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# Discovery of Potent and Selective Phenylalanine Derived CCR3 Antagonists. Part 1

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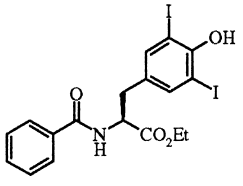
**Abstract**—The discovery of a series of phenylalanine derived CCR3 antagonists is reported. Parallel, solution-phase library synthesis has been utilized to delineate the structure–activity relationship leading to the synthesis of highly potent, CCR3-selective antagonists. 

The chemokine superfamily of secreted proteins exert their biological activity by binding to and activating seven-transmembrane domain G-protein coupled cell surface receptors (GPCRs) present on a variety of cells including immune and inflammatory cells.[1] To date, over 20 distinct chemokines have been characterized[2] and have been shown to be involved in the highly specific trafficking of pro-inflammatory leukocytes important in a number of disease states.[3] Eosinophils are proinflammatory granulocytes that are thought to play a major role in allergic diseases, such as bronchial asthma, allergic rhinitis, pruritis and atopic dermatitis.[4] Upon activation, eosinophils release lipid mediators, cytotoxic proteins, oxygen metabolites, and cytokines, all of which have the potential to produce the clinically observed pathophysiology. Recently, a CC chemokine, eotaxin, has been shown to mediate eosinophil infiltration in vivo by the activation of a novel CC chemokine receptor, designated CCR3, present on peripheral blood eosinophils.[5] Studies with CCR3 monoclonal antibodies have demonstrated that receptor expression is primarily restricted to eosinophils and a subset of Th2 T-cells and that this restricted expression may be responsible for the selective recruitment of eosinophils and Th2 T-cells in allergic inflammation.[6] Our interest in this area stems from the potential therapeutic application of selective CCR3 antagonists[7] as antiinflammatory agents and we report herein the discovery and initial structure–activity relationships of a series of highly selective and potent phenylalanine derived CCR3 antagonists. The cloning and functional expression of the human CCR3 (hCCR3) has been reported by a number of laboratories[8] and we have utilized a stable RBL-2H3 cell line expressing the receptor to identify antagonists that are able to block the human eotaxin-induced intracellular calcium mobilization in these cells. High-throughput screening of our proprietary compound collection using a fluorescence imaging plate reader (FLIPR) to track the intracellular calcium changes identified *N*-benzoyl-3,5-diiodotyrosine ethyl ester **1** as a modestly effective antagonist ($IC_{50} = 2.3$ μM). Consistent with its functional activity, in a binding assay using [$^{125}$I]human eotaxin as the radioligand and purified human eosinophils as the CCR3 source, **1** was determined to have reasonable CCR3 affinity ($IC_{50} = 535$ nM).[9] Although the presence of the metabolically labile ester precluded in vivo evaluation, we felt that **1** had sufficient biological activity to warrant further chemical optimization with the aim of improving initially the CCR3 affinity and ultimately also identifying an acceptable ester surrogate. The structure of **1** suggested that the compound was amenable to rapid synthesis of analogues, prompting us to investigate a solution-based parallel synthesis approach towards the optimization of CCR3 affinity.




**1**, CCR3 $IC_{50} = 535$ nM

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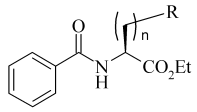
## Chemistry[10]

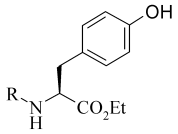
The compounds described in Tables 1 and 2 were either commercially available or prepared by the benzoylation of the corresponding amino acid esters.[11] Reaction of tyrosine ethyl ester hydrochloride with a variety of electrophiles according to Scheme 1 gave access to the compounds of Table 3. The substituted alkyl and benzyl esters of Table 4 were synthesized using literature procedures.[12] Racemic **24** (Table 3) was obtained in excellent yield via a modified Dakin–West reaction[13] and finally the amides **25** and **26** were prepared using standard peptide coupling conditions.[11]

