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Bioorganic & Medicinal Chemistry Letters
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Bioorganic & Medicinal Chemistry Letters 14 (2004) 1619–1624

Discovery and SAR of trisubstituted thiazolidinones as CCR4 antagonists

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Received 25 November 2003; revised 23 January 2004; accepted 23 January 2004

Abstract—Substituted thiazolidinones were identified as CCR4 antagonists from high throughput screening. Subsequent lead optimization efforts resulted in defined structure–activity relationships and the identification of potent antagonists (compounds **90** and **91**) that inhibited the chemotaxis of Th2 T-cells in vitro.
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Allergic inflammation diseases, such as asthma, atopic dermatitis and allergic rhinitis are increasing in prevalence in the world and represent significant health care expenses. Asthma affects greater than 15 million people in the US[1] and although less serious, approximately 15 million people are affected by atopic dermatitis and allergic rhinitis.[2] Current treatments for allergic inflammation include antihistamines and bronchodilators, which control symptoms but not disease progression, in addition to corticosteroids which specifically target the disease but present certain safety concerns. There is a need for novel therapies that provide safe and effective treatments for these diseases.

CCR4 belongs to a family of CC chemokine receptors which act through G-protein-coupled receptors with a characteristic seven-transmembrane structure. CCR4 is selectively expressed on Th2-type $CD4^+$ T-cells, which play a pivotal role in driving the allergic inflammation response. There are two ligands which bind CCR4 exclusively[3] with high affinity: Macrophage Derived Chemokine (MDC; MW = 8081 Daltons; $K_d = 120$ pM) and Thymus and Activation Regulated Chemokine (TARC; MW = 8083 Daltons; $K_d = 400$ pM). It has been demonstrated that antagonism of MDC or TARC can reduce the migration of T-cells into sites of inflammation, positing that CCR4 antagonists may be effective therapeutics for the treatment of allergic inflammation.[4] This report focuses on our efforts to discover novel, selective, small molecule CCR4 antagonists and subsequent validation that such antagonists can halt T-cell migration.

The use of small heterocyclic rings has become very important to medicinal chemists since they act as rigid cores which can be readily prepared and functionalized to serve as peptidomimetics and thus exhibit wide ranging biological activity. Thiazolidinones exhibit interesting biological activity profile as anti-inflammatories,[5] anti-bacterials[6] and anti-histamines.[7] As such, many synthetic strategies for thiazolidinones have been described, most employing a one-pot three-component condensation, and these have gained utility in combinatorial and parallel synthesis.[8]

Screening of a thiazolidinone-based diversity library against a MDC-CCR4 binding assay[9] resulted in a number of structurally related hits with IC_{50}'s determined to be in the low micromolar range. Initial SAR profiling quickly led to compound **1**, a mixture of 2-aryl-thiazolidinone diastereomers containing a terminal morpholine and naphthalene group ($IC_{50} = 2.4$ μM; see Fig. 1). Resynthesis, purification and separation of the diastereomeric mixture led to the identification of the

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doi:10.1016/j.bmcl.2004.01.072

