Novel 2-(Substituted phenyl)benzimidazole Derivatives with Potent Activity against IgE, Cytokines, and CD23 for the Treatment of Allergy and Asthma

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Received August 31, 2004

Abstract: The effectiveness of the injectable anti-IgE antibody omalizumab has validated IgE as an important target for allergic diseases, thus spawning the development of small-molecule IgE inhibitors. Herein, a brief SAR is described for novel phenylbenzimidazole compounds that potently suppress IgE responses. In addition to IgE, these agents inhibit other targets critical for allergic response. The profile of orally active AVP-13358, the lead compound of this series currently in clinical trials, is described.

About 5% of Americans suffer asthma, and the incidence is expanding in epidemic proportions. The cost of hospitalization in the U.S. alone is $1.6 billion, and the cost of prescription asthma medications is $1.1 billion annually.[1] The paucity of novel pharmacological approaches introduced in the past 30 years for the treatment of asthma is a striking contrast to the plethora of new medicines for diseases such as cancer or arthritis. Moreover, the drugs currently available for the treatment of asthma are largely palliative; i.e., they treat the symptoms rather than the underlying disorder. Drugs such as antihistamines and leukotriene antagonists target single effecter molecules and provide some relief, but the heterogeneous nature of asthma makes this approach unsuccessful for most patients.[2] Corticosteroids act in a multifactorial manner, are very effective at suppressing allergic symptoms, and remain the mainstay for treating serious asthma.[3] However, the long-term morbidity associated with daily use severely limits their use, particularly in children and for less serious allergic manifestations in adults.

An important drug recently approved for the treatment of asthma is the injectable humanized anti-IgE antibody omalizumab (Xolair).[4] The effectiveness of this antibody validates IgE as an important mediator in atopic diseases and has led to an influx of small-molecule IgE inhibitors under development for the treatment of asthma and allergic rhinitis.[5] The latter compounds work via various mechanisms to suppress IgE responses such as inhibiting the release of the Th2 cytokine IL-4.[6] While their oral bioavailability provides an advantage over the anti-IgE antibody, previous work with omalizumab has shown that optimal therapeutic effectiveness requires greater than 95% suppression of IgE,[4] a level that has been unachievable in vivo by small-molecule IgE inhibitors.

Our objective was to address one of the foremost challenges in the field of asthma pharmacotherapy, to suppress disease development at its foundation without imparting significant liability to the patient. Herein, we describe a group of compounds that is novel in structure and apparent mechanism. These agents appear to act via a single target shared by multiple cell types to suppress their activation to allergic stimuli, including production of IgE and Th2 cytokines and expression of CD23 and IL-4 receptor-α. The lead compound in this series **1i** (AVP-13358) is orally active in mouse models of asthma and currently in phase I clinical trials for the treatment of asthma and allergic rhinitis.

Identification of these compounds started with the premise that since IgE is central to allergic manifestations, a compound that interferes with the IgE response would act on a target that is fundamentally linked to its development. This allergic cascade was experimentally reconstituted in mice by immunizing BALB/c mice with an antigen (DNP-KLH) and adjuvant (alum) followed 2 weeks later by removing the spleen and establishing cultures of lymphocytes with specific antigen, in the presence and absence of drug.[7] Allergen sensitization in vivo initiates the cascade of events that prepares the animal to produce IgE upon subsequent challenge. The response to allergen challenge (exposure), which is pertinent to the clinical situation, is thus reconstituted in the spleen cell culture, wherein equal numbers of B and T cells act in concert to produce IgE. In this system, B cells likely function to present the introduced antigen (DNP-KLH) to T cells, which respond by producing IL-4 (and/or IL-13) and expressing costimulatory molecules such as the T cell receptor and CD40 ligand, which in turn activate B cells/plasma cells to produce IgE. Thus, although IgE is the downstream product of this experimental system, the assay identifies drug candidates that act on numerous potential targets within either of two cell types that are involved in the generation or propagation of allergic responses. To identify lead structures that would potentially interfere with this process, an in-house universal informer library (developed from over 300 000 compounds) was screened for biological activity using a cell-based ex vivo IgE response assay. This effort resulted in the identification of compounds with the 2-phenylbenzimidazole core structure and modest activity against the IgE response. This core structure was chosen for follow-up.

