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Discovery of Potent, Selective, and Orally Bioavailable Alkynylphenoxyacetic Acid CRTH2 (DP2) Receptor Antagonists for the Treatment of Allergic Inflammatory Diseases

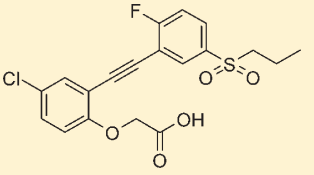
Stefano Crosignani,*[,†] Adeline Prêtre,[†] Catherine Jorand-Lebrun,[†] Gaële Fraboulet,[†] Jeyaprakashnarayanan Seenisamy,[‡] John Kallikat Augustine,[‡] Marc Missotten,[†] Yves Humbert,[†] Christophe Cleva,[†] Nada Abla,[†] Hamina Daff,[†] Olivier Schott,[†] Manfred Schneider,[†] Fabienne Burgat-Charvillon,[†] Delphine Rivron,[†] Ingrid Hamernig,[†] Jean-François Arrighi,[†] Marilène Gaudet,[†] Simone C. Zimmerli,[†] Pierre Juillard,[†] and Zoe Johnson[†]

[†]Merck Serono S.A., 9 Chemin des Mines, CH-1202 Geneva, Switzerland

[‡]Syngene International Ltd., Biocon Park, Plot No. 2 & 3, Bommasandra IV Phase, Bommasandra, Jigani Link Road, Bangalore 560 099, India

Supporting Information

ABSTRACT: New phenoxyacetic acid antagonists of CRTH2 are described. Following the discovery of a hit compound by a focused screening, high protein binding was identified as its main weakness. Optimization aimed at reducing serum protein binding led to the identification of several compounds that showed not only excellent affinities for the receptor (41 compounds with K_i < 10 nM) but also excellent potencies in a human whole blood assay (IC_{50} < 100 nM; PGD2-induced eosinophil shape change). Additional optimization of the PK characteristics led to the identification of several compounds suitable for in vivo testing. Of these, **19k** and **19s** were tested in two different pharmacological models (acute FITC-mediated contact hypersensitivity and ovalbumin-induced eosinophilia models) and found to be active after oral dosing (10 and 30 mg/kg).



19s Ki 3.4 nM
Whole blood IC_{50} 23 nM
Mouse PK: Cl 0.4 L/kg/h; Fz 63%

INTRODUCTION

Prostaglandin D2 (PGD2, **1**, Figure 1) is the major prostanoid species produced by mast cells in response to stimulation by allergens and plays a key role in inflammatory processes. PGD2 exerts its effect through two high affinity G-protein-coupled receptors: the classical DP receptor and the more recently discovered chemoattractant receptor-homologous expressed on Th2 lymphocytes (CRTH2 also known as DP2). In humans, CRTH2 is predominantly expressed by Th2 cells, eosinophils, and basophils, all known to play a key role in allergic diseases.[1] Activation of the Gi-coupled CRTH2 by PGD2 or the selective agonist DK-PGD2 stimulates chemotaxis of human Th2 cells, eosinophils, and basophils in vitro and in vivo,[2,3] suggesting that the CRTH2 receptor may directly mediate the recruitment of inflammatory cells in allergic diseases such as asthma, allergic rhinitis, and atopic dermatitis. Severity of atopic dermatitis has been correlated with increased numbers of circulating Th2 cells.[4] Atopic dermatitis subjects or those with sensitivity to pollen or to house dust mite antigens have significantly increased levels of CD4+ T cells expressing CRTH2 receptors.[2,5,6] CRTH2 is expressed by Ag-specific Th2 cells in allergic individuals, supporting the notion that CRTH2 receptor is important in the recruitment of Th2 in allergic diseases in humans.[7] Finally, sequence variants of the CRTH2 receptor that confer increased mRNA stability are associated with a higher degree of bronchial hyper-responsiveness and the occurrence of fatal asthma.[8]

Additional data in animal models likewise point to a central role for CRTH2 in allergic disorders. CRTH2 receptor activation induces the release of eosinophils from guinea pig bone marrow.[9] In mouse models of allergic asthma and atopic dermatitis, CRTH2 receptor activation promotes eosinophilia and exacerbates pathology.[10] Intratracheal administration of PGD2 in rats, with or without pretreatment with systemic IL-5, induces eosinophil trafficking into the airways. This effect is mimicked by selective CRTH2 receptor agonists but not by a DP (DP1) selective agonist.[3,11] Furthermore, inhibition by selective small molecules of the CRTH2 receptor but not of the DP or TP receptor abolishes inflammatory responses in mouse models of acute and chronic contact hypersensitivity as well as eosinohilic airway inflammation.[12] Recently, it has been postulated that DP and CRTH2 might have opposing effects in complex inflammatory processes, suggesting that inhibition of CRTH2 may have the added benefit of promoting DP induced anti-inflammatory effects.[13]

PGD2 is enzymatically and nonenzymatically metabolized into many different products, several of which are biologically active.[13,14] The thromboxane A2 antagonist ramatroban (**2**), a drug marketed for allergic rhinitis, was shown to be also a potent CRTH2 antagonist. In addition, the finding that indomethacin

