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Synthesis and structure–activity relationships of γ-carboline derivatives as potent and selective cysLT$_1$ antagonists

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ABSTRACT

A γ-carboline series of cysLT$_1$ receptor antagonists has been prepared. Some of the compounds show good potencies both, in vitro and in vivo, compared to the standard compounds.

Asthma is one of the most rapidly growing therapeutic markets, the disease affecting over 300 million people worldwide.[1] Cysteinyl leukotrienes (LTC$_4$, LTD$_4$ and LTE$_4$) are products of the 5-lipoxygenase pathway of arachidonic acid metabolism and play a crucial role in asthma pathophysiology by causing bronchoconstriction, mucus production and an increase in vascular permeability.[2] They represent one of the most effective approaches to the treatment of asthma[3] and several compounds with this mechanism of action have reached the market.[4] In recent years there has been particular interest in searching for dual H$_1$/cysLT$_1$ antagonists in the hope of managing asthma by synergistic effects.[5]

One of the chemical series we have designed in order to achieve this goal is based on the antiH$_1$ derivative mebhydroline.[6] Taking into account the pharmacophoric model for cysLT$_1$ antagonists,[7] we expected that the introduction of a quinoline-type substituent in the benzyl group of mebhydroline and an acid group branching from the piperidine moiety (see Fig. 1) would confer a cysLT$_1$ antagonistic character to the resulting structure (e.g. **1**, see Fig. 2).

Although the first compounds synthesized in this series (**1** and related tetrahydro-β-carbolines) lacked the parent anti H$_1$ activity, the anticysLT$_1$ activities were so interesting as to encourage us to pursue our efforts in this field.[8] In this Letter we describe our studies in developing a new series of cysLT$_1$ antagonists based on our first mebhydroline derivative, compound **1**.

The basic pathway to the target compounds is depicted in Scheme 1. Starting from the phenylhydrazines **2** the corresponding tetrahydrocarbazoles **3** were prepared by Fischer indolization of 1-benzyloxycarbonyl-4-piperidone. Compounds **3** were alkylated at the indole nitrogen and then the BOC group was removed in acidic conditions. After alkylation of the carboline nitrogen in **4** and subsequent hydrolysis (or reaction of the corresponding nitrile with tributyltin azide) the target carbolines **1** and **5**–**30** were obtained.[9] The hexahydrocarboline derivative **33** were prepared through a similar synthetic pathway from the corresponding compounds **32**, which were in turn synthesized by cyanoborohydride reduction of the indole compound **3** (R^1 = H).

The indole derivatives **35** and **36** (Scheme 2) were obtained by alkylation and subsequent saponification of the butyric esters **34**, which are commercially available (when R = H) or easily prepared from phenylhydrazine and ethyl 6-oxoheptanoate (when R = Me).

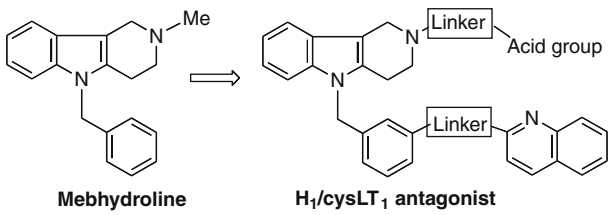

Figure 1.

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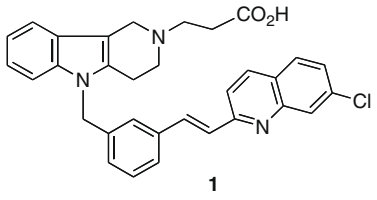
The open chain amino derivatives **37** and **38** were prepared from tryptamine and indole, respectively, following the same reaction procedure as above (Scheme 2).

The carbazole **39** was prepared through a synthetic pathway involving Fries-type and Willgerodt–Kindler transpositions, whilst the tetrahydrocarbazole **40** was synthesised again through a Fischer-indole cyclization. The β-carboline derivatives **41** and **42** were prepared from the commercially available tetrahydro-β-carboline (Scheme 3).

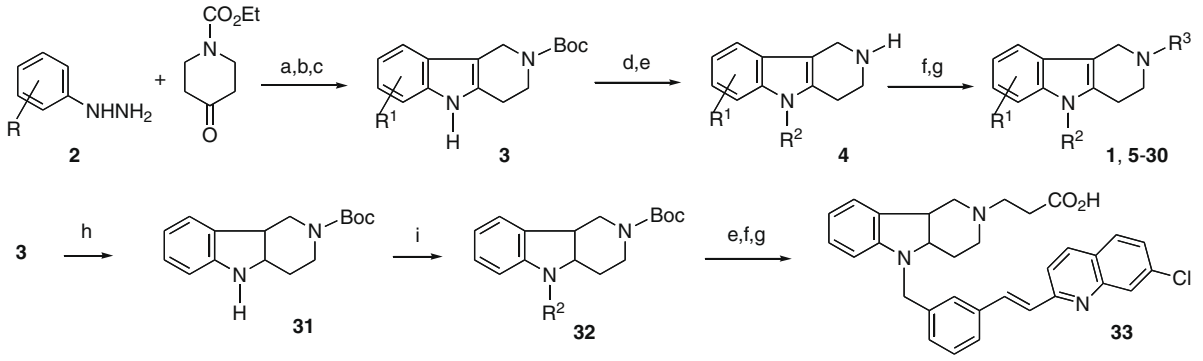
All synthesized compounds were tested in a binding assay in guinea pig lung using [^{3}H]LTD$_4$ as a radioligand,[10] and in the inhibition of LTD$_4$-induced airway microvascular permeability at 4 h, also in guinea pigs.[11] First of all, the influence of the carboxylic side chain R in γ-carbolines (**1**, **5**–**12**) was assessed (Table 1). All compounds tested showed moderate to good in vitro activity, while the best in vivo compound was our first derivative, the propionic acid **1**. By inserting a double C=C bond, a cyclopropyl or a phenyl group the affinity improved significantly but not the oral activity.

The nature of the linker between the phenyl spacer and the lipophilic group was also examined (Table 2). Substitutions of the vinyl bridge for methoxy (**13**), ethylene (**15**), or acetylene (**16**) or inclusion in a benzofuran ring (**14**) afforded compounds with poorer oral activities in all cases. Similar results were obtained when changing the position of the nitrogen atom to a β-carboline, modifying the acid group to a tetrazole, saturating the system to indoline or 'opening' the carboline system (Table 3), in spite of the improvement in some affinity values. The role of the carboline nitrogen atom was also assessed. In all the analogs lacking it—open or cyclic derivatives—the oral activities decreased in spite of the improvement in affinity (Table 4). The improvement of oral activity related to the ω-aminoalkyl carboxylic chain motif has also been observed with many of the 2nd generation antiH$_1$ derivatives. In this type of compounds the presence of a carboxylic chain attached to the nitrogen atom keeps or sometimes enhances oral activity compared to the parent amine compound, even though in some cases the H$_1$ affinity decreases.[12] The next point of variation concerns the lipophilic moiety, for which a number of substituted quinolines and other heterocycles were tested (Table 5). In this case a significant improvement was achieved with the 6,7-difluoroquinoline derivative, a compound that shows an ED$_{50}$ value for inhibition of LTD$_4$ extravasation of 0.04 mg/kg. Finally, a few substitutions at the carboline phenyl system were tested. A methoxy group did not improve oral activity on parent compound **1**, but

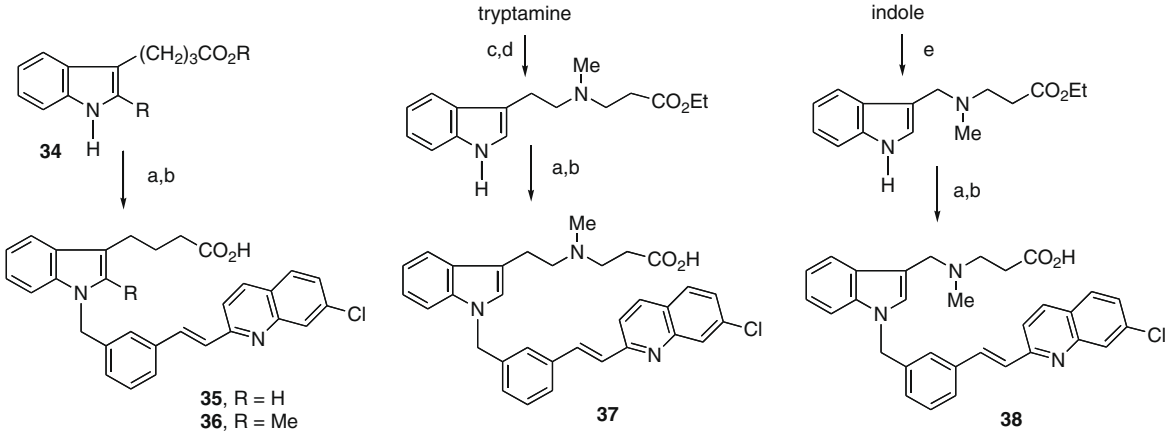


1

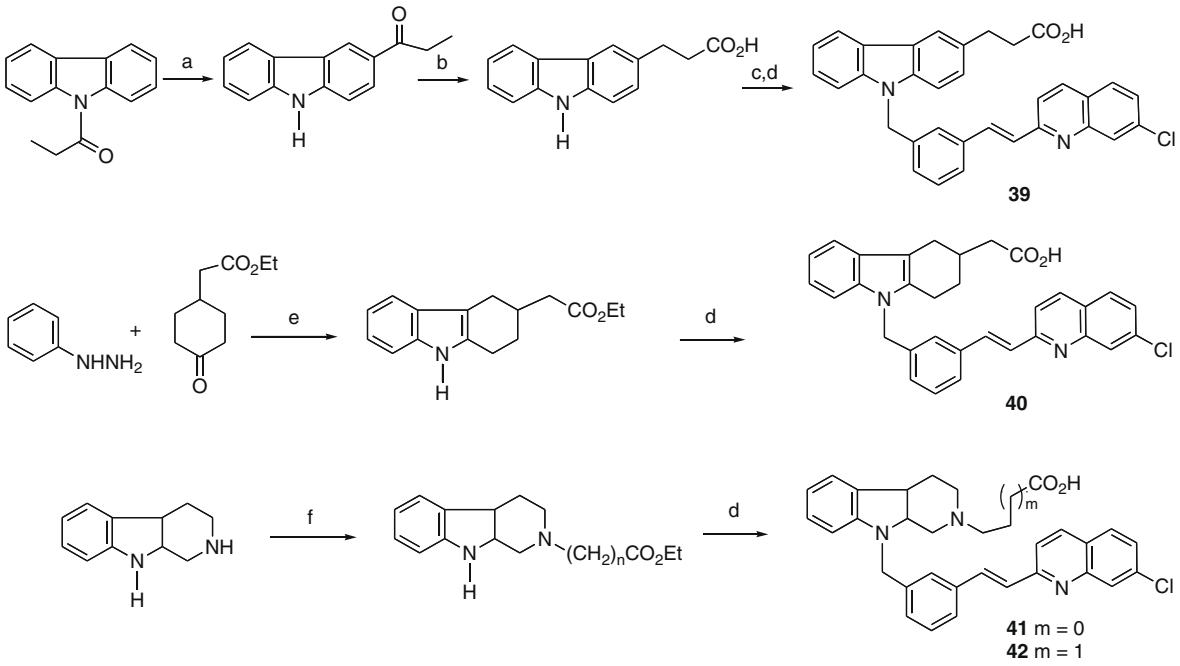
Figure 2.



Scheme 1. Reagents and conditions: (a) AcOH, rfx, 3 h (88%); (b) KOH/IPA, rfx, 16 h (87%); (c) (Boc)$_2$O, THF, 5 °C to rt (98%); (d) NaH, DMF; then R^{2}X (69–95%); (e) TFA, CH_2Cl_2, 1 h rt or aq HCl, EtOH, rfx, 1 h (69–97%); (f) ethyl acrylate or acrylonitrile, EtOH, rfx 1 h or R^{3}X, K_2CO_3, MIK, rfx, 16 h; (g) 5 N NaOH, EtOH or LiOH, THF, H_2O, 1 h, rt or $SnBu_3N_3$, 110 °C, 3 h (28–97% for the two steps); (h) $NaCNBH_3$, AcOH, rt (30%); (i) (*E*)-7-chloro-2-[3-(chloromethyl)styryl]quinoline, Et_3N, DMF, 60 °C (40%).

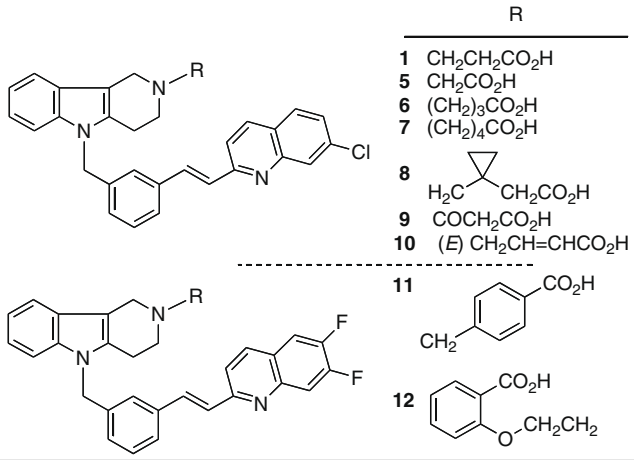


Scheme 2. Reagents and conditions: (a) NaH/DMF, then (*E*)-7-chloro-2-(3-(chloromethyl)styryl)quinoline; (b) NaOH 5 N/EtOH or LiOH/THF/H_2O, 1 h, rt (13–86% overall yield); (c) ethyl acrylate, EtOH, rfx, 1 h (quantitative); (d) MeI, K_2CO_3, CH_3CN, rt, 5 h (51%); (e) ethyl 3-methylaminopropanoate/HCHO, MeOH, 60 °C, 5 h (36%).



Scheme 3. Reagents and conditions: (a) $AlCl_3$ neat, 115 °C, 2 h; (b) S_8, morpholine, rfx, 16 h, then KOH/EtOH, rfx, 5 h (38%); (c) EtOH/HCl rfx, 5 h; (d) NaH, (*E*)-7-chloro-2-(3-(chloromethyl)styryl)quinoline; DMF, rt; then NaOH 5 N, EtOH, THF, rt (23–42% overall yield); (e) ethyl (4-oxocyclohexyl)acetate, AcOH, 100 °C, 1 h (32%); (f) ethyl acrylate, EtOH, rfx, 1 h (quantitative) for **41**; $Br(CH_2)_3CO_2Et$, K_2CO_3, KI, MIK, 90 °C, 2 h (62%) for **42**.

Table 1
Binding and oral activities for different acid side-chains of γ-carbolines (**1**, **5**–**12**)



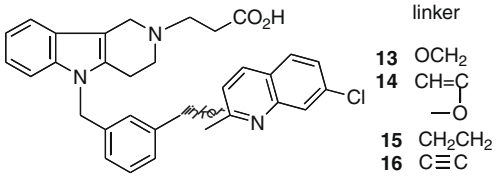
Compd	LTD_4 binding IC_{50}[a] (nM)	Inhibition of LTD_4 extravasation[b]
1	16 (6)	72 (0.1); 100 (1)
5	17 (6)	22 (0.1); 43 (1)
6	30 (12)	25 (0.1); 80 (1)
7	16 (6)	13 (0.1); 77 (1)
8	3.2 (1.1)	20 (0.1); 64 (1)
9	26 (11)	1 (0.1); 26 (1)
10	4.1 (1.5)	22 (0.1); 52 (1)
11	3.0 (1.8)	34 (0.1); 79 (1)
12	0.91 (0.43)	0 (0.1); 46 (1)

[a] Values are means of three experiments, standard deviation is given in parentheses.
[b] % Inhib (dose mg/kg).

a fluorine atom at 9 position on parent difluoro derivative **21** did (Table 6).

In order to assess the duration of action of this type of compounds, the inhibition of LTD_4 extravasation in guinea-pig was also performed at 1 and 8 h for some selected compounds (see results in Table 7). The assayed compounds showed a sustained duration of action in vivo, similar to that observed for Zafirlukast or Montelukast, with potencies lying between those observed for these two standard LTD_4 antagonists. The pharmacokinetics of **21** in rat

Table 2
Binding and oral activities for different quinoline/spacer linkers of γ-carbolines (**13**–**16**)



Compd	LTD_4 binding IC_{50}[a] (nM)	Inhibition of LTD_4 extravasation[b]
13	42 (13)	24 (0.1); 55 (1)
14	14 (4)	15 (0.1); 30 (1)
15	25 (1)	7 (0.1); 19 (1)
16	1.3 (0.08)	0 (0.1); 60 (1)

[a,b] See footnotes at Table 1.

Table 3
Binding and oral activities for miscellaneous compounds (**17**, **33**, **37**, **38**, **41**, **42**)

Compd	LTD_4 binding IC_{50}[a] (nM)	Inhibition of LTD_4 extravasation[b]
41	20 (5.4)	10 (0.1); 43 (1)
42	15 (3.4)	16 (0.1); 39 (1)
17[c]	1.2 (1.7)	3 (0.1); 14 (1)
33	12 (4.5)	22 (0.1); 66 (1)
37	9.3 (2.7)	55 (0.1); 67 (1)
38	61 (19)	—

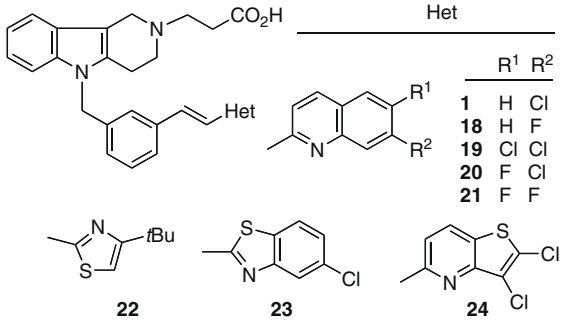
[a,b] See footnotes at Table 1.
[c] Compound **17** has a structure as **1** exchanging the carboxyl by a 5-tetrazolyl group.

Table 4
Binding and oral activities for compounds lacking the carboline N-atom (**35**, **36**, **39**, **40**)

Compd	LTD_4 binding IC_{50}[a] (nM)	Inhibition of LTD_4 extravasation[b]
39	4.8 (1.4)	29 (0.1); 52 (1)
40	4.9 (2.1)	14 (0.1); 66 (1)
35	4.4 (2.8)	24 (0.1); 31 (1)
36	3.4 (3)	0 (0.1); 15 (1)

[a,b] See footnotes at Table 1.

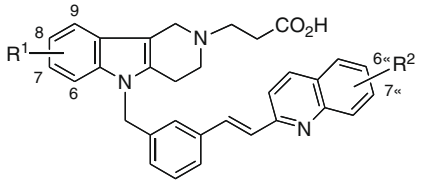
Table 5
Binding and oral activities for different lipophilic moieties (**1**, **18**–**24**)



Compd	LTD_4 binding IC_{50}[a] (nM)	Inhibition of LTD_4 extravasation[b]
1	16 (6)	72 (0.1); 100 (1)
18	3.4 (2.2)	42 (0.1); 100 (1)
19	21 (6.8)	0 (0.1); 86 (1)
20	1.7 (0.5)	58 (0.1); 97 (1)
21	1.0 (0.4)	48 (0.03); 96 (0.1); 100 (1)
22	37 (9)	—
23	112 (52)	—
24	50 (29)	—

[a,b] See footnotes at Table 1.

Table 6
Binding and oral activities for some phenyl-substituted γ-carbolines (**1**, **21**, **25**–**30**)



	R1	R2
1	H	7«Cl
25	6-OEt	7«Cl
26	7-OMe	7«Cl
27	8-OMe	7«Cl
21	H	6«7«diF
28	7-F	6«7«diF
29	8-F	6«7«diF
30	9-F	6«7«diF

Compd	LTD_4 binding IC_{50}[a] (nM)	Inhibition of LTD_4 extravasation[b]
1	16 (6)	72 (0.1); 100 (1)
25	150 (74)	—
26	16 (5)	29 (0.1); 53 (1)
27	7.5 (2,5)	45 (0.1); 74 (1)
21	1.0 (0.1)	0.04
28	4.5 (3.4)	0.05
29	2.5 (0.6)	0.07
30	1.2 (0.8)	0.02

[a] See footnotes at Table 1.
[b] % Inhib (dose mg/kg) or ED_{50}, mg/kg.

shows a 47% of bioavailability, an intermediate value between the 63% for montelukast and the 25% for zafirlukast.

In conclusion, we have developed a SAR around the tetrahydrocarbazole scaffold, which led to the selection of compound **21**. Further studies will provide a better understanding of its long term efficacy and potential for human treatment.

Table 7
Duration of action in vivo of selected compounds

Compound	Oral activity[a]		
	1 h	4 h	8 h
1	0.06	0.041	0.075
21	0.02	0.04	0.03
30	0.014	0.02	0.05
A[b]	0.14	0.43	0.39
B[b]	0.26	2.0	1.0
C[b]	0.027	0.008	0.006

[a] Inhibition of LTD_4 extravasion: ED50, mg/kg.
[b] **A**:{3-[2-Methoxy-4-toluene-2-sulfonylaminocarbonyl)benzyl]-1-methyl-1*H*-indol-5-yl}carbamic acid cyclopentyl ester (zafirlukast); **B**: 8-[4-(4-phenylbutyloxy)benzoyl]amino-2-(tetrazol-5-yl)-4-oxo-4*H*-1-benzopyran (pranlukast); **C**: [1-(1-{3-[2-(7-chloroquinolin-2-yl)-vinyl]-phenyl}-3-[2-(1-hydroxy-1-methyl-ethyl)-phenyl]-propylsulfanylmethyl)-cyclopropyl]-acetic acid (montelukast).

Acknowledgments

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9. In the case of the isomeric fluoro derivatives **28** and **30** the separation was achieved through chromatography on silica gel of the mixture of the corresponding amino antecedents **4** using a mixture of CH_2Cl_2/MeOH/NH_4OH (40:2.5:0.1).
10. The assays were performed using guinea-pig lung membranes as a source of receptors and [^{3}H]LTD_4 from NEN in a modification of the previously described method (Aharony D.; Falcone, R. C.; Krell, R. D. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 921). The assay mixture contained 200 μg of membranes per assay in a final volume of 10 mM PIPES pH 7.5 containing 10 mM $CaCl_2$, 50 mM NaCl, 2 mM L-cysteine, 2 mM Glycine and 300 pM [^{3}H]LTD_4. Non specific binding was determined in the presence of zafirlukast 10 μM. The assays were directly performed on GF/B Millipore Multiscreen 96 well plates, pre-soaked with 200 μl/well of assay buffer, filtered, washed three times with 175 μl of 10 mM TRIS buffer pH 7.5 containing 100 mM NaCl at 4 °C, dried and read in a TRILUX Microbeta Counter of Wallac.
11. Male Dunkin–Hartley guinea-pigs were administered with the test compounds by oral gavage at the indicated time-points before being anesthetized. Then, the left jugular vein was cannulated and the animals received the Evans blue dye intravenously. After 5 min, LTD_4 was administered to the animals in order to induce airway microvascular leakage. After another period of 5 min, animals were exsanguinated and the vascular bed was rinsed. Then the trachea was excised and incubated in formamide for 20 h at 55 °C to extract the Evans blue dye from the tissue. Microvascular permeability was determined by light spectrophotometry at 620 nm. The number of animals was 6 per dose or 8 in the case of the complete dose–response curve.
12. Inter alia, see: (a) Iwasaki, N.; Sakaguchi, J.; Ohashi, T.; Takahara, E.; Ogawa, N.; Yasuda, S.; Koshinaka, E.; Kato, H.; Ito, Y.; Sawanishi, H. *Chem. Pharm. Bull.* **1994**, *42*, 2276; (b) Iwasaki, N.; Sakaguchi, J.; Ohashi, T.; Yamazaki, M.; Ogawa, N.; Yasuda, S.; Koshinaka, E.; Kato, H.; Ito, Y.; Sawanishi, H. *Chem. Pharm. Bull.* **1994**, *42*, 2285.