The synthesis of the 2-(substituted phenyl)benzimidazoles is indicated in Scheme 1.[8] Appropriate structural modifications followed by SAR analyses of this scaffold yielded compounds with higher potency and eventually with oral bioactivity (Table 1).[8] SAR studies of bis-substituted phenylbenzimidazole series revealed that when R1 and R2 are small aliphatic or aromatic groups, low potency resulted (IC_{50} > 100 nM).[9] The potency significantly increased when aliphatic cycloalkyl groups or aromatic groups were inserted in R1 and R2. Combining aliphatic and aromatic groups yielded com-

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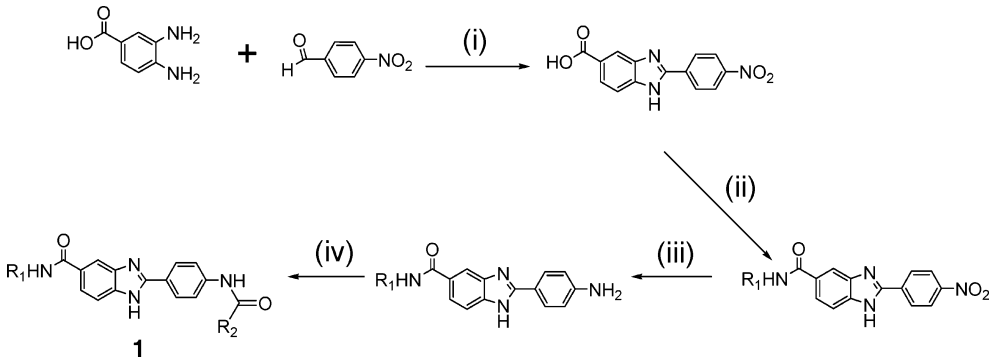
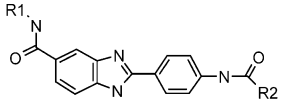
10.1021/jm049288j CCC: $27.50
Published on Web 11/13/2004

Scheme 1. Synthesis of 2-(Substituted phenyl)benzimidazole Derivatives[a]



[a] Reagents: (i) $C_6H_5NO_2$ at 155−160 °C for 18 h, 79%; (ii) CDI/DMF at 80 °C for 3 h, then amine/DMAP at 100 °C for 18 h or EDC/DMF at room temp for 3 h, then amine and DMAP at room temp for 18 h, 65%; (iii) MeOH/DMF (9:1)/10% Pd/C, 80%; (iv) dry THF, dry Py, acid chloride, room temp for 18 h, 45%.

Table 1. Biological Activity of Phenylbenzimidazole Compounds



Compound	R1	R2	Formula	*ex vivo* IC50 (nM)	*in vitro* IC50 (nM)	Oral Bioavailability relative to 1i[a]
1a		CH_3	$C_{26}H_{28}N_4O_2$	25	90	<0.1
1b			$C_{31}H_{36}N_4O_2$	2	7	<0.1
1c			$C_{27}H_{32}N_4O_2$	5	8	<0.1
1d			$C_{27}H_{26}N_4O_2$	8	24	<0.1
1e			$C_{31}H_{32}N_4O_2$	60	40	<0.1
1f			$C_{32}H_{38}N_4O_2$	1	2	<0.1
1g			$C_{31}H_{28}Cl_2N_4O_2$	0.4	2	<0.1
1h			$C_{32}H_{31}N_5O_4$	1	5	0.35
1i			$C_{30}H_{29}N_5O_2$	3	8	1
1j			$C_{30}H_{27}N_5O_3$	10	40	0.5
1k			$C_{30}H_{29}N_5O_3$	1	8	<0.1
1l			$C_{30}H_{29}N_5O_3$	1	15	<0.1
1m			$C_{26}H_{19}N_5O_2$	30	300	0.8

[a] Includes the combined levels of parent and active metabolite. Relative oral bioavailability was determined from the levels of active drug/metabolite in mouse serum samples taken 1 and 4 h following oral gavage of 40 mg/kg doses of drug.

pounds (e.g., **1d** and **1e**) with intermediate potency, although dichlorophenyl substitution at R2 together with an adamantyl at R1 yielded the most potent compound of the series (**1g**). In general, modifications of the 2-phenylbenzimidazole/bis-amide backbone ablated the bioactivity, including replacement of amide linkages or alternative amide substitution sites on the benzimidazole and phenyl ring structures.[9] The core 2-phenylbenzimidazole/bis-amide structure also was found to have little tolerance for substitution except for small halogens such as fluorine.[9] Potency was optimized when large hydrophobic residues were placed on the amide linkage; preferred substitutions at R1 and R2 are bulky fused cycloalkyl groups (**1f**: R1 = R2 = adamantyl; IC_{50} = 1 nM).