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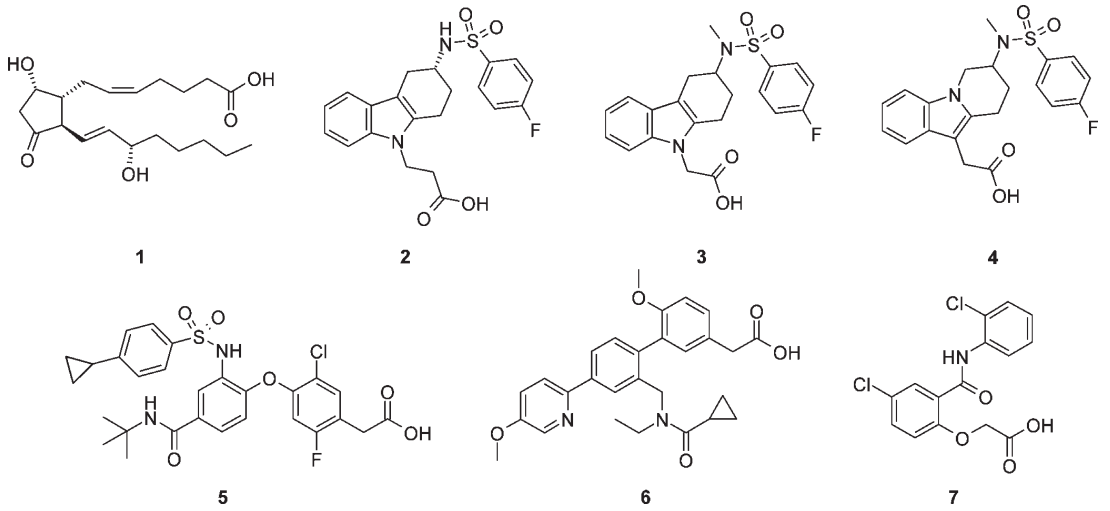


Figure 1. Structures of prostaglandin D2 (PGD2) (**1**), ramatroban (**2**), CRTH2 antagonists TM30089 (**3**),[17a] MK-7246 (**4**),[17i] AMG853 (**5**),[16c] and AM432 (**6**),[16b] and screening hit **7**.

was able to activate CRTH2, among other pharmacological actions, was the starting point for the discovery of other indole acetic acid derivatives and further spurred much effort in the pharmaceutical industry aimed at discovering selective agents to modulate its action.[15] Several classes of potent CRTH2 antagonists have been described, for example, arylacetic acids,[16] various heteroarylacetic acids,[17] phenoxyacetic acids,[18] and tetrahydroisoquinolines.[19] The availability of highly selective ligands will provide new insights on the role of CRTH2 in inflammatory processes.

We set out to discover and develop a new class of potent, selective, and orally active DP2 antagonists as potential new agents for the pharmacological management of allergic and inflammatory diseases.

RESULTS AND DISCUSSION

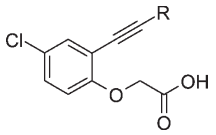
Biological Assays. The affinity of the compounds for the receptor was determined using a ^{3}H-PGD2 competition binding assay. To determine the functional behavior of the compounds, several different assays were used: the GTPγS binding assay to monitor an early event in the signaling cascade (G-protein activation) and a cellular dielectric impedance assay to monitor a cell shape change induced by ligand binding on CHO-CRTH2 cells (the change of impedance being linked to a cytoskeletal rearrangement, a necessary step for PGD2-induced chemotaxis). These two assays proved to be complementary. Indeed, the cellular dielectric impedance assay was more adapted to the discovery of compounds showing partial agonist activities, as E_{max} values observed were often higher than in the corresponding GTPγS binding assay. This effect might be due to an amplification mechanism in the signaling cascade or to the potential integration signals induced by activation of the G-protein dependent and/or independent signaling pathways. Conversely, the GTPγS binding assay allowed us to detect compounds having an inverse agonist behavior. Some of the compounds were further characterized in an endogenously receptor expressed cellular assay, namely, the PGD2-induced eosinophil chemotaxis assay. This assay was used to verify the ability of these compounds to interfere with one of the main pathophysiologically relevant results of CRTH2 activation.

Finally, it was decided to set up a whole blood assay in order to test the activity of the compounds in physiological conditions, including the presence of plasma proteins and red blood cells. The assay that was selected is a flow-cytometry-based detection of the change of shape of eosinophils in response to challenge with PGD2 (at 10 nM) in whole blood from healthy volunteers.[20] The assay proved to be sufficiently high-throughput and valuable in establishing a ranking order among the compounds. It is emphasized that the IC_{50} values were found to vary among the different donors (a compound used as an internal standard in all experiments showed IC_{50} values varying from 25 to 190 nM across 31 different donors). All compounds were tested on at least two donors (four to six donors for all most active compounds), and in each case the ranking order of the compounds did not change. Nevertheless, the IC_{50} data should be treated with caution.