**Table 1.**



| Compound | R | *n* | CCR3 $IC_{50}$ (nM) |
|---|---|---|---|
| **1** | 3,5-$I_2$-4-OH-Ph | 1 | 535 |
| **2** | 4-OH-Ph | 1 | 383 |
| **3** | Ph | 1 | 190 |
| **4** | H | 1 | 9000 |
| **5** | L-4-$NO_2$-Ph | 1 | 49 |
| **6** | D-4-OH-Ph | 1 | >50,000 |
| **7** | L-3-Indolyl | 1 | 325 |
| **8** | (±)-Ph | 0 | >20,000 |
| **9** | (±)-Ph | 2 | 5950 |

**Table 2.**

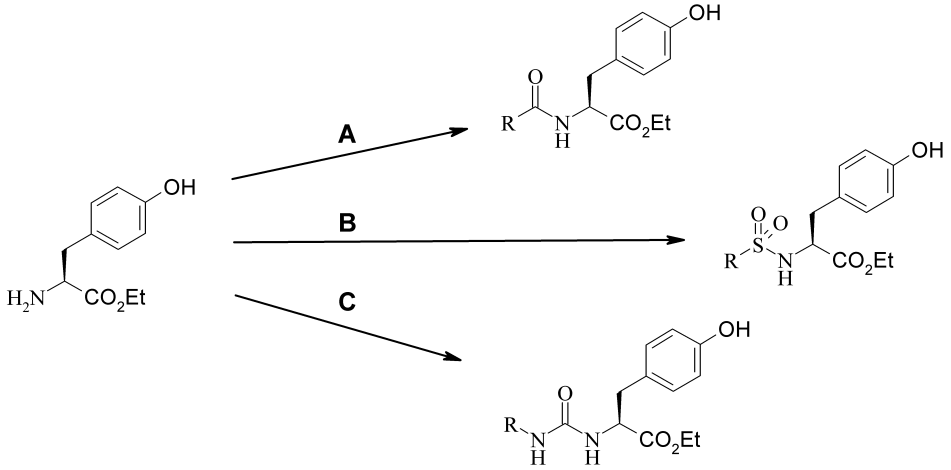


| Compound | R | CCR3 $IC_{50}$ (nM) |
|---|---|---|
| **2** | COPh | 383 |
| **10** | CONHPh | >33,000 |
| **11** | $SO_2$(4-Me)Ph | 16,000 |
| **12** | CO-*Z*-L-Asn | >25,000 |
| **13** | CO-*Z*-L-Phe | 6250 |
| **14** | CO-*Z*-L-Ser | >25,000 |
| **15** | CO-*Z*-L-Gln | >25,000 |
| **16** | CO-1-napthyl | 80 |
| **17** | CO-2-pyridyl | 1700 |
| **18** | $COCH_2Ph$ | 2600 |

Our initial efforts focused on investigating alternatives to the diiodotyrosine side chain in **1** while maintaining both the *N*-benzoyl group and ethyl ester (Table 1). Removal of the two iodine atoms either alone (**2**) or in combination with the phenolic hydroxyl (**3**) not only retained but led to a small increase in CCR3 affinity. Less conservative changes, for example, removal of the aromatic system (**4**), were not tolerated. The CCR3 affinity of **3** could be further improved by substitution, and of a number of groups surveyed, a 4-nitro group was found to be associated with particularly high receptor affinity. A high degree of enantiospecificity in the CCR3/antagonist interaction was apparent since the correct amino acid stereochemistry was critical for good receptor affinity with only the natural L-stereochemistry having significant activity. For example, the L-enantiomer **2** had >200-fold higher CCR3 affinity than its corresponding D-isomer **6** (Table 1) and a similar trend was observed for other analogues evaluated. Among heterocyclic analogues of the phenylalanine ring, the tryptophan derivative **7** was found to be approximately equipotent to the carbocyclic parent.

The effect of varying the length of the linker between the α-carbon and the aryl side chain in **3** was equally dramatic and either deletion or extension of the methylene chain to the phenylglycine **8** or to the homophenylalanine **9**, respectively, was detrimental for CCR3 affinity.