The oral bioavailability of an asthma/allergy drug is of paramount importance for application of that compound in the clinical setting. However, the highly lipophilic character that defines compound potency in this series is diametrically opposed to achieving good bioavailability after oral administration. Addition of polar groups to improve solubility and gastrointestinal absorption results in an increased IC_{50} for IgE inhibition. In the absence of −OH conjugation sites (**1h**, **1j**), the addition of a pyridine group on the benzimidazole side of the molecule (**1i**) substantially improved oral bioavailability without a significant loss of activity. Moreover, the pyridinephenylbenzimidazole core structure is suitable for salt formation. The best counterion was found to be methansulfonic acid, which improved the apparent oral bioavailability approximately 3-fold more than that achieved by the free base (data not shown).

To address the question of which cell types respond to compound, individual cell populations were isolated from the spleens of naive mice and tested for bioactivity. IgE responses were elicited from B-lymphocytes that were isolated from spleens of naive BALB/c mice and cultured 5 days with interleukin-4 (IL-4) and anti-CD40 antibody (in vitro IgE assay). The latter stimuli are normally provided by T cells and are critical for B cell production of IgE. As shown in Figure 1 and Table 1, the activities of these compounds against IgE in both the ex vivo and in vitro assays roughly follow in parallel, demonstrating that the compounds are acting directly on the B cell. The higher potency in the ex vivo assay suggests that these compounds may also have an effect on the T cell. This question was addressed directly by isolating T lymphocytes from mouse spleen and testing for cytokine responses to various stimuli. The observation that IL-4, IL-5, and IL-13 production/release were potently suppressed by **1i** demonstrated a direct action on the T cell.[10] The benzimidazole compounds also target other markers important for development of allergic response, including suppression of the B cell IgE receptor (CD23) on human monocytes[11] and of CD23 and IL-4 receptor-α on murine B cells.[12] Finally, the revers-

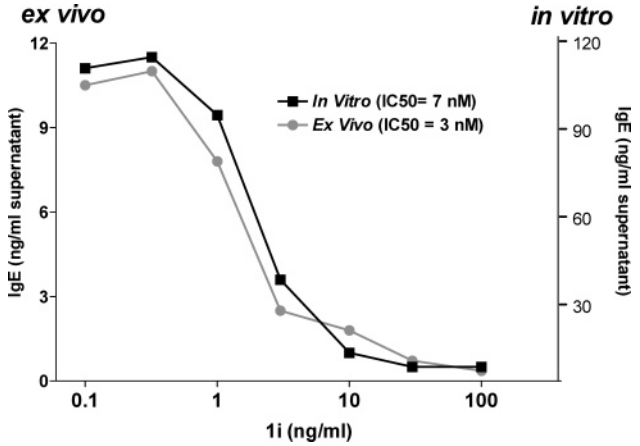


Figure 1. IgE Response. Cultures of spleen cells from antigen-sensitized mice were established ex vivo in the presence and absence of antigen and **1i**, and antigen-specific IgE response was measured as described.[7] In vitro assays were performed on B cells that were isolated from naive BALB/c mice and cultured for 5 days with IL-4 (10 ng/mL) and antimouse CD40 antibody (100 ng/mL). The level of IgE in the supernatants of both assays was quantified by ELISA and by comparing with a standard curve using a DNP-specific mouse monoclonal antibody.