Hit Discovery. In an effort to discover other series of CRTH2 antagonists, a focused screening of 8500 compounds was performed. The screening was performed using a competitive ^{3}H-PGD2 binding assay, and the compounds were selected on the basis of substructure similarity with known CRTH2 antagonists. The relevance of the selection was highlighted by the high hit rate of the screening (8% of compounds with >50% inhibition and 5% of compounds with >80% inhibition at 10 μM). Among other positives, compound **7** was identified, which showed a high potency (K_i = 22 nM). Our attention was particularly drawn to the amide group linking the two aromatic groups. On the basis of pharmacophore analysis of the receptor, we could envision both possible conformations of the amide (cis and trans) as possible binding modes. In order to establish which of the conformations of the amide was indeed active, we set out to prepare rigidified analogues of both conformations. While compounds mimicking the cis conformation turned out to be inactive, the corresponding alkynyl derivative **8a**, which positions the two aromatic groups in a similar way to the trans amide, was found to be active on the receptor, with only a moderate decrease in potency (K_i = 108 nM). This established not only the trans as the active conformation of the amide but also that both the hydrogen-bond acceptor and donor functions of the amide are not essential for binding.

A small number of analogues of **8a** were also prepared to have an initial idea of the SAR, and several compounds with K_i < 100 nM

Table 1. Initial SAR of Alkynylphenoxyacetic Acids



compd	R	K_i (nM)
8a	phenyl	108
8b	2-chlorophenyl	28
8c	3-chlorophenyl	29
8d	4- chlorophenyl	60
8e	2-methoxyphenyl	190
8f	2-fluorophenyl	47
8g	2-trifluorophenyl	24
8h	3-trifluorophenyl	92
8i	2,4-difluorophenyl	101
8j	5-chlorothiophene-2-yl	183
8k	1-methyl-1*H*-imidazol-2-yl	1470

could be identified (Table 1). The beneficial effect on potency of having a halide or CF_3 substituent in the 3- or preferably 2-position of the aromatic ring (R in Table 1) could be identified, whereas an alkoxide in the same position had a slightly detrimental effect. Also, substituting the aromatic ring with various five-membered heterocycles led to analogues with reduced potency.

Of these analogues, **8c** was selected for further testing to determine the potential for this chemical series for optimization (Table 2). Testing of the compound in a GTPγS binding assay showed antagonist activity with a potency similar to that in the binding assay (IC_{50} = 104 nM). In contrast, the potency in a cellular dielectric impedance assay was found to be reduced by around 1 order of magnitude (IC_{50} = 1.2 μM). This discrepancy was found to be linked to a difference in the conditions among the assays: while the binding and GTPγS binding assays were run in the absence of BSA (bovine serum albumin), the cellular dielectric impedance assay was run in the presence of 0.1% BSA. In fact, if the binding assay was run in the presence of 0.1% BSA, the IC_{50} of the compound could be shifted from 29 to 896 nM, while performing the cellular dielectric impedance assay in the absence of BSA gave an IC_{50} of 114 nM. This very marked difference due to the presence of a small amount of BSA could be explained by the very high protein binding of the compounds, with the unbound fraction (F_u) found to be below the detection limit (which in this case was around 0.3%) for all three species tested. In accordance with these data, the potency of the compound in the whole blood assay was found to be moderate (IC_{50} = 6.9 μM). In addition, the efficacy of close analogue **8b** was also measured in an eosinophil chemotaxis assay, in which **8b** was able to inhibit PGD2-induced chemotaxis of eosinophils with an IC_{50} of 123 nM, confirming its effect on one of the main mechanisms of action purported for CRTH2 antagonists. Finally, **8c** was also tested in a competition binding assay on the mouse CRTH2 receptor, and good cross-reactivity was observed (K_i = 37 nM).

The in vitro early ADME profile of **8c** showed a good stability toward oxidative metabolism in both rat and human liver microsomes and a high permeability in the Caco-2 cell assay. Almost no inhibition effect on five major cytochrome P450 isoforms could be observed at 10 μM, with the only exception being 2C9 for

Table 2. Pharmacological and Early in Vitro ADME Profile of Hit 8c

parameter	
K_i binding (hCRTH2) (nM)	29
MW	321.2
BEI	23.2
K_i binding (mCRTH2) (nM)	37.4
IC_{50} GTPγS (hCRTH2) (nM)	104
IC_{50} CDI, 0.1% BSA (hCRTH2) (nM)	1200
IC_{50} CDI, no BSA (hCRTH2) (nM)	114
IC_{50} WBA (nM)	6900
Cl (HLM) (μL min^{-1} (mg protein)$^{-1}$)	12
Cl (RLM) (μL min^{-1} (mg protein)$^{-1}$)	27
kinetic solubility (PBS, 2% DMSO) (μM)	>200
Caco-2 P_{app} (cm/s)	18.4×10^{-6}
PPB F_u, h, m, and r (%)	<0.3%
log *D* (pH 7.4)	0.5
selected PK parameter (mouse)	
Cl (L kg^{-1} h^{-1})	1.0
F_z (%)	82
$t_{1/2}$, iv/po (h)	0.3/1.6
V_{ss} (L/kg)	0.3