Having established that a substituted phenylalanine was the simplest minimal requisite for good CCR3 affinity, we next evaluated potential alternatives to the amide group in **2**. In order to rapidly survey a number of diverse combinations of linker alternatives and aryl substituents, a solution-based parallel-synthesis approach was adopted in which the active tyrosyl and ethyl ester side chains of the lead were retained and the




**Scheme 1.** Reagents: (A) 1 equiv $RCO_2H$, 3 equiv NMM, 1.2 equiv HOBT, 1.2 equiv EDCI, DMF; (B) 1 equiv $RSO_2Cl$, 3 equiv $Na_2CO_3$, $CHCl_3$/$H_2O$; (C) 1 equiv RNCO, dioxane.

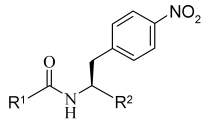
amino acid nitrogen derivatized. Reaction of tyrosine ethyl ester under standard conditions with a range of carboxylic acids, sulfonyl chlorides, and isocyanates generated a variety of the corresponding amides, sulfonamides, and ureas (Scheme 1). Of these, the amide-linked derivatives were of most interest since the level of potency displayed was markedly superior to that of the other linkers surveyed. The urea analogues were found to be devoid of CCR3 affinity and were not pursued. Several of the sulfonamides, however, retained some receptor binding activity although the structure–activity relationship appeared to be divergent to that of the amides. Further details of these studies will be the subject of future publications. Consistent with the presence of a largely lipophilic binding domain in the vicinity of the amide moiety, lipophilic aryl amides were generally associated with the highest CCR3 affinity and the introduction of either polar or nonaryl moieties was poorly tolerated, leading to virtually complete abolition of activity. Extended aromatic systems were highly favoured with the 1-naphthoyl derivative **16** being among the most potent analogues identified. Interestingly, amino acid derived amides were particularly poor receptor ligands regardless of the nature of the amino acid side chain, suggesting that the presence of the additional carboxylate functionality was disfavored (Table 2).

We found that combining the best substituents discovered from the independent optimization of the phenylalanine side chain and the aryl group resulted in the identification of potent antagonists such as **20** and **27**, whose receptor affinity was comparable to that of the chemokine agonist, eotaxin (Tables 3 and 4).

As shown in Table 3, in contrast to the highly specific requirements shown for the amide side chain, a greater variety of esters was tolerated without significant loss of receptor affinity. Linear alkyl derivatives such as methyl **20** and ethyl **19** analogues along with the branched $^{i}$Pr analogue **21** were all found to retain high CCR3 affinity. However, additional steric encumbrance as in the $^{t}$Bu analogue **22** led to some loss of CCR3 potency, indicating an overall size limitation. A change from an alkyl to an aromatic ester was well tolerated with the benzyl ester **23** being equipotent to **21**. The lack of discrimination for the alkyl part of the ester moiety suggested that this part of the molecule was not involved in making critical receptor interactions and may well be exposed to solvent when bound to the receptor. Consequently, we anticipated that replacement of the ester with other metabolically more stable groups to provide analogues suitable for in vivo evaluation would not be problematic providing that any important interactions of the ester heteroatoms were retained. We selected **19** as the template to base ester replacement structure–activity relationship on because the potency of this compound was sufficiently high that small losses in CCR3 affinity introduced by these changes should not lead to complete loss of affinity. However, we quickly realized that the ester group in this series of antagonists played a key role in CCR3 binding and even conservative modifications to this group led to huge decreases in receptor affinity. For example, either removal of the ester carbonyl to give the corresponding ether or replacement with a simple alkyl eliminated CCR3 affinity (data not shown). Surprisingly, the ketone **24** was devoid of all CCR3 affinity and conversion of the ester to the corresponding secondary (**25**) or tertiary (**26**) amide was similarly not tolerated (Table 3). This exquisite sensitivity of the ester moiety for CCR3 binding is in contrast to that reported for tryptophan ester derived antagonists of the NK1 receptor where the ketone derivatives retained very high receptor affinity although interestingly, even in that case, the amide and ether analogues demonstrated much lower affinity.[14]

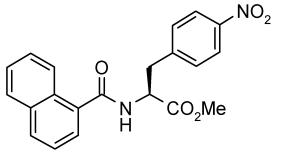
Although such reactions are not well precedented with GPCRs, the apparent absolute requirement for an ester moiety for CCR3 antagonism prompted us to investigate whether the receptor/antagonist interaction involved an irreversible transesterification of a critical residue in the protein. Incubation of CCR3 (primary human eosinophils) with **5** for 30 min at room temperature followed by extensive washing completely restored both the binding affinity and calcium mobilization activity of eotaxin, suggesting that the compound was a simple, freely reversible antagonist. Clearly, the limited experiments carried out so far would not preclude a rapid acylation/deacylation of the receptor.

**Table 3.**




| Compound | $R^1$ | $R^2$ | CCR3 $IC_{50}$ (nM) |
|---|---|---|---|
| **19** | 1-Naphthyl | $CO_2Et$ | 8 |
| **20** | 1-Naphthyl | $CO_2Me$ | 5 |
| **21** | 1-Naphthyl | $CO_2^{i}Pr$ | 21 |
| **22** | 1-Naphthyl | $CO_2^{t}Bu$ | 57 |
| **23** | 1-Naphthyl | $CO_2CH_2Ph$ | 18 |
| **24** | 1-Naphthyl | (±)COPr | >33,000 |
| **25** | 1-Naphthyl | $CONHCH_2Ph$ | 4000 |
| **26** | 1-Naphthyl | $CONMe_2$ | >33,000 |
| **27** | 2,4-Dimethylphenyl | $CO_2Et$ | 3 |

**Table 4.**




**20**

| Assay | Agonist | $IC_{50}$ (nM) |
|---|---|---|
| CCR3 binding | [$^{125}$I]-Eotaxin | 5 |
| | [$^{125}$I]-MCP-4 | 7 |
| $Ca^{2+}$ mobilization (RBL-2H3-CCR3 cells) | Eotaxin | 38 |
| | Eotaxin-2 | 35 |
| | MCP-4 | 20 |
| Eosinophil chemotaxis | Eotaxin | 32 |
| | Eotaxin-2 | 25 |
| | MCP-4 | 55 |
| | C5a | >330 |

The functional activity of the CCR3 binding derivatives was confirmed by assaying their ability to block the eotaxin-mediated increases in intracellular calcium mobilization either in HEK293 cells transfected with human CCR3 or in primary human eosinophils.[15] In an alternative assay, the inhibition of eotaxin induced chemotaxis of primary human eosinophils from allergic donors was determined.[16] In both assays, the prototypical inhibitor **20** was shown to be a potent antagonist with $IC_{50}$ values consistent with those determined in the binding assay (Table 4). In addition, **20** also effectively inhibited the binding of [$^{125}$I]MCP-4 to human eosinophils, strongly suggesting that **20** mediated its effects by binding to CCR3 and not the chemokine agonist.[15]

The chemokine receptors are known to bind and interact with multiple agonists. CCR3 for example has been shown to bind not only eotaxin but also the chemokines eotaxin-2, RANTES, MCP-3, and MCP-4.[17] Compound **20** effectively blocked the calcium mobilization and chemotaxis mediated by eotaxin and MCP-4 with potency identical to inhibition of eotaxin (Table 4), indicating it will block all CCR3 ligand interactions. The chemokine receptor selectivity of small molecule antagonists is therefore critical in obtaining a desired pharmacological effect whilst minimizing the potential for undesired side effects. The CCR3 selectivity of **20** against a panel of related chemokine and non-chemokine 7TM GPCRs was determined and the compound found to be $>2500$-fold selective for CCR3 over the other 10 receptors in the screen. Compound **20** represents one of the most potent and selective CCR3 antagonists reported.

A phenylalanine-based antagonist of the human CCR3 has been discovered and initial structure–activity relationship studies revealed a key role for both the *N*-acyl and ester moieties. Parallel, solution-phase library synthesis was utilized to delineate the structure–activity relationship and highly potent, CCR3-selective antagonists have been synthesized. In contrast to other small molecule CCR3 antagonists reported,[18] the presence of a basic nitrogen functionality within the molecule is not a requirement for good CCR3 binding affinity in this series, suggesting a unique binding site.

## References and Notes

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