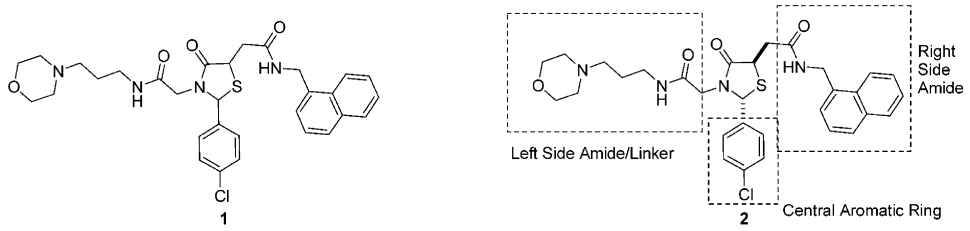


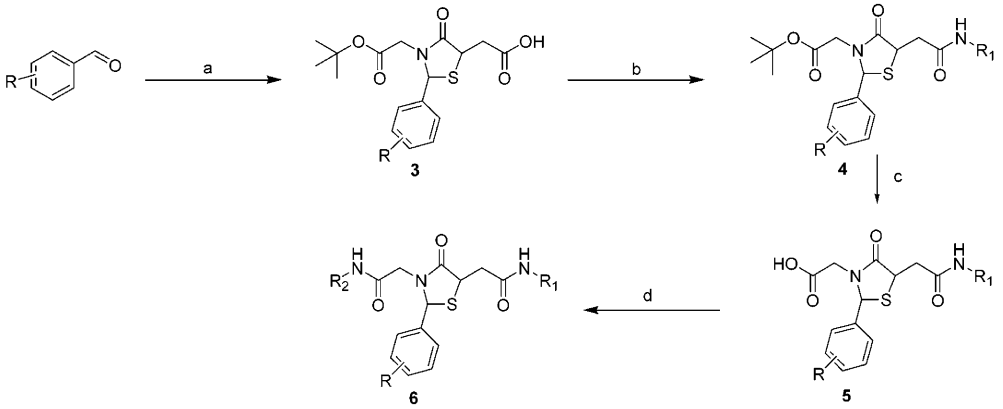
Figure 1. CCR4 antagonist screening hit.

more potent *trans* diastereomer, **2** ($IC_{50} = 0.8$ μM). NMR studies confirmed the *trans* configuration for the active diastereomer[10] and the corresponding *cis* diastereomer was found to be much less potent ($IC_{50} \sim 6$ μM). Although the molecular weight (595) and calculated lipophilicity (clogP 4.8) were high and the molecule contained three amide linkages, it was decided to explore the SAR against CCR4 antagonism within this series with the goal of demonstrating functional inhibition of T-cell migration. Indeed, many functionally active chemokine receptor antagonists have recently appeared in the literature with similar profiles.[11] Accordingly, the lead compound **2** was divided into three distinct areas for subsequent SAR investigation; the central angular aryl ring, the right side amide and the left-side tethered amide.

A convergent synthetic route to access thiazolidinones was identified (Scheme 1). A one-pot three component reaction involving imine formation between glycine *t*-butyl ester and substituted benzaldehydes followed by treatment with mercaptosuccinic acid provided rapid access to differentially protected thiazolidinones **3** as a mix of diastereomers (cis/trans ~2:1). Subsequent CDI-mediated amide coupling afforded right-side amide **4**, which, after deprotection, facilitated a left-side amide coupling and diastereomer separation to produce the fully functionalized thiazolidinones, **6**. The minor *trans* diastereomers were consistently 2-5 times more potent than their *cis* counterparts and while stereoselective thiazolidin-4-one synthesis has been reported,[12] this was not pursued because of the ease of separation of diastereomers.

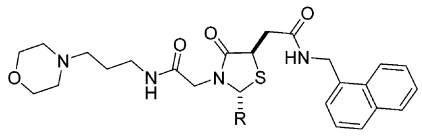
The SAR of the central aryl ring was explored first (Table 1) and, in general, reasonable receptor affinity at this position was only obtained with substituted benzenes (compounds **7**–**21**). Substitution was poorly tolerated at the 2- and 3-positions, somewhat preferred at the 4-position and was optimal when disubstituted at the 2- and 4-positions (compound **16**). All other substitution patterns that were explored returned reduced binding potency. The nature of the substituent was also important. Small electron withdrawing groups were generally well tolerated, particularly halogens, whereas introduction of an electron donating group led to much lower potencies even with the preferred substitution pattern (compound **16**, **19** vs **17**, **20**). Incorporation of heterocycles (compounds **22**–**26**) was detrimental to affinity while removal of aromaticity (compound **29**) or homologation (compounds **27**, **28**) led to a complete loss of binding.

The SAR around the left side tethered amine portion of analogue **16** was then explored (Table 2). Replacement of the glycine linker was attempted in the hope of reducing the peptidic nature of the molecule and imparting more drug-like characteristics, but this was not successful. Direct attachment of the basic amine through an all carbon chain (compounds **30**–**34**) led to decreased potency while introduction of a sulfonamide (compound **35**) or relocation of the amide (compounds **36**–**38**) gave similar results. Direct replacement of the amide with a phenyl ring resulted in complete loss of activity (compound **39**), suggesting that the amide is participating in a required hydrogen-bonding interaction, although the *N*-methyl amide (compound **40**) indicates the



Scheme 1. Reagents: (a) Glycine Ot-Bu, toluene, reflux, then mercaptosuccinic acid, Dean–Stark (70–80%); (b) CDI, CH_2Cl_2, then R_1NH_2, rt (50–80%); (c) TFA, CH_2Cl_2 (100%); (d) CDI, CH_2Cl_2, then R_2NH_2, rt (23–50%).

