Letter

Discovery of 3-substituted 1H-indole-2-carboxylic Acid Derivatives as a Novel Class of CysLT1 Selective Antagonists

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Discovery of 3-substituted 1*H*-indole-2-carboxylic Acid Derivatives as a Novel Class of $CysLT_1$ Selective Antagonists

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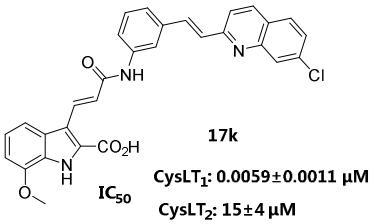
KEYWORDS: Cysteinyl leukotrienes, $CysLT_1$, $CysLT_2$, selective antagonists, asthma

ABSTRACT: The indole derivative, 3-((*E*)-3-((3-((*E*)-2-(7-chloroquinolin-2yl)vinyl)phenyl)amino)-3-oxoprop-1-en-1-yl)-1*H*-indole-2-carboxylic acid (**17k**), was identified as a novel and highly potent and selective $CysLT_1$ antagonist with IC_{50} values of 0.0059 ± 0.0011 μM and 15 ± 4 μM for $CysLT_1$ and $CysLT_2$, respectively.



Cysteinyl-leukotrienes (CysLTs) are potent inflammatory lipid mediators synthesized from arachidonic acid.[1,2] CysLTs include leukotriene C_4 (LTC_4), leukotriene D_4 (LTD_4), and leukotriene E_4 (LTE_4). They play established or evolving roles in asthma, allergic rhinitis, and other inflammatory conditions, such as cardiovascular diseases, cancer, and atopic dermatitis.[3] CysLTs activate at least 2 receptors, designated as $CysLT_1$[4] and $CysLT_2$,[5] which belong to the G protein-coupled receptor (GPCR) superfamily.

$CysLT_1$ receptors are mainly expressed in the lung, peripheral blood leukocytes and the spleen.[4] Most of the pathophysiological effects of CysLTs in asthma are mediated by the $CysLT_1$ receptor.[6] Several $CysLT_1$ selective antagonists have been launched for treating bronchial asthma and allergic rhinitis, such as montelukast, pranlukast and zafirlukast (Figure 1).[6]

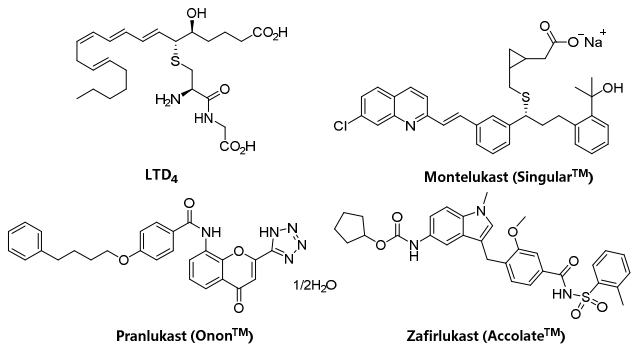


Figure 1. LTD_4 and launched drugs selective targeting $CysLT_1$

$CysLT_2$ receptors are highly expressed in the peripheral blood leukocytes, spleen and lymph nodes and are uniquely expressed in the heart, brain and adrenal glands.[5] Recently, Sekioka, T. et al. reported the expression of $CysLT_2$ receptors in asthmatic lungs and investigated their possible role in bronchoconstriction.[7] However, the pharmacological roles of $CysLT_2$ are less well defined, and there is no specific antagonist being marketed as a therapeutic agent so far.[6] Wunder et al. reported the identification of the first potent and selective $CysLT_2$ antagonist, HAMI3379 (Figure 2), which was shown to inhibit the cardiovascular effects mainly through mediation of $CysLT_2$ receptors.[8] Meanwhile, a highly similar compound, $BayCysLT_2$ (Figure 2), was also reported to be a potent and selective $CysLT_2$ antagonist.[9]

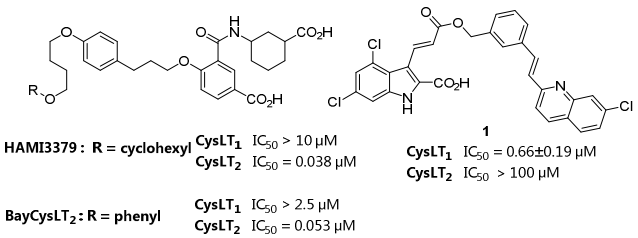


Figure 2. Selective CysLT receptor antagonists.

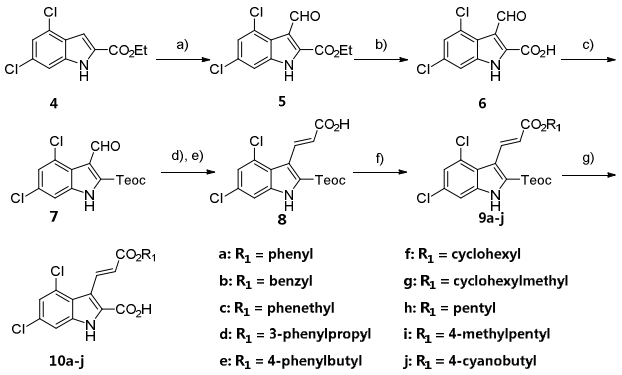
Although the marketed $CysLT_1$ selective antagonists are effective therapeutics for the general treatment of mild to moderate bronchial asthma, it is known that the current $CysLT_1$ antagonists do not have sufficient effects for some nonresponsive patients. A recent report demonstrated that $CysLT_1$ may also play a role in multiple sclerosis; blocking this receptor protects the integrity of the blood-brain barrier and reduces infiltration of pathogenic T cells into the central nervous system.[10] More recently, Ludwig et al reported that anti-asthmatic drug montelukast, which antagonizes $CysLT_1$, reduces neuroinflammation, promotes hippocampal neurogenesis and improves learning and memory in old animals.[11] Therefore, the development of new CysLT antagonists is still of great interest.

A high-throughput screening (HTS) campaign of our compound library yielded indole derivative **1**, which showed mi-

cromolar $CysLT_1$ antagonist activity with an IC_{50} value of 0.66 ±0.19 μM and exhibited no $CysLT_2$ antagonist activity (Figure 2). Compound **1** shares same hydrophobic (*E*)-3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl group as montelukast, but it has a unique and essential indole-2-carboxylic acid moiety, which is different from the corresponding counterparts of known $CysLT_1$ antagonists. The structural novelty of compound **1** encouraged us to further optimize and develop more potent $CysLT_1$ antagonists.

First, we investigated the effects of the ester groups and designed compounds **10a-10j**. The syntheses of compounds **10a-10j** are described in Scheme 1. Ethyl-4,6-dichloro-1*H*-indole-2-carboxylate **4** was prepared from 3,5-dichloroaniline in a two-step synthetic procedure using a Japp-Klingemann condensation followed by a Fischer indole (aza-Cope) ring closure reaction. Vilsmeier-Haack formylation of **4** afforded aldehyde **5**.[12] Subsequent ester hydrolysis of **5** afforded carboxylic acid **6**, which upon esterification, afforded **7**. Compound 7 was reacted with tert-butyl(triphenylphosphoranylidene)acetate in a Horner-Wadsworth-Emmons reaction followed by the chemoselective deprotection of the *tert*-butyl ester group, which yielded the α, β-unsaturated acid intermediate **8**.[13] Esterification of **8** generated compounds **9a-9j**, which were then converted to compounds **10a-10j** using TBAF in THF.

Scheme 1. Syntheses of Compounds 10a-10j[a]

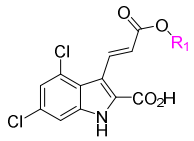


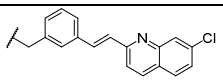
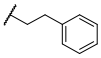
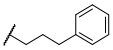
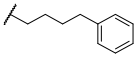
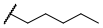
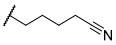
[a]Reagents and conditions: (a) DMF, $POCl_3$, DCE, reflux, 7 h; (b) EtOH, $LiOH·H_2O$, 50 °C, 2 h; (c) 2-(trimethylsilyl)ethan-1-ol, EDCI, DMAP, DCE, rt, overnight; (d) tert-butyl (triphenylphosphoranylidene)acetate, toluene, reflux, overnight; (e) 98% HCOOH, rt, overnight; (f) R_1Br, Cs_2CO_3, DMF or R_1OH, DCC/EDCI, DMAP, DMF; (g) TBAF, THF, rt, 2 h.

As shown in Table 1, the results revealed that the (*E*)-3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl group was necessary for the antagonist activity of the compounds; replacing it with other ester groups eliminated antagonist activity against both $CysLT_1$ and $CysLT_2$. Therefore, we attempted to modify the functional groups at other positions of hit compound **1** while retaining the (*E*)-3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl group to increase its antagonist activity against $CysLT_1$.

The chlorine atoms in the indole ring of compound **1** were removed and proposed compound **2** (Table 2) was synthesized following the previously described method. We removed the carboxylic acid group of compound **1** and yielded compound **3**, which was synthesized following the method shown in Scheme 2. Decarboxylation of **6** provided aldehyde **11**,[14] which was subsequently converted to **12** using a Doebner Knoevenagel modification reaction;[15] finally, esterification of **12** provided **3.**

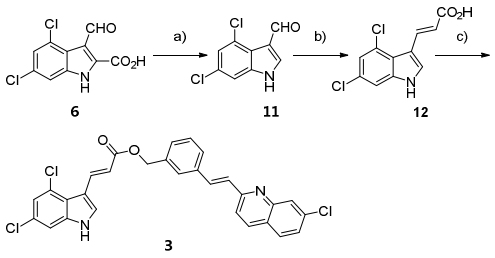
Table 1. Effects of Ester Groups on Activity Profiles



Cmpd	R_1	IC_{50}(μM)[a]	
		$CysL_1$	$CysLT_2$
1		0.66±0.19	>100
10a		>100	>100
10b		>100	~100
10c		>100	>100
10d		>100	~100
10e		>100	~100
10f		>100	>100
10g		>100	>100
10h		>100	~100
10i		>100	>100
10j		>100	>100
montelukast		0.31±0.09	27±6
pranlukast		0.023±0.006	43±5
zafirlukast		0.014±0.003	58±3

[a]Assay protocols are provided in the Supporting Information. IC_{50} values were obtained from one experiment with 3 replicates.

Scheme 2. Synthesis of 3[a]



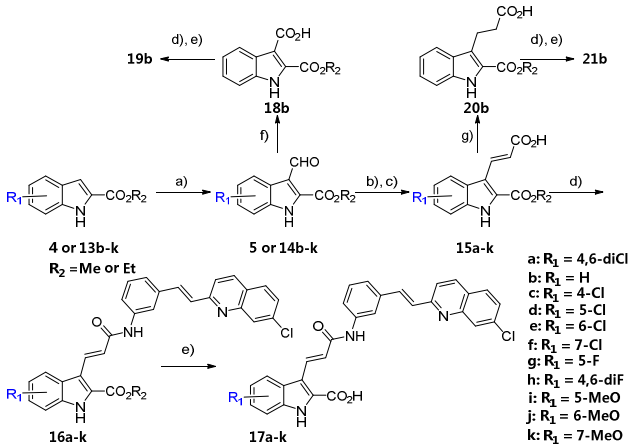
[a]Reagents and conditions: (a) CuCl, quinoline, microwave, 200 °C, 10 min; (b) malonic acid, pyridine, piperidine, 50 °C , 12 h; (c) (*E*)-2-(3-(bromomethyl)styryl)-7-chloroquinoline, Cs_2CO_3, DMF, rt, overnight.

In order to replace the ester bond of compound **1** with an amide bond, we intended to synthesize (*E*)-3-(2-(7-chloroquinolin-2-yl)vinyl)benzylamine, unfortunately, we failed to obtain the intermediate after several synthetic attempts; considering the easy availability of (*E*)-3-(2-(7-

chloroquinolin-2-yl)vinyl)aniline, compound **17a** was prepared (Scheme 3). As shown in Table 2, compounds **2** and **17a** exhibited slightly improved antagonist activities against $CysLT_1$ compared to hit compound **1**, which suggested that derivatives with no substituents in the indole ring were better than derivatives with chlorine atoms and that amide bonds were superior to ester bonds in the internal chain. However, compound **3**, which lacked the indole-2-carboxylic acid moiety was approximately 47-fold less potent than hit compound **1**; this demonstrated that the carboxylic acid group at position 2 of the indole ring was necessary. These results further supported the common features of $CysLT_1$ antagonists, namely, they all contained a lipophilic region which incorporates into the lipophilic pocket of the $CysLT_1$ receptor and an acidic moiety modelling the C1-carboxylic acid of LTD4.[16-18] Furthermore, these results were in accordance with the essential structural elements for $CysLT_1$ receptor ligands.[16-17, 19] The activity results of compounds in Table 1 and compound **3** demonstrated that the indole ring, the carboxylic acid function, and the (*E*)-3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl group were the three necessary pharmocophores for the novel series, the activity results of compounds **2** and **17a** revealed that removement of chlorine atoms in the indole ring and replacement of the ester bond with the amide function were favorable for improvement of the potency, therefore, compound **17b** (Scheme 3) was suggested based on the structure-activity relationships (SARs) in the present study. Satisfactorily, **17b** demonstrated significantly better antagonist activity against $CysLT_1$ than hit compound **1**, and its antagonist activity against $CysLT_2$ remained very low. Subsequently, to explore the effects of the α, β-unsaturated double bond at position 3 of the indole ring of **17b** on activity profiles, **17b** was modified leading to **19b** and **21b** (Scheme 3). As shown in Table 2, **19b** and **21b** were approximately 4 and 6-fold less potent against $CysLT_1$ than **17b**, and they demonstrated stronger antagonist activities against $CysLT_2$ than **17b**, which revealed that the importance of the α, β-unsaturated double bond .

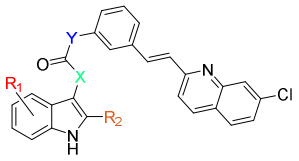
Finally, we investigated the effects of the substituents of the indole ring on the activity profiles with compounds **17c-17k**. The syntheses of **17a-17k, 19b**, and **21b** are described in Scheme 3. Vilsmeier-Haack formylation of **4** and **13b-13k** afforded aldehydes **5** and **14b-14k**. The Wittig-type olefination of the latter compounds and the subsequent deprotection of the tert-butyl ester group with TFA afforded compounds **15a-15k**. Oxidation of **14b** afforded **18b**. Reduction of **15b** by catalytic hydrogenation afforded **20b**. Condensation of **15a-15k**, **18b**, and **20b** with (*E*)-3-(2-(7-chloroquinolin-2-yl)vinyl)aniline and subsequent hydrolysis provided compounds **17a-17k**, **19b**, and **21b**, respectively.

Scheme 3. Syntheses of 17a-17k, 19b, and 21b[a]



[a]Reagents and conditions: (a) DMF, $POCl_3$, DCE, reflux, 7 h; (b) tert-butyl (triphenylphosphoranylidene)acetate, toluene, reflux, overnight; (c) TFA, DCM, rt, 3 h; (d) (*E*)-3-(2-(7-chloroquinolin-2-yl)vinyl)aniline, HATU, DIPEA, DMF, rt, overnight; (e) NaOH aq, EtOH, 50 °C, overnight; (f) 30% H_2O_2, $NaClO_2$, $NaH_2PO_4 \cdot 2H_2O$, CH_3CN, rt, overnight; (g) Pd/C, H_2, EtOH-THF, rt, 7 h.

Table 2. Activity Profiles of Indole Derivatives

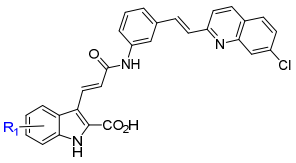


Cmpd	R_1	R_2	X	Y	$IC_{50}(\mu M)$[a]	
					$CysLT_1$	$CysLT_2$
1	4,6-diCl	-COOH	-CH=CH-	$-OCH_2-$	0.66±0.19	>100
2	H	-COOH	-CH=CH-	$-OCH_2-$	0.12±0.02	>100
3	4,6-diCl	H	-CH=CH-	$-OCH_2-$	31±13	>100
17a	4,6-diCl	-COOH	-CH=CH-	-NH-	0.29±0.14	>100
17b	H	-COOH	-CH=CH-	-NH-	0.0090±0.0043	58±26
19b	H	-COOH	-	-NH-	0.035±0.005	23±1
21b	H	-COOH	$-CH_2CH_2-$	-NH-	0.058±0.014	50±18

[a]Assay protocols are provided in the Supporting Information. IC_{50} values were obtained from one experiment with 3 replicates.

As shown in Table 3, the fluorine substituted derivatives were more potent than the chlorine substituted derivatives (**17d** vs **17g** and **17h** vs **17a** (Table 2)); **17c**, **17d**, **17e**, and **17f** demonstrated that substitution at position 4 of the indole ring was the least favorable. Moreover, the methoxy group substituted derivatives exhibited substitution at position 7 of the indole ring was the most favorable. Among these derivatives, compounds **17b**, **17g**, and **17k** showed comparable low nanomolar level potencies against $CysLT_1$, while some derivatives exhibited weak (**17b**, **17g** and **17k**) to no (**17j**) $CysLT_2$ antagonist activities.