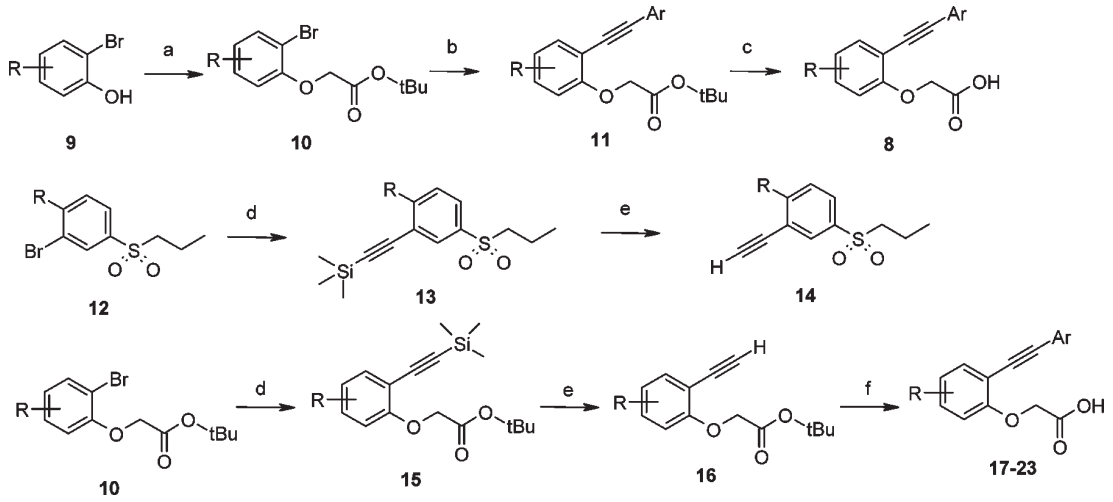
which an IC_{50} of 1.44 μM could be observed. The compound was shown to be very selective; out of a panel of 50 receptors and ion-channel pharmacological targets (including other prostanoid receptors) the compound showed some affinity only for the Cl^- channel (GABA gated, rat, 76% inhibition) and EP2 receptor (66% inhibition, binding assay) at 10 μM. The compound was also tested in a low dose cassette PK experiment in the mouse (0.2 mg/kg iv and 1 mg/kg po) and was found to have a reasonable pharmacokinetic profile for a hit compound, with a moderate clearance (1.0 L kg^{-1} h^{-1}) and excellent oral bioavailability (F_z = 82%). The compound was also shown to have a fairly small volume of distribution (V_{ss} = 0.3 L/kg), in keeping with the high protein binding, and a consequent short half-life ($t_{1/2}$ iv of 0.3 h, $t_{1/2}$ po of 1.6 h).

On the basis of these data, **8c** was considered as a suitable starting point for lead optimization. In the following sections, we describe the SAR of these compounds, regarding potency and key pharmacokinetic parameters and the pharmacological activity of the two optimized compounds in two different animal models.

Chemistry. All the compounds were prepared via a Sonogashira reaction to install the diarylalkyne moiety. Initially, the coupling was performed between *tert*-butyl 2-bromophenoxyacetates **10** and an arylacetylene, using standard Sonogashira conditions (Scheme 1). The Sonogashira couplings could also be performed on nonprotected 2-bromophenoxyacetic acid, but the yields were less satisfactory and the purifications more tedious than using a protecting group. The *tert*-butyl ester group was routinely used as protective group by virtue of the easy cleavage and the stability of the diphenylalkyne moiety in the deprotection conditions, especially when HCl in dioxane was used instead of TFA in DCM.

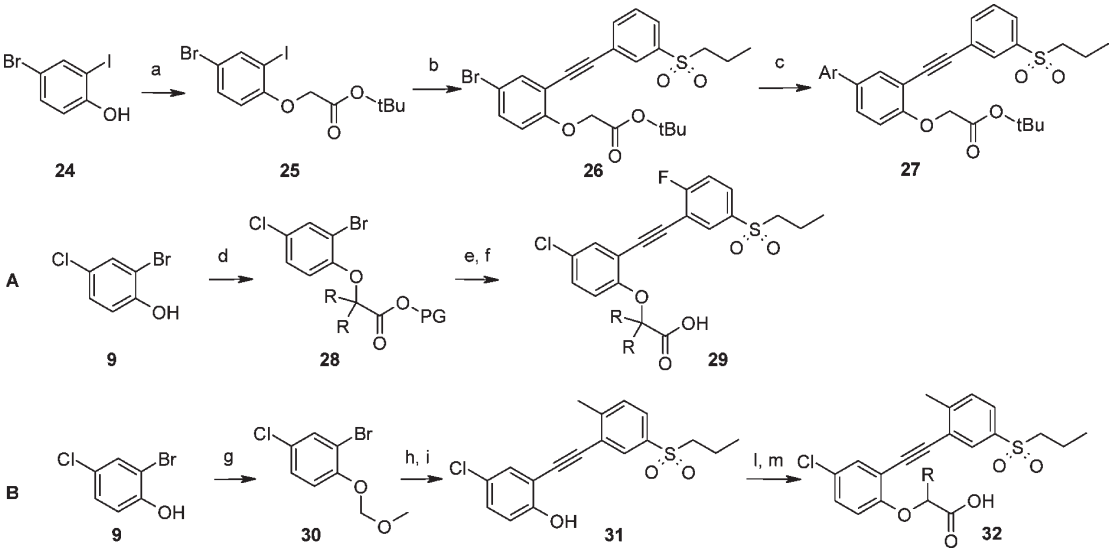
The reduced number of available arylacetylenes led us to build this key connection starting from easily prepared *tert*-butyl 2-alkynylphenoxyacetates **16** (Scheme 1) and more readily available

