5-HETE was carried out by UV absorbance at 130 nm. Compound **4** presented interesting activity (Table 1) and was submitted to IC_{50} determination (Scheme 5). Compound **4** is as active as phenidone, but less potent than NDGA in vitro.

The results offer several conclusions concerning pharmacomodulations. Replacement of indoline (**1**) by a non-cyclic skeleton (**2**) does not provide a more active compound, and the presence of a sulfonyl group (**3**) instead of its bioisosteric carbonyl group (**2**) seems to increase the inhibition of 5-LO. Compound **4** is more potent than the non-hydroxylated analogue **1**: C_5 substitution by a hydroxyl enhances 5-LO inhibition and this group seems to be essential as regards enzyme binding since compound **5**, substituted at C_5 by a methoxy group, is less active. The introduction of the *cis* hexenoic chain (**6**) has no influence. On the other hand, amide **7**, tested at 1 μM concentration because of low solubility, could be attractive for further investigations.

Table 1.

Compound	Inhibition% 10 μM		IC_{50} (μM)
NDGA	73±8[a]		0.41±0.20
Phenidone	—		2.54±0.53
1	15±15	$n=3$	—
2	28±3	$n=3$	—
3	53±19	$n=4$	—
4	80±8	$n=4$	3.47±0.93
5	20±18	$n=4$	—
6	18±9	$n=3$	—
7	57±8.5[b]	$n=2$	—

[a]Tested at 0.5 μM.
[b]Tested at 1 μM.



Scheme 5. Inhibition of 5-lipoxygenase activity by **4**. Points represent means±SEM of at least three determinations.

In conclusion, the prepared *N*-aminoindoline derivatives are only moderately potent in inhibiting 5-LO. However, further studies in the aminoindoline and benzothiophene series (relative to Zileuton for example[18]) are underway taking into account the preliminary and encouraging results obtained on compounds **4** and **7**, and considering the fact that a sulfonyl group could induce an increase in activity.

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7. Compound **7** possesses, like some compounds developed by Pfizer, an *N*-decylamide function (Kadin, S. B. Eur. Pat. Appl. EP 50,977 (Cl. C07C103/30); *Chem. Abstr.* **1982**, *97*, 144598b).
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