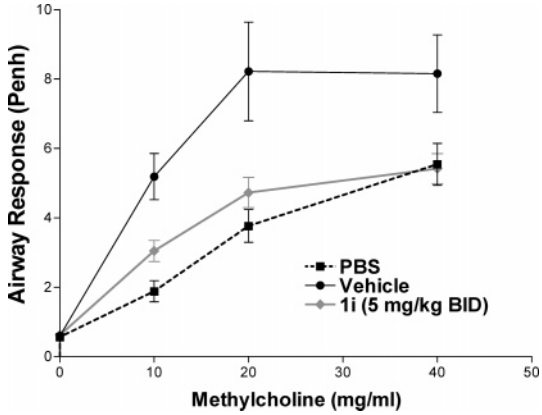


Figure 2. In vivo efficacy model was performed similarly to that in previous reports.[15] Female BALB/c mice were injected with 10 μg of ovalbumin (OVA) in 4 mg of alum intraperitoneally. Two weeks hence, the mice were exposed to OVA by nebulization (2% in PBS) for 20 min on 2 consecutive days and treated with **1i** or vehicle orally twice daily for 3 days. After 2 additional weeks, the mice were re-administered OVA by nebulization on 4 consecutive days while receiving drug or vehicle as above. Control mice were nebulized with PBS only. One day after the last OVA nebulization and drug dose, mice were placed in a chamber and tested for airway responsiveness to increasing concentrations of methacholine by a Buxco whole-body plethysmograph. The graph compares the data from mice that received 5 mg/kg AVP-13358 orally twice daily; the ED_{50} of **1i** in this assay system was previously determined to be 2.5 mg/kg when administered as a suspension in a 0.2 M citric acid formulation (pH 4) by mouth twice daily.

ibility of the biological activity of the phenylbenzimidazoles suggests that their activity is not a result of nonspecific toxicity; i.e., lymphocytes respond normally to stimulus following exposure (up to 24 h) and subsequent removal of the drug **1i**.[8] Thus, these compounds have a direct action on several individual lymphoid cell types to inhibit their activation by a variety of polyclonal stimuli.

The similar potency of these compounds in suppressing multiple targets in diverse cell types suggests that they act on a specific target that is shared by these cells. Investigations into the mechanism of action of the phenylbenzimidazoles have revealed a novel effect on the processing of proteins in the Golgi.[13] This activity is consistent with the observed effects on IgE, cytokines, and CD23, all of which putatively require Golgi processing for cellular release or membrane expression.[14] Moreover, the mechanism of **1i** was clearly shown to be distinct from the action of other agents known to perturb the Golgi, such as brefeldin A and monensin.

The in vivo efficacy of **1i** was tested in a murine model of airway hyper-responsiveness.[15] As shown in Figure 2, mice that had been sensitized to an allergen (ovalbumin, OVA) develop significantly greater airway resistance in response to methacholine than mice that have not been exposed to OVA (PBS group). Mice that were exposed to OVA and treated with **1i** responded to methacholine in a similar manner as the PBS mice, indicating that the compound is protecting against the allergic response elicited by OVA sensitization and challenge. It is noted that a majority of the in vivo activity of this compound is contributed by an active metabolite **1k** that has been identified and synthesized;[8] **1k** has about twice the potency of the parent **1i** and is present at about 2-fold higher serum concentrations.

In conclusion, new 2-(substituted phenyl)benzimidazoles with potent anti-IgE activity and Th-2 cytokine inhibitory activity have been identified. These agents are unique in terms of their chemical structure, high potency, and putative mechanism of action. The inhibition of IgE production in cultures of B-lymphocytes and in mixed cultures of mouse spleen cells is complemented by activity against other targets within the allergy cascade, thus vastly improving their opportunity for efficacy in asthma. The breadth of in vitro activities is reflected in the efficacy of **1i** in a murine model of asthma. The structure−activity relationships thus offer a focus for the discovery and dissection of a potentially important target for IgE response regulation, which can be exploited therapeutically in allergic diseases.

Acknowledgment. The authors thank Homayon Banie for excellent technical contributions.

Supporting Information Available: Experimental procedures and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Data not shown here. Detailed SARs of the series are being compiled for publication.

(10) T cells were isolated from the spleens of female BALB/c mice and cultured with ConA (5 μg/mL) or anti-CD3 antibody (1 μg/mL) for 16 h. The IC_{50} of **1i** for IL-4, IL-5, and IL-13 was 30−40 nM in the presence of either stimulus. While Th2 cytokines were predominantly affected, the Th1 cytokine IFNγ also was suppressed with equivalent potency. While the role of IFNγ in asthma is controversial, its suppression might contribute to the therapeutic efficacy of **1i** in vivo.

(11) Peripheral blood lymphocytes were isolated from healthy human donors and cultured in the presence of IL-4 (50 ng/mL) and anti-CD40 antibody (200 ng/mL) for 5 days before analysis of cell surface proteins by FACS. CD23 on CD14-positive cells was suppressed by **1i** with an IC_{50} of 20 nM.

(12) CD23 and IL-4 receptor-α were measured on mouse splenocytes following a 24−48 h culture with IL-4 (10 ng/mL) and anti-CD40 antibody (100 ng/mL). IC_{50} values of **1i** versus CD23 and IL-4Rα are 40 and 100 nM, respectively.

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JM049288J