Scheme 1[a]



[a] Reagents and conditions: (a) *tert*-butyl bromoacetate, K_2CO_3, acetone, reflux, 86−95%; (b) arylalkyne, $PdCl_2(PPh_3)_2$, CuI, TEA, THF or ACN; (c) HCl, dioxane, av yield 12% over two steps; (d) trimethylsilylacetylene, $Pd(dppf)Cl_2$, CuI, TEA, THF, 60 °C, 51−93%; (e) TBAF, THF, room temp, 37−71%; (f) various aryl halides, $PdCl_2(PPh_3)_2$, CuI, TEA, THF, 60 °C, or $PdCl_2(PPh_3)_2$, piperidine (3−5 equiv) (alternatively, TEA; see text), 60 °C, then HCl, dioxane, av yield 29% over two steps.

Scheme 2[a]



[a] Reagents and conditions: (a) *tert*-butyl bromoacetate, K_2CO_3, acetone, reflux, quant; (b) **14a**, $PdCl_2(PPh_3)_2$, piperidine, 70 °C, 52%; (c) $ArB(OR)_2$, $PdCl_2(PPh_3)_2$, CsF, dioxane, H_2O, 120 °C (microwave), 53−99%; (d) methyl 2-bromopropionate or ethyl 2-bromoisobutyrate, K_2CO_3, DME or DMF, reflux, 40−82%; (e) **14c**, $PdCl_2(PPh_3)_2$, PPh_3, CuI, TEA, 90 °C; (f) HCl, dioxane or NaOH, ethanol, H_2O, room temp, 7−12% over two steps; (g) MOMCl, DIEA, DCM,room temp, 90%; (h) **14b**, $PdCl_2(PPh_3)_2$, PPh_3, CuI, TEA, 90 °C, 70%; (i) HCl, dioxane, 91%; (l) different 2-bromo-2-alkyl acetates, K_2CO_3, DME, reflux; (m) HCl, dioxane or NaOH, ethanol, H_2O, room temp, 7−22% over two steps.

aryl halides. This coupling was at first performed using $PdCl_2(PPh_3)_2$ (5 mol %), CuI (5 mol %), and triethylamine in acetonitrile or THF. However, it soon became evident that the yields were not satisfactory, and a large amount of homocoupling of the alkyne moiety occurred. A screening of different Sonogashira coupling conditions[21] led to the identification of reaction conditions that gave improved yields and a much reduced amount of homocoupling, using $PdCl_2(PPh_3)_2$ and a base (3 equiv of piperidine) without solvent and heating at 70 °C for 16−18 h.[22] These conditions allowed, in our experience, for the coupling of electron-poor aryl bromides (such as the sulfone-containing derivatives **12**) or of aryl iodides. Generally piperidine was found to be a good base, but when a fluorine atom was also present on electron-poor aryl halides, we found a significant amount of S_NAR reaction taking place, with the piperidine taking the place of the fluorine. In these cases, the use of triethylamine in place of piperidine solved the problem and restored good yields. For some reactions, however, this protocol failed to give the desired

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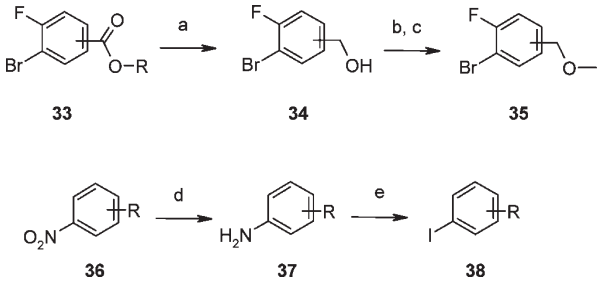
products and more adapted reaction conditions had to be found. For example, the coupling to obtain naphthyl derivative **22g**, for which the standard procedure did not afford the desired product, was performed using $PdCl_2$, PPh_3, and piperidine in an acetone/water mixture, heating at 60 °C for 2 days. Some of the fluorine-containing products and the hindered **23e** were prepared using $Pd(dppf)_2Cl_2$, CuI, and TEA in THF at 70 °C for 16−18 h.

Arylacetylenes **14** bearing alkylsulfone groups in the 3-position could be easily prepared starting from 3-bromoarylsulfones **12** in two steps, using conditions similar to those employed for the preparation of *tert*-butyl 2-alkynylphenoxyacetates **16** (Scheme 1).

Scheme 3[a]



[a] Reagents and conditions: (a) $LiAlH_4$, Et_2O, room temp or BH_3−THF, room temp, 83−94%; (b) $MeSO_2Cl$, TEA, DCM, 0 °C to room temp; (c) MeOH, 2,6-lutidine, room temp, 25−43% over two steps; (d) H_2, Pd/C, MeOH, room temp, 80−99%; (e) $NaNO_2$, HCl (aq), then KI, room temp, 27−73%.