Table 3. Effects of the Substituents of the Indole Ring on Activity Profiles



Cmpd	R_1	IC_{50}(μM)	
		$CysLT_1$	$CysLT_2$
17b	H	0.0090±0.0043	58±26
17c	4-Cl	0.067±0.022	36±13
17d	5-Cl	0.017±0.002	>100
17e	6-Cl	0.022±0.002	87±17
17f	7-Cl	0.016±0.004	>100
17g	5-F	0.0078±0.0013	46±20
17h	4,6-diF	0.038±0.001	76±14
17i	5-MeO	0.025±0.007	46±7
17j	6-MeO	0.012±0.007	>100
17k	7-MeO	0.0059±0.0011	15±4

[a]Assay protocols are provided in the Supporting Information. IC_{50} values were obtained from one experiment with 3 replicates.

CysLTs have been reported to induce chemotactic activity in eosinophils[20] and monocytes[21]. We previously found that LTD_4 could also induce chemotaxis of splenocytes isolated from mice with EAE (experimental autoimmune encephalomyelitis), an animal model of multiple sclerosis,[10] and this effect could be blocked by montelukast.[10] We then tested compounds **1**, **17b**, **17g**, **17j** and **17k** with the chemotaxis assay. We found that these compounds could also block LTD_4-induced chemotaxis of leukocytes isolated from the spleen of EAE-mice in a dose-dependent manner (Figure 3). Compound **1** exhibited similar activity as montelukast (Figure 3 A and B), and both compounds displayed ~50% inhibition of chemotaxis at concentrations of 1 μM. Compounds **17b**, **17g**, **17k** and **17j** showed much better inhibitory effects, and all these compounds displayed ≥50% inhibition at concentrations of 100 nM (Figure 3C, D, E and F). In particular, compounds **17g** and **17k**, which displayed best antagonist activity in calcium assay (Table 3), showed significant inhibition of chemotaxis at 10 nM concentration (Figure 3D and E).

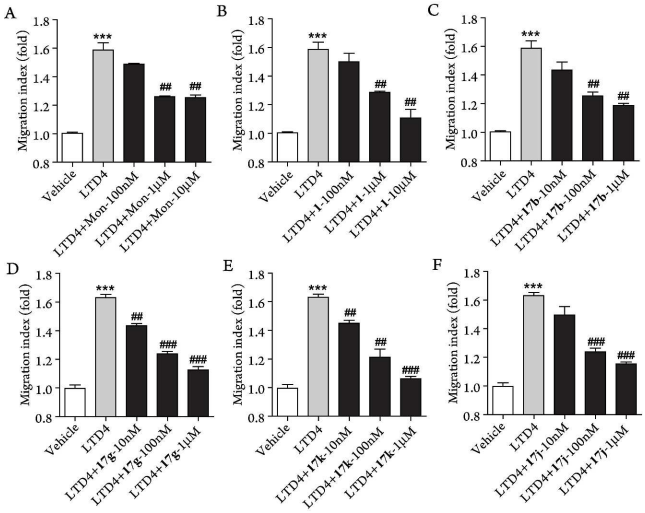


Figure 3. Selective $CysLT_1$ antagonists inhibit leukocyte chemotaxis induced by LTD_4. Data are from three independent experiments (means ± SEM). ***p < 0.001, versus vehicle control, ##p < 0.01, ###p < 0.001 versus LTD_4 (100 nM) treatment group (Student t test).

In summary, we have discovered a novel class of selective $CysLT_1$ antagonists. Our results indicated that it is essential that such antagonists possess (*E*)-3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl and indole-2-carboxylic acid moieties for effective $CysLT_1$ antagonist activity. Additionally, α, β-unsaturated amide moieties at position 3 of the indole rings were also important factors. The most potent compound (**17k,** IC_{50} value of 0.0059 ± 0.0011 μM ($CysLT_1$)) demonstrated significantly more potent $CysLT_1$ antagonist activity than the known selective $CysLT_1$ antagonist, montelukast, both in calcium mobilization assay and chemotaxis assay. The further optimization and development including the pharmacokinetic profile of the most potent antagonists and their pharmacological effects evaluated in relevant animal models are in progress in our lab.

ASSOCIATED CONTENT

Supporting Information

Synthetic procedures, analytic data, procedures for the biological assays, and NMR spectras of the key compounds (PDF). This material is available free of charge on the ACS Publications website at DOI: XXX.

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Author Contributions

[b]These authors (H. C. and H. Y.) contributed equally to this work.

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Notes

The authors declare no competing financial interest.

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