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CCR3 Antagonists: A Potential New Therapy for the Treatment of Asthma. Discovery and Structure–Activity Relationships

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Abstract—CCR3 antagonist leads with IC_{50} values in the μM range were converted into low nM binding compounds that displayed in vitro inhibition of human eosinophil chemotaxis induced by human eotaxin. In particular, 4-benzylpiperidin-1-yl-*n*-propylureas and *erythro*-3-(4-benzyl-2-(α-hydroxyalkyl)piperidin-1-yl)-*n*-propylureas (obtained via Beak reaction of *N*-BOC-4-benzylpiperidine) exhibited single digit nanomolar IC_{50} values for CCR3. © 2002 Published by Elsevier Science Ltd.

Asthma is an inflammatory disease of the lungs, which is characterized by the restriction of airflow and bronchial hyperresponsiveness. A hallmark of the disease is the selective localization of eosinophils to the lung tissue of allergic asthmatics.[1] Clinical studies have implicated eotaxin as the primary chemokine associated with the recruitment of eosinophils to the airways of asthmatic patients.[2,3] The eotaxin receptor has been identified as CC chemokine receptor 3 (CCR3)[4–6] and has been demonstrated to be the dominant functional chemokine receptor on eosinophils.[7] This growing body of clinical studies and animal models suggest that eotaxin and CCR3 play a primary role in the recruitment of eosinophils in allergic asthma and therefore, small molecule antagonists[8–15] of CCR3 may provide a novel mechanism of inhibiting this recruitment process.

High-throughput screening of the corporate compound library yielded several lead structures, including a series of indolinopiperidines which are summarized in Table 1.[16] The screening data indicated that the potency of the leads could be affected by increasing the alkyl chain linker (**1**–**3**) or by incorporating a carbonyl into the linker (**4**). With the identification of a site in which we could change the binding potency, we began our SAR investigation by replacing the carbonyl group with the amide, sulfonamide, and urea moieties since (1) they closely resembled a carbonyl group and (2) permitted the rapid synthesis of both linker and phenyl analogues.

Unfortunately, we were not able to increase potency over our lead compounds **1**–**4** (Table 2). However, we did learn that there are a few SAR trends. A four carbon chain linker was the best for amides (**8**–**10**) and sulfonamides (**11** and **12**). Adamantyl is better than cyclohexyl, which in turn is better than a *t*-butyl amide (**15** > **14** > **13**). Moving a methoxy group around the phenyl ring did not lead to a preferred position for enhanced binding (**16**–**18**). Keeping the optimal chain length (6 atoms) the same as for the butylamides and sulfonamides, the propylureas were investigated. Although a complete systematic SAR study was not done, the data suggested that unlike for amides **16**–**18**, there was preference for a H-bond acceptor in the 3-position on the phenylureas as evidenced by the contribution to binding affinity of the 3-cyano group **21**.

During the synthesis of the tetracycles, it was discovered in our group that a series of *N*-substituted 4-benzylpiperidines (not shown) also displayed micromolar binding affinities for the CCR3 receptor.[17] We hypothesized that the 4-(*N*-phenylamino)-piperidine portion of the

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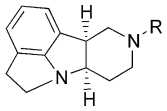
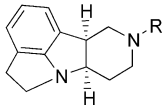
Table 1. Binding affinities of CCR3 leads obtained from screening of the corporate compound library: all compounds are racemic



Compd	R	% Inh.[a]	IC_{50} (μM)
1	$(CH_2)_1$-Ph-4-F	15	—
2	$(CH_2)_2$-Ph-4-F	50	—
3	$(CH_2)_3$-Ph-4-F	100	0.5±0.8
4	$(CH_2)_3$-CO-Ph-4-F		1.1±0.3

[a]Percent inhibitions are at 10 μM substrate.