Products bearing an aromatic or heteroaromatic group in position 4 of the first aromatic ring could be obtained by Suzuki coupling on the protected *tert*-butyl esters bearing a bromine atom (Scheme 2).

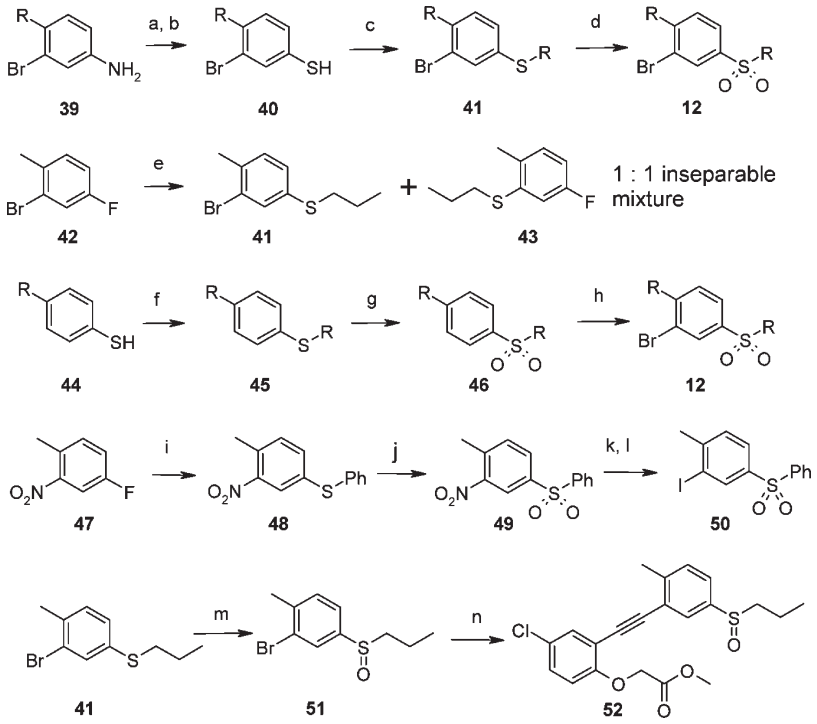
Finally, compounds substituted in α to the carboxylic acid moiety could be obtained by alkylation of the phenol moiety prior to the Sonogashira reaction (Scheme 2A), or the Sonogashira reaction could be carried out first, using MOM-protected 2-bromo-4-chlorophenol followed by deprotection with HCl and alkylation (Scheme 2B).

For the synthesis of the noncommercial aryl halide building blocks, they were prepared according to a variety of synthetic strategies. The noncommercial alcohol-containing building blocks **34a,b** were prepared starting from the corresponding carboxylic acids or esters by reduction with $LiAlH_4$ or BH_3. The methyl ethers **35a,b** were obtained by tosylation followed by methanolysis in the presence of lutidine (Scheme 3).

Several aryl halide building blocks were easily accessible, as the corresponding nitro compounds are commercially available (Scheme 3): reduction of the nitro group by catalytic hydrogenation to the corresponding aniline followed by a Sandmeyer reaction ($NaNO_2$, KI, aqueous HCl) gave the desired iodoaryl compounds.

For the sulfone derivatives, several alternatives were explored (Scheme 4). First, a strategy starting from the corresponding 3-bromothiophenol via alkylation (Cs_2CO_3 in DMF) followed by oxidation (*m*CPBA or Oxone) was employed. However, for the methyl substituted derivatives, the synthesis of the corresponding 3-bromo-4-methylthiophenol (from 3-bromo-4-methylaniline by reaction with sodium nitrite and potassium

Scheme 4[a]



[a] Reagents and conditions: (a) $NaNO_2$, HCl (aq), then KS_2COEt, 80 °C; (b) KOH EtOH 70% over two steps; (c) RI, NaH, DMF, 25−70%; (d) Oxone, THF, H_2O, room temp, 69−92%; (e) NaSPr, DMF, room temp; (f) RI, NaOH, MeOH, H_2O, room temp, 81−95%; (g) *m*CPBA, DCM, room temp, 78−88%; (h) NBS, conc H_2SO_4, room temp, 55−91%; (i) PhSH, K_2CO_3, DMSO, 150 °C (microwave), 47%; (j) Oxone, MeOH, H_2O, room temp, 82% (k) H_2, Pd/C, MeOH, 94%; (l) $NaNO_2$, HCl (aq), then KI, room temp, 47%; (m) $NaIO_4$, MeOH, MeOH, H_2O, 47%; (n) **16a**, $Pd_2(PPh_3)_2$, TEA, 60 °C, 24%.