Table 1. The effect of central aromatic ring variations



Compd	R[a]	IC_{50} (μM)[b]
1	4-Cl-Phenyl	0.83
7	4-F-Phenyl	4.0
8	2-Cl-Phenyl[c]	11
9	3-Cl-Phenyl[c]	7.0
10	3-CF_3-Phenyl[c]	12
11	3,4-di-Cl-Phenyl[c]	>25
12	3,5-di-Cl-Phenyl	9.0
13	2,3-di-Cl-Phenyl	18
14	2,5-di-Cl-Phenyl	12
15	2,6-di-Cl-Phenyl	8.0
16	2,4-di-Cl-Phenyl	0.69
17	2,4-di-MeO-Phenyl	33
18	2,4-di-CF_3-Phenyl	>100
19	2,4-di-F-Phenyl	1.0
20	2,4-di-Me-Phenyl	11
21	2-Cl, 4-F-Phenyl	1.0
22	2-Thienyl	33
23	3-Thienyl	66
24	2-Furyl	29
25	3-Furyl	100
26	4-Pyridyl[c]	34
27	Benzyl[c]	>100
28	Phenethyl[c]	100
29	Cyclohexyl[c]	100

[a] Compounds are racemic *trans* diastereomers unless otherwise labelled.
[b] Inhibition of binding of ^{125}I-MDC to L1.2 engineered murine pre-B cells.
[c] Racemic mixture of diastereomers.

importance of an acceptor. Truncation of the amine tether via cyclic amines was attempted to reduce molecular weight and rotatable bonds[13] (compounds **41**–**43**) with limited success. A study of linker length to the basic amine was performed (compounds **44**–**46**) and the optimal chain length was determined to be 3–4 carbon atoms. The nature of the basic amine was also examined (compounds **47**–**50**) and superior binding potencies were obtained when more basic amines were employed, particularly piperidine (compound **49**, 0.20 μM) which also demonstrated enhanced in vitro PK properties (data not shown) and was selected as the preferred basic amine in later studies.

Finally, the SAR of the right side amide portion was explored (Table 3). The naphthalene moiety can be highly susceptible to metabolism through ring oxidation,[14] therefore efforts were focused on replacing this group. Non-aromatic functionalities (compounds **53**–**55**) were not tolerated at this position, neither were heteroaromatic groups (compounds **56**–**59**). A substituted benzyl amide was essential for binding potency (compounds **60**–**80**) and, while 3-substituents were preferred (compounds **62**–**65**) and 2-substituents were not tolerated well in the monosubstituted series (compounds **60**, **61**), a cumulative effect was observed with 2,3-disubstitution (compounds **73**–**76**). Perhaps the 2,3 substitution mimics the naphthalene unit. Biphenyls or benzhydryl moieties did not improve receptor affinity (compounds **78**–**81**). Other analogues of interest included indole (compound **82**) and benzothiophene (compound **85**), which were shown to be reasonable naphthalene replacements. Both *N*-methylation and replacement of the amide were not tolerated (data not shown), emphasizing the importance of this pharmacophoric element similar to the results for the left side amide.

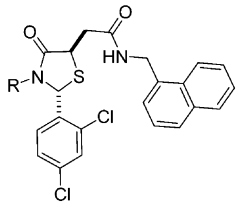
Introduction of a third chiral center via α-methyl substituted benzylamines and naphthylamines (compounds **86**–**89**) demonstrated the importance of stereochemistry within this series. In both cases, the diastereomers derived from coupling with the (*R*)-isomer of the amine demonstrated significantly higher affinity for the receptor than the (*S*)-counterpart. Subsequently, compound **89** was separated by HPLC to afford small quantities of each enantiomerically pure diastereomer in order to verify the importance of enantiopurity in CCR4 binding. While the binding potency of the active enantiomer (0.25 μM) was comparable to **89**, the other enantiomer exhibited a much lower binding potency (8 μM).[15]