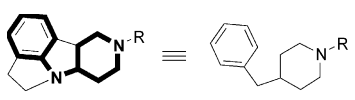
Table 2. Binding affinities of indolinopiperidinylalkylureas and amides: all compounds are racemic



Compd	R	% Inh.[a]	IC_{50} (μM)
5	$(CH_2)_2$-NH_2	15	—
6	$(CH_2)_3$-NH_2	14	—
7	$(CH_2)_4$-NH_2	5	—
8	$(CH_2)_2$-NHCO-Ph-4-F		8.3±1.9
9	$(CH_2)_3$-NHCO-Ph-4-F		5.5±1.5
10	$(CH_2)_4$-NHCO-Ph-4-F		3.9±1.8
11	$(CH_2)_3$-$NHSO_2$-Ph-4-F		10.1±0.1
12	$(CH_2)_4$-$NHSO_2$-Ph-4-F		6.4±6.2
13	$(CH_2)_4$-NHCO-t-butyl	45	25.6±2.9
14	$(CH_2)_4$-NHCO-cyclohexyl	45	11.0±0.0
15	$(CH_2)_4$-NHCO-adamant-1-yl	73	6.6±2.1
16	$(CH_2)_4$-NHCO-Ph-2-OMe		6.8±0.4
17	$(CH_2)_4$-NHCO-Ph-3-OMe		8.1±1.2
18	$(CH_2)_4$-NHCO-Ph-4-OMe		5.1±0.7
19	$(CH_2)_3$-NHCONH-Ph-4-NMe_2		4.8±3.1
20	$(CH_2)_3$-NHCONH-Ph-4-CF_3		3.7±2.1
21	$(CH_2)_3$-NHCONH-Ph-3-CN		0.7±0.1
23	$(CH_2)_3$-NHCONH-Ph-3-I		1.1±0.5
24	$(CH_2)_3$-NHCONH-Ph-4-I		1.5±0.4

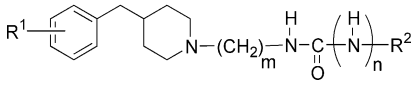
[a]Percent inhibitions are at 10 μM substrate.

tetracycle was mimicking a 4-benzylpiperidine as shown below. We also hypothesized that perhaps the tetracyclic indolinopiperidine was too rigid to allow for an optimal binding conformation to the CCR3 receptor. Therefore, we replaced the indolinopiperidine with a 4-benzylpiperidine and these new analogues are summarized in Table 3.



From Table 3 we see that the benzylpiperidines were 3–7 times more potent than the indolinopiperidines (**10** vs **63**, **17** vs **61**, **19** vs **31**, **20** vs **30**, and **21** vs **27**). A chain length of 3 carbon atoms was optimal as exhibited by the 3-cyanophenylureas **26**–**29**. On the left-hand side of the molecule, the addition of a fluorine to the benzylpiperidine increased potency by 10-fold (**32** vs **24** and **35** vs **27**). Of all of the substituents investigated on the benzylpiperidine's phenyl, the 4-F and 4-Cl substituents were the most potent (**35** vs **56**–**59**). On the right-hand side of the molecule, the phenylureas were more potent than the alkylureas (**32** vs **33**, **34**, **64**, **65**). The acetyl group on the phenylurea preferred the 3- position to the 4-position (**43** vs **44**). The methylsulfide, sulfoxide, and sulfone also preferred the 3-position (**45** vs **48**, **46** vs **49**, and **47** vs **50**), although they are less potent than the acetyl group. The nitro and cyano groups showed a preference for the 3- and 4-positions on the phenylurea (**35**, **36**, and **40**–**42**). H-bond acceptor substituents such as CN, NO_2, Ac, and tetrazole at the 3- and 4-positions were generally more potent than more lipophillic substituents such as CF_3, methyl, methylthio, furan, and thiophene. Finally, the ureas were more potent than the corresponding amides (**60** vs **24**; and **61** vs **25**).

Table 3. Binding affinities 4-benzylpiperidinealkylureas and amides



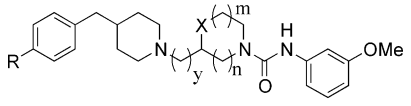
Compd	R^1	M	*n*	R^2	IC_{50} (μM)
24	H	3	1	Ph	0.4±0.1
25	H	3	1	3-MeO-Ph	0.3±0.1
26	H	2	1	3-CN-Ph	0.5±0.1
27	H	3	1	3-CN-Ph	0.2±0.1
28	H	4	1	3-CN-Ph	0.5±0.1
29	H	5	1	3-CN-Ph	1.2±0.4
30	H	3	1	4-CF_3-Ph	1.2±0.2
31	H	3	1	4-Me_2N-Ph	1.6±0.2
32	4-F	3	1	Ph	0.03±0.02
33	4-F	3	1	-CH_2Ph	0.4±0.02
34	4-F	3	1	-CH_2CH_2Ph	0.3±0.1
35	4-F	3	1	3-CN-Ph	0.02±0.01
36	4-F	3	1	4-CN-Ph	0.02±0.004
37	4-F	3	1	2-CF_3-Ph	0.2±0.1
38	4-F	3	1	3-CF_3-Ph	0.07±0.01
39	4-F	3	1	4-CF_3-Ph	0.06±0.002
40	4-F	3	1	2-NO_2-Ph	0.089±0.002
41	4-F	3	1	3-NO_2-Ph	0.009±0.002
42	4-F	3	1	4-NO_2-Ph	0.007±0.002
43	4-F	3	1	3-Ac-Ph	0.01±0.004
44	4-F	3	1	4-Ac-Ph	0.2±0.1
45	4-F	3	1	3-MeS-Ph	0.02±0.01
46	4-F	3	1	3-MeSO-Ph	0.05±0.0
47	4-F	3	1	3-$MeSO_2$-Ph	0.02±0.0005
48	4-F	3	1	4-MeS-Ph	0.04±0.03
49	4-F	3	1	4-MeSO-Ph	0.2±0.05
50	4-F	3	1	4-$MeSO_2$-Ph	0.07±0.03
51	4-F	3	1	3-MeO-Ph	0.03±0.01
52	4-F	3	1	3-(Furan-2-yl)-Ph	0.1±0.01
53	4-F	3	1	3-(Thiophen-2-yl)-Ph	0.2±0.05
54	4-F	3	1	3-(Imidazol-2-yl)-Ph	0.1±0.03
55	4-F	3	1	3-(1-Me-tetrazol-5-yl)-Ph	0.005±0.003
56	2-F	3	1	3-CN-Ph	0.2±0.1
57	4-Cl	3	1	3-CN-Ph	0.02±0.01
58	4-CF3	3	1	3-CN-Ph	52% @ 5 μM
59	4-Me	3	1	3-CN-Ph	0.5±0.3
60	H	4	0	Ph	0.8±0.3
61	H	4	0	3-MeO-Ph	1.2±0.5
62	H	4	0	4-CN-Ph	1.3±0.8
63	H	4	0	4-F-Ph	1.0±0.8
64	4-F	3	1	Adamant-1-yl	0.09±0.02
65	4-F	3	1	Cyclohexyl	0.1±0.05

Concurrent with the alkyl linker efforts, a series of heterocyclic linkers was investigated (Table 4). We

hypothesized that a more rigidified linker would provide compounds with improved potency for CCR3. The SAR efforts utilized the 3-methoxyphenylurea for direct comparison to the alkyl linker compounds. The 4-piperidine **66** and 4-methylpiperidine **67** were less potent than the alkyl linker **25**. However, the 3-methylpiperidine linker **68** was equipotent with **25**. Further optimization of the heterocyclic linker was carried out with 4-fluorobenzylpiperidine because it proved to be about 6-fold more potent than the non-fluorinated analogue (**69** vs **68**). Optimization first consisted of shortening **70** and lengthening **71** the spacer of the 3-substituted piperidine linker, which reduced the potency of the compounds by about 5- to 10-fold. The linker was moved to the 2-postion of the piperidine **72** and again the potency was reduced by about 3-fold. Contraction of the 3-piperidine ring to a pyrrolidine ring **73** reduced the potency by 5-fold, making it clear that the 3-substituted methylpiperidine ring was the optimal arrangement for the linker. Incorporation of an O or N heteroatom into the piperidine ring to form a morpholine **74** or piperazine **75** and **76** increased the potency only marginally over the piperidine analogue. The morpholine linker was synthesized enantiomerically pure by the procedures of Brown et al.[18] yielding **77** and **78** proving that the *R*-stereoisomer was the most potent. All of the heterocyclic-linked compounds exhibited similar, but not improved, potencies to the alkyl linkers.

The heterocyclic linker compounds were utilized to characterize our compounds for their functional response at CCR3 by using an eotaxin induced Ca^{2+} mobilization assay.[19] Several compounds (Table 4) inhibited eotaxin induced Ca^{2+} mobilization with IC_{50}s between 139 and 822 nM. The compounds by themselves did not produce a functional response in the Ca^{2+} mobilization assay at concentrations up to 10 μM (results not shown). These experiments indicate that this series of molecules is acting as functional antagonists of CCR3.

Table 4. Structure–activity relationships for *p*-fluorobenzylpiperidine heterocyclic CCR3 antagonists



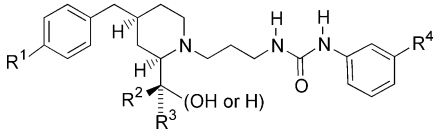
Compd	Ent.	R	X	m	*n*	y	CCR3 IC_{50} (μM)[a]	Ca^{2+} IC_{50} (μM)[a]
66		H	CH_2	0	2	0	2.3	
67		H	CH_2	0	2	1	1.6	
68	(±)	H	CH_2	1	1	1	0.33±0.05	
69	(±)	F	CH_2	1	1	1	0.05±0.01	0.82±0.08
70	(±)	F	CH_2	1	1	0	0.53±0.04	
71	(±)	F	CH_2	1	1	2	0.25±0.01	
72	(±)	F	CH_2	2	0	1	0.17±0.03	
73	(±)	F	CH_2	0	1	1	0.24±0.03	
74	(±)	F	O	1	1	1	0.05±0.01	0.22±0.03
75	(±)	F	NBOC	1	1	1	0.04±0.01	0.20±0.03
76	(±)	F	NH	1	1	1	0.03±0.01	0.14±0.02
77	*R*	F	O	1	1	1	0.03±0.01	0.19±0.03
78	*S*	F	O	1	1	1	0.26±0.06	

[a]Values are means of $n \geq 2$.

With knowledge that piperidine ring conformation and substitution pattern can affect binding of compounds to 7-transmembrane receptors, we began an investigation using the Beak reaction to substitute at the piperidine 2-position.[20] These compounds are summarized in Table 5. Allyl bromide alkylation of *N*-Boc-4-benzylpiperidine led to both *cis* and *trans* compounds **79** and **80**, both sharing the same binding affinity. Alkylation using benzyl bromide led to only the *cis* compound **81**, also with moderate binding affinity. Quenching the *N*-Boc-piperidinyl anion with aldehydes yielded only *cis* alkylated products with a 1:1 *erythro*/*threo* mixture at the alcohol carbon. The *erythro* diastereomer was always more potent (**85** vs **86**). The presence of the newly introduced OH group increased binding affinity 10-fold (**85** vs **79**). The *erythro* isomer can be separated via chiral HPLC into its enantiomers, one being more potent than the other, such as with **87a** versus **87b** and **93a** versus **93b**. The longer the alkyl chain, the more potent the binding. Branching in the alkyl side chain lowered binding affinity (**89**, **91**, and **92**). A pentanol side chain yielded compounds with binding affinities in the 1 nM range (**93b** and **94**). Elimination of the chiral center via oxidation[21] of alcohol **82** to ketone **83** or via gem disubstitution (**84**: via Beak reaction with acetone) lowered affinity. A fluoro-substituent in this series (**95**) did not increase affinity as previously observed.

Finally, a representative set of compounds was characterized for their ability to block the in vitro functional

Table 5. Binding affinities for the 'Beak' alkylated benzylpiperidine analogues: compounds are racemic unless otherwise specified



Compd	R^1	R^2	R^3	OH or H	R^4	IC_{50} (μM)[a]
79	H	Et	H	H	Ac	0.1±0.02
80	H	Et (*trans*)	H	H	Ac	0.2±0.01
81	H	Ph	H	H	Ac	0.2±0.1
82[c]	H	Me	H	OH	Ac	0.02±0.01
83	H	Me	OH	OH	Ac	0.05±0.02
84	H	Me	Me	OH	Ac	0.2±0.05
85	H	Et	H	OH	Ac	0.006±0.003
86	H	H	Et	OH	Ac	0.05±0.01
87a[b]	H	Et	H	OH	Ac	40%
87b[b]	H	Et	H	OH	Ac	0.008±0.003
88	H	*i*-Pr	H	OH	Ac	0.05±0.01
89	H	H	*i*-Pr	OH	Ac	0.1±0.08
90[c]	H	*n*-Pr	H	OH	Ac	0.007±0.002
91	H	*i*-Bu	H	OH	Ac	0.02±0.01
92	H	H	*i*-Bu	OH	Ac	0.06±0.004
93a[b]	H	*n*-Bu	H	OH	Ac	0.08±0.002
93b[b]	H	*n*-Bu	H	OH	Ac	0.001±0.0005
94[b]	H	*n*-Bu	H	OH	Tet[d]	0.001±0.0007
95	F	*n*-Bu	H	OH	Ac	0.005±0.002

[a]Percent inhibitions are at 0.5 μM substrate.
[b]Compounds **87a** and **87b**, **93a** and **93b** are enantiomers of one another and **94** is a single enantiomer, all of which have the above assigned relative stereochemistry, but whose absolute configuration is unknown.
[c]Compounds **82** and **90** are a mixture of *erythro*/*threo* diastereomers.
[d]Tet = 1-methyltetrazol-5-yl.

Table 6. Binding affinities and human eosinophil chemotaxis inhibition data

Compd	Binding IC_{50} μM	Chemotaxis IC_{50} μM
35	0.02±0.01	0.08±0.05
57	0.02±0.01	0.2±0.15
87b	0.008±0.003	0.02

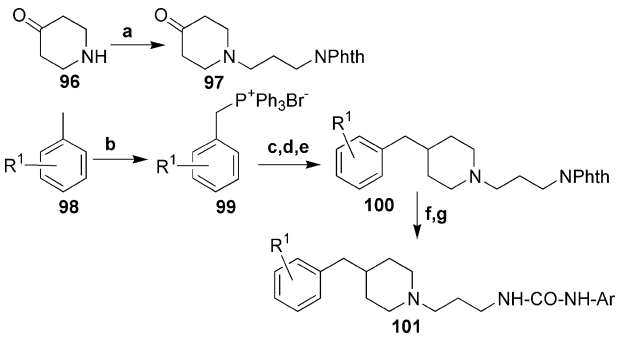
activity of CCR3 by using an eotaxin induced human eosinophil chemotaxis assay (Table 6).[22] These compounds proved to be potent inhibitors of chemotaxis and the potency correlated to the binding assay results. The compounds by themselves did not cause chemotaxis and thus indicates these molecules are functional antagonists of CCR3.

The synthesis of the piperidines, including the tetracyclic indolino-piperidines, was accomplished by alkylating with *N*-(bromoalkyl)-phthalimide in the presence of K_2CO_3, KI and refluxing MEK. Subsequent hydrazinolysis followed by reaction of the amine with the appropriate isocyanate or phenylcarbamate yielded the desired ureas. Tetracyclic indolino-piperidines were synthesized via a Fisher–Indole reaction employing *N*-aminoindoline and 4-piperidone hydrate hydrochloride in refluxing IPA. Reduction of the indole double bond with sodium borohydride pellets (Caution! Use pellets) in TFA yielded the tetracycle, which was alkylated as described above.

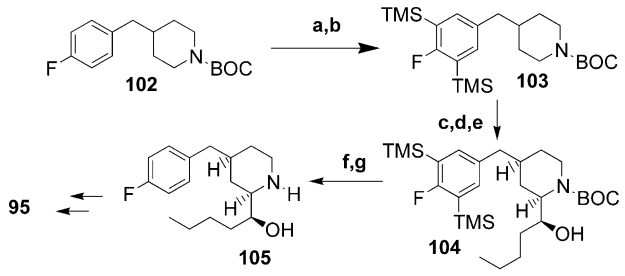
Substituted benzylpiperidines **56–59** were synthesized via Wittig reaction with *N*-(phthalimidopropyl)-4-piperidone **97** as shown in Scheme 1. Compounds **52** and **53** were made via Stille[23] coupling of 2-tributyltinfuran/thiophene to 1-iodo-3-nitrobenzene. Imidazole **54** was made via reaction of 3-nitro-benzaldehyde with glyoxal and ammonia.[24] Tetrazole **55** was synthesized via the method of Thomas[25] (solvent–AcCN) from *N*-methyl-3-nitrobenzamide, triflic anhydride and NaN_3. The synthesis of compound **95** (Scheme 2) exemplifies the use of the Beak reaction which was employed to make all of the compounds in Table 5. TMS protection was necessitated in the case of **95** due to competing metallation *ortho* to the fluorine.

Exploration of the heterocyclic benzylpiperidines was facilitated by the synthesis outlined in Scheme 3. The heterocyclic aldehydes were synthesized from their commercially available precursor alcohols or, in the case of morpholine analogues, according to literature procedures.[18] The alcohols were reacted with di-*t*-butyl dicarbonate in CH_2Cl_2 and then oxidized with tetrapropylammoniumperruthenate and *N*-methyl-morpholine oxide in CH_2Cl_2 to produce the carbonyl compounds **106**. The oxidation products **106** were reductively alkylated with 4-benzylpiperidine and $NaBH(OAc)_3$ in $ClCH_2CH_2Cl$ to yield the amines **107**. Removal of the amine protecting group with HCl in dioxane and then treatment with triethylamine and the desired isocyanate afforded the final products.

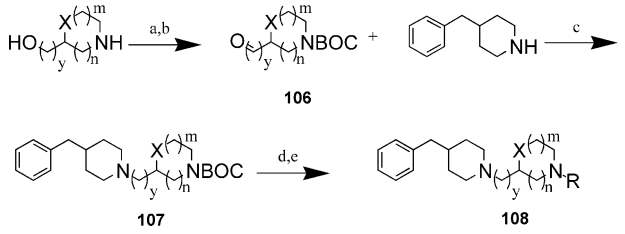
Figure 1 summarizes what is required for high affinity binding to the CCR3 receptor: (1) a chain length of 6



Scheme 1. (a) *N*-(3-bromopropyl)phthalimide, DBU, DMF, 100 °C; (b) NBS, AIBN, CCl_4, reflux; (c) *n*-BuLi, THF, −78 °C; (d) **90**; (e) H_2, 10% Pd/c, MeOH; (f) N_2H_4, EtOH, reflux; (g) ArNCO, THF, rt.



Scheme 2. (a) 10 equiv LDA, benzene, 0 °C; (b) 10 equiv TMSCl, THF, −70 °C, 65%; (c) *s*-BuLi, TMEDA, −78 to −30 °C; (d) RCHO, warm to 0 °C, 18%; (e) H_2O; (f) NaOH, EtOH, reflux; (g) CsF, DMF, H_2O, 79%.



Scheme 3. (a) $(BOC)_2O$, CH_2Cl_2, rt, 1 h, 95%; (b) TPAP, NMO, CH_2Cl_2, 3 Å MS, 1 h, 70–90%; (c) $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, 50–85%; (d) 4 M HCl, dioxane, 99%; (e) acyl chlorides, sulfonyl chlorides, alkyl bromides, or isocyanates, TEA, CH_2Cl_2, 5 h, 70–90%.

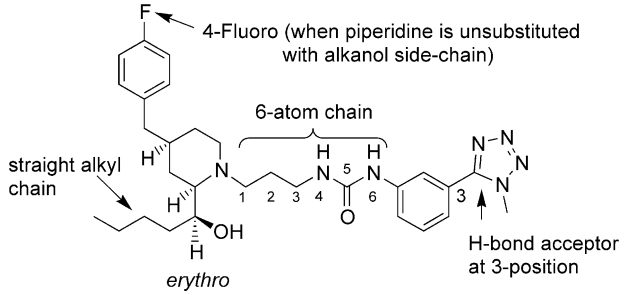


Figure 1. Structural requirements for high affinity binding to CCR3.

atoms between the piperidine and the phenyl, (2) a H-bond accepting substituent such as a tetrazole, acetyl, cyano, etc. at the 3-position of the phenyl, (3) a fluorine or chlorine at the 4-position on the benzyl group, or (4) an optional *erythro*-pentanol side chain at the piperidine 2-position. Thus we have taken micromolar binding

leads and through structure–activity relationships managed to increase affinity to the single digit nanomolar range. These binding affinities also translate into a pharmacological effect, namely the in vitro inhibition of human eosinophil chemotaxis or eotaxin induced Ca^{2+} mobilization. It remains to be seen whether in human clinical trials, compounds of this class will alleviate the symptoms of asthma via the in vivo inhibition of eosinophil chemotaxis.

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References and Notes

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