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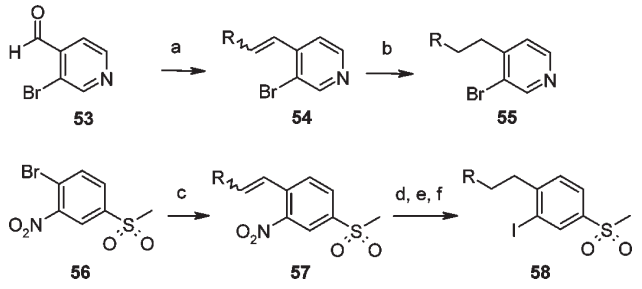
O-ethylxanthate) proved to be challenging, especially in terms of scale-up. A second strategy involved a S_NAr reaction of an alkylthiol on 2-bromo-4-fluorotoluene, which unfortunately resulted in a 1:1 ratio of substitution on the bromine and fluorine atoms. Finally, the best and most versatile strategy proved to be the one starting from a 4-substituted thiophenol, which was first alkylated under basic conditions, oxidized using *m*CPBA, and finally brominated using NBS in concentrated H_2SO_4. This sequence gave the desired intermediates in good yields, could be used with different groups in the position para to the sulfone (methyl, fluorine, chlorine), did not require chromatography or complex purifications, and was easily scalable to 30 g scale. Those sulfonamide derivatives that were not commercially available (such as the fluoro-substitued ones) were prepared in a similar way, by formation of the sulfonamide followed by the bromination, under the same reaction conditions.

For the preparation of sulfoxide **21e**, a similar strategy could not be followed, as the bromination reaction in the presence of the sulfoxide was not successful. A variant of strategy I was followed by alkylating 3-bromo-4-methylthiophenol followed by careful oxidation with $NaIO_4$. Sonogashira coupling was then performed on a methyl ester protected building block rather than the usual *tert*-butyl protected one, as the deprotection in acidic medium had resulted in decomposition of the product. In contrast, deprotection of the methyl ester via basic hydrolysis was successful. Phenylsulfone derivative **52** was obtained by S_NAr reaction of thiophenol on 4-fluoro-2-nitrotoluene, followed by oxidation, reduction of the nitro group, and Sandmeyer reaction to afford the desired iodide.

The 3-bromo-4-alkylpyridine building blocks were prepared according to Scheme 5, starting from 3-bromo-4-pyridinecarboxaldehyde. A Wittig reaction with the appropriate alkylphosphonium bromide installed an alkenyl group (as a mixture of *E*/*Z* isomers) that could be reduced by hydrogenation using PtO_2 as catalyst. Similarly, for the preparation of 2-iodo-4-(methylsulfonyl)-1-alkylbenzene derivatives the diversity was installed by Suzuki reaction between 2-bromo-5-methylsulfonylnitrobenzene and different alkenylboronic acids or esters. Reduction of the nitro group with iron in acetic acid, then of the double bond by hydrogenation followed by Sandmeyer reaction gave the desired building blocks.

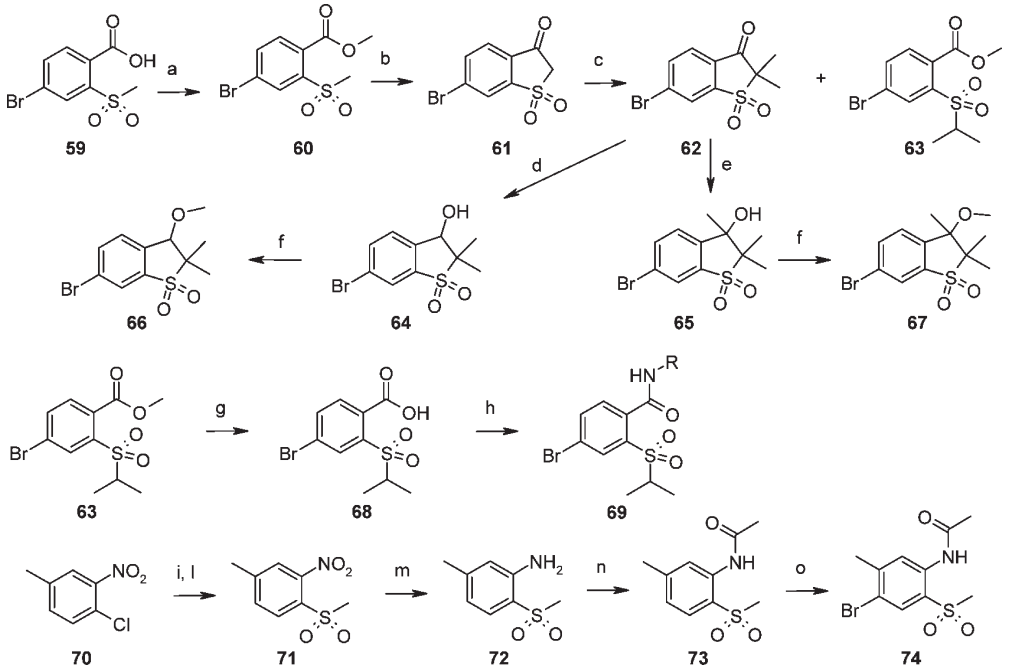
Intermediates **62**−**67** could be prepared starting from methyl 4-bromo-2-(methylsulfonyl)benzoate: treatment with NaH afforded the cyclized compound **61** (Scheme 6). Alkylation with

Scheme 5[a]



[a] Reagents and conditions: (a) various alkyltriphenylphosphonium bromides, BuLi, THF, 0 °C, 46−62%; (b) H_2, PtO_2, EtOAc, room temp, 19−79%; (c) various alkenylboronic acids, $PdCl_2(PPh_3)_2$, CsF, dioxane, H_2O, 80 °C, 63−89%; (d) Fe, AcOH, 90 °C, 64−93%; (e) H_2, Pd/C, MeOH, room temp, 81−99%; (e) $NaNO_2$, HCl (aq), then KI, room temp, 44−83%.