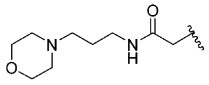
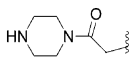
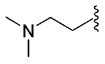
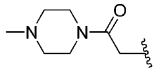
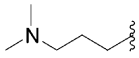
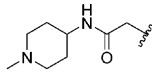
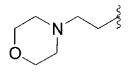
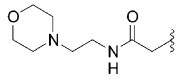
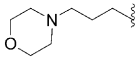
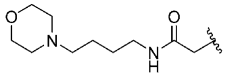
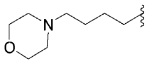
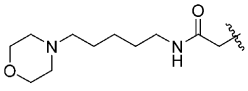
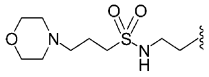
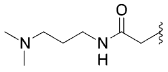
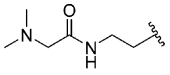
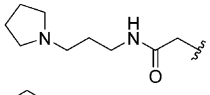
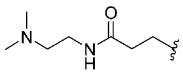
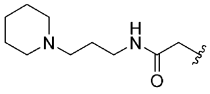
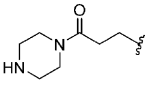
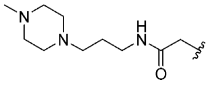
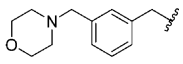
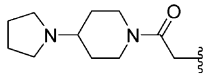
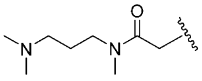
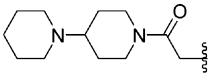
Ultimately, the optimal groups identified in Tables 1–3 were combined to produce several compounds with excellent CCR4 binding affinities (see Fig. 2). Compounds **90** (IC_{50} = 0.11 μM) and **91** (IC_{50} = 0.14 μM) both incorporate the 2, 4-dichlorophenyl ring in the central position along with the terminal piperidine on the left side. The α-methyl naphthyl and 3-chloro-2-methyl-phenyl moieties were preferred on the right side. These compounds were selected for evaluation in a functional chemotaxis assay.

Using a transwell chemotaxis system[16] involving a transfected murine cell line expressing human CCR4, these compounds demonstrated dose-dependent, potent inhibition of MDC-stimulated chemotaxis (**90**, EC_{50} = 0.6 μM; **91**, EC_{50} = 2.0 μM). Although these compounds have similar potencies in the binding assay, the threefold difference in their EC_{50} values may reflect the stability and/or solubility of these compounds in different whole cell systems (chemotaxis assay conditions: 4 h at 37 °C with 0.1% final DMSO concentration; binding assay conditions: 1 h at 16 °C with 1% final DMSO concentration). Similar functional results were obtained with $CCR4^+$ Th2 cell lines derived from primary human T-cells (data not shown) and with the use of TARC instead of MDC (i.e., compound **90**, TARC-stimulated ED_{50} = 0.3 μM). Finally, these compounds do not directly promote chemotaxis of $CCR4^+$ cells or enhance random chemokinesis and are, therefore, excluded as being agonists at this receptor.

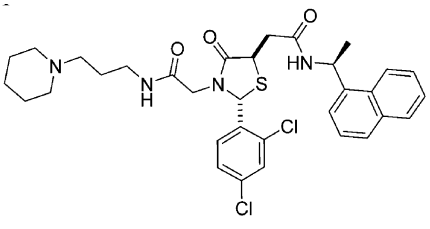
In summary, we have discovered a series of trisubstituted thiazolidinones as novel antagonists of CCR4. Optimization of library hit **1** led to inhibitors with potency in the 100–200 nM range. Important pharmacophoric elements were defined, including requirements for a glycine amide linked via 3–4 carbons to a cyclic tertiary amine, preferably a piperidine. A central 2,4-disubstituted phenyl group is optimal for binding while the original naphthyl amide was replaced by isosteric

Table 2. The effect of left side variations



Compd[a]	R	IC_{50} (μM)[b]	Compd[a]	R	IC_{50} (μM)[b]
16		0.67	**41**		1.4
30		36	**42**		20
31[c]		5.6	**43**		8
32[c]		9	**44**		3.5
33[c]		33	**45**		0.38
34[c]		18	**46**		2.4
35		24	**47**		0.36
36		11	**48**		0.31
37		9	**49**		0.20
38[c]		3.5	**50**		0.57
39[c]		100	**51**		0.60
40		0.36	**52**		0.33

[a] Compounds are racemic *trans* diastereomers unless otherwise labeled.
[b] Inhibition of binding of ^{125}I-MDC to L1.2 engineered murine pre B cells.
[c] Racemic mixture of diastereomers.



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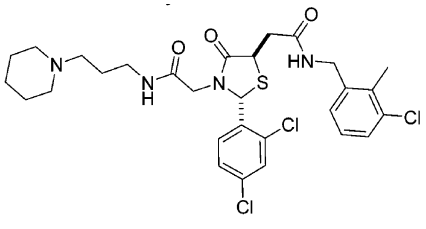

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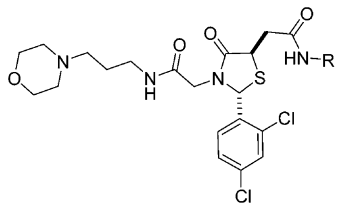
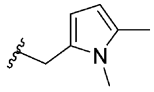
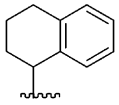
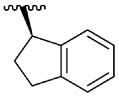
Figure 2. Potent, functionally active CCR4 antagonists.

Table 3. The effect of various right side amides



Compd	R	IC_{50} (μM)[a]	Compd	R	IC_{50} (μM)[a]
16	CH_2(2-naphthyl)	0.67	**71**	CH_2(2,5-di-Cl-Phenyl)	4
53	$CH_2CH_2C(CH_3)_3$	21	**72**	CH_2(2,4-di-Cl-Phenyl)	3
54	$CH_2CH_2CH(CH_3)_2$	19	**73**	CH_2(2,3-di-Cl-Phenyl)	0.79
55	Cyclohexyl	7	**74**	CH_2(2,3-di-Me-Phenyl)	0.44
56	CH_2(2-Furan)	7	**75**	CH_2(2-Me, 3-Cl-Phenyl)	0.40
57	2-Thiazole	11	**76**	CH_2(2-F, 3-CF_3-Phenyl)	2.5
58	CH_2(2-Thiophene)	11	**77**	CH_2CH_2(3,4-di-Cl-Phenyl)	13
59		2.5	**78**	CH_2(2-Ph-Phenyl)	6
60	CH_2(2-CH_3-Phenyl)	11	**79**	CH_2(3-Ph-Phenyl)	1.3
61	CH_2(2-Cl-Phenyl)	10	**80**	CH_2(4-Ph-Phenyl)	2.5
62	CH_2(3-Me-Phenyl)	2.3	**81**	$CHPh_2$	6
63	CH_2(3-Cl-Phenyl)	0.93	**82**	CH_2(5-indole)	1
64	CH_2(3-F-Phenyl)	1.5	**83**		6.7
65	CH_2(3-CF_3-Phenyl)	1	**84**		2.9
66	CH_2(2,4-di-Cl-Phenyl)	2	**85**	CH_2(3-benzo[*b*]thiophene)	0.34
67	CH_2(2,4-di-OMe-Phenyl)	32	**86**	*S*-CH(Me)-1-Naphthalene	8
68	CH_2(3-CF_3-4-Cl-Phenyl)	6	**87**	*R*-CH(Me)-1-Naphthalene	0.25
69	CH_2(2,3,4-tri-OMe-Ph)	11	**88**	*S*-CH(Me)-Phenyl	12
70	CH_2(3,5-di-Cl-Phenyl)	1.5	**89**	*R*-CH(Me)-Phenyl	0.25

[a] Inhibition of binding of ^{125}I-MDC to L1.2 engineered murine pre B cells.

2,3-disubstituted phenyl groups with improved potency. Incorporation of a third chiral center facilitated testing of enantiomerically pure analogues and highlighted the importance of stereochemistry and enantiopurity. The activity of these analogues in cell-based functional chemotaxis assays demonstrates that CCR4 binding is related to chemotaxis and supports the hypothesis that CCR4 antagonists may be effective therapeutics for the treatment of allergic inflammation.

References and notes

1. Barnes, P. J.; Jonsson, B.; Klim, J. B. *Eur. Respir. J.* **1996**, *9*, 636.
2. Beasley, R. *Lancet* **1998**, *351*, 1225.
3. Chvatchko, Y.; Hoogewerf, A. J.; Meyer, A.; Alouani, S.; Juillard, P.; Buser, R.; Conquet, F.; Proudfoot, A. E.; Wells, T. N.; Power, C. A. *J. Exp. Med.* **2000**, *191*, 1755.
4. (a) Godiska, R.; Chantry, D.; Raport, C. J.; Sozzani, S.; Allavena, P.; Leviten, D.; Mantovani, A.; Gray, P. W. *J. Exp. Med.* **1997**, *185*, 1595. (b) Chantry, D.; DeMaggio, A. J.; Brammer, H.; Raport, C. J.; Wood, C. L.; Schweickart, V. L.; Epp, A.; Smith, A.; Stine, J. T.; Walton, K.; Tjoelker, L.; Godiska, R.; Gray, P. W. *J. Leukoc. Biol.* **1998**, *64*, 49. (c) Imai, T.; Chantry, D.; Raport, C. J.; Wood, C. L.; Nishimura, M.; Godiska, R.; Yoshie, O.; Gray, P. W. *J. Biol. Chem.* **1998**, *273*, 1764. (d) Wakugawa, M.; Nakamura, K.; Kakinuma, T.; Tamaki, K. *Drug News Perspect.* **2002**, *15*, 175.
5. Look, G. C.; Schullek, J. R.; Holmes, C. P.; Chinn, J. P.; Gordon, E. M.; Gallop, M. A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 707.
6. Küçükgüzel, S. G.; Oruç, E. E.; Rollas, S.; Şahin, F.; Özbek, A. *Eur. J. Med. Chem.* **2002**, *37*, 197.
7. Diurno, M. V.; Mazzoni, O.; Piscopo, E.; Calignano, A.; Giordano, F.; Bolognese, A. *J. Med. Chem.* **1992**, *35*, 2910.
8. (a) Holmes, C.P.; Chinn, J. P.; Look, G. C.; Gordon, E. M. ; Gallop, M. A. *J. Org. Chem.* **1995**, *60*, 7328. (b) Holmes, C.P. WO 96/00148. (c) Srivastava, T.; Haq, W.; Katti, S. B. *Tetrahedron* **2002**, *58*, 7619. (d) Munson, M. C.; Cook, A. W.; Josey, J. A.; Rao, C. *Tetrahedron Lett.* **1998**, *39*, 7223.
9. Assay measures inhibition of binding of ^{125}I-MDC (assay concentration: 100 pM) to murine pre-B cell line L1.2 over-expressing CCR4 [see reference 3(c)]. IC_{50} values are

shown as the mean of duplicate determinations and possess a standard error of $<20\%$.

10. Stereochemistry across the 1-thia-3-azolidin-4-ones at locations 2,5 was determined via nuclear Overhauser experiments. These experiments took the form of a Double Pulsed Field Gradient Spin Echo-Nuclear Overhauser Enhancement (DPFGSE-NOE) pulse sequence (See Stott, K.; Keeler, J.; Vand, Q. N.; Shaka, A. J. *J. Magn. Reson.* **1997**, *125*, 302). Thus, utilizing a Varian 500 MHz Unity Inova System (Palo Alto, CA) equipped with linear amplifiers, Z-axis gradients and a waveform generator on channel 1, selective excitation of the methine at C-2 resulted, in the *cis*-2,5-disubstituted series, in strong NOE effect to the opposing C-5 methine.
11. For example Millenium's CCR1 antagonists (Example 1 in Luly, J. R.; Nakasato, Y.; Ohshima. E.; Sone, H.; Kotera, O.; Harriman, G. C. B.; Carson, K. G.; Brown, J. A.; WO0109119), Roche Bioscience's CCR3 antagonists (see: Bryan, S. A.; Jose, P. J.; Topping, J. R.; Wilhelm, R.; Sodeberg, C.; Kertesz, D.; Barnes, P. J.; Williams, T. J.; Hansel, T. T.; Sabroe, I. *Am. J. Respir. Crit. Care Med.* **2002**, *165*, 1602) and Schering-Plough's CCR5 antagonists (see: Palani, A.; Shapiro, S.; Clader, J. W.; Greenlee, W. J.; Cox, K.; Strizki, J.; Endres, M.; Baroudy, B. M. *J. Med. Chem.* **2001**, *44*, 3339).
12. Tanabe, Y.; Kubota, Y.; Sanemitsu, Y.; Itaye, N.; Suzukamo, G. *Tetrahedron Lett.* **1991**, *32*, 383.
13. Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615.
14. Whited, G. M.; Downie, J. C.; Hudlicky, T.; Fearnley, S. P.; Dudding, T. C.; Olivo, H. F.; Parker, D. *Bioorg. Med. Chem.* **1994**, *2*, 727.
15. This finding is congruent with previous work regarding enantiomerically pure thiazolidin-4-ones as PAF antagonists. See: Tanabe, Y.; Suzukamo, G.; Komuro, Y.; Imanishi, S.; Morooka, S.; Enomoto, M.; Kojima, A.; Sanemitsu, Y.; Mizutani, M. *Tetrahedron Lett.* **1991**, *32*, 379.
16. Brown, N. S.; Bicknell, R. *Methods in Molecular Medicine* **2001**, *58*, 47.