Scheme 6[a]



[a] Reagents and conditions: (a) H_2SO_4, MeOH, reflux, 72%; (b) NaH, THF, room temp quant; (c) NaH, MeI, DMF, room temp, 52%; (d) $NaBH_4$, MeOH, DCM, 0 °C, 97%; (e) MeMgBr, Et_2O, room temp, 0 °C, 97%; (f) NaH, MeI, DMF, 0 °C, 60−97%; (g) NaOH, H_2O, THF, room temp, 78%; (h) RRNH, PS-Mukaiyama reagent, TEA, room temp, 33−81%; (i) PrSH, K_2CO_3, DMF, 70 °C, 91%; (l) *m*CPBA, DCM, room temp, 64%; (m) H_2, Pd/C, room temp, 94%; (n) AcCl, NMM, DCM, room temp, 70%; (o) NBS, H_2SO_4, room temp, 62%.

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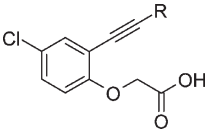
MeI in the presence of NaH gave **62** as the major product intermediate but also a sizable amount of ring-opened product **63**. Reduction of the keto group gave alcohol **64**, which could then be methylated (MeI, NaH) to get **66**. Addition of MeMgBr gave **65**, which could also be methylated to give **67**. Byproduct **63** could be hydrolyzed to give the corresponding acid **68**, which could be then coupled with a variety of amines, using polymer-supported Mukaiyama reagent, to give the corresponding amides **69a**−**c**.

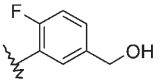
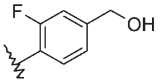
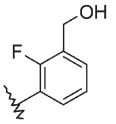
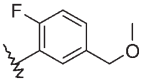
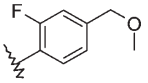
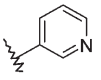
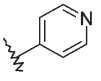
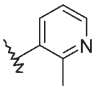
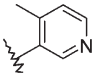
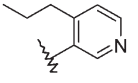
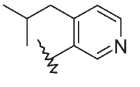
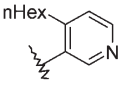
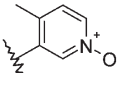
The reverse amide building block **74** could be obtained by reacting 4-chloro-3-nitrotoluene with propanethiol in the presence of a base (K_2CO_3), followed by oxidation with *m*CPBA, reduction of the nitro group via hydrogenolysis, acetylation of the aniline group using acetyl chloride, and finally regioselective bromination using NBS in concentrated H_2SO_4.

Biological Activity. Given the effect of the very high protein binding of **8c**, the increase of the unbound fraction of our compounds was the focus of the first stages of optimization. In order to be able to quickly estimate the extent of protein binding of a large number of compounds, it was decided to first measure the retention time of the compounds on an HPLC column coated with human serum albumin. Derived capacity factors K_{HSA} constitute then a chromatographic expression of the fraction of compound bound to albumin. Since albumin is the most abundant protein in plasma, K_{HSA} values can be used to rank compounds according to their potential to bind to plasma proteins in general.[23] Protein binding values by ultrafiltration were then measured for the most promising compounds. Given the nature of **8c**, it was hypothesized that increasing the polarity of the second aromatic ring (i.e., the one not bearing the phenoxyacetic acid moiety) could be advantageous in this respect (Table 3). Introduction of various groups containing either an alcohol or methyl ether group led to a number of analogues of similar potency to **8c** (for example, **17b** and **17e**). However, the protein binding of these derivatives, albeit improved, was still quite high as judged by their K_{HSA} values, which were between 0.97 and 0.98 (K_{HSA} > 0.99 for **8c**). The unbound fraction of **17b** in human plasma was confirmed at 0.4% in a classical protein binding experiment, and the compound showed an IC_{50} of 680 nM in the human whole blood assay, with an improvement of approximately 1 order of magnitude compared to **8c**. The mouse PK profile of **17b** was less satisfactory, showing a higher clearance (Cl = 2.1 L kg^{-1} h^{-1}) and very poor oral bioavailability (F_z = 3%).

Interesting compounds were also found replacing the second phenyl ring with a pyridine (Table 3). While the 2- and 4-pyridyl compounds were found to be only moderately potent, the 3-pyridiyl derivative **18b** was found to retain most of the potency of the hit compound, while the K_{HSA} for this derivative was found to be significantly lower at 0.91 (F_u = 2.9% in human plasma). Having determined earlier the positive effect of a lipophilic substituent in the ortho position relative to the triple bond, a methyl group was inserted in either the 2 or 4 position of the 3-pyridyl group (respectively, **18d** and **18e**). Compound **18d** was found to be the most active of the pair, with a K_i of 25 nM, indicating that the better relative position of the lipophilic group and the polar nitrogen atom was on the opposite sides of the ring. The positive effect of the lipophilic group could be leveraged by using a bigger alkyl group instead of the methyl, with propyl (**18f**) or isobutyl groups (**18g**) giving rise to single-digit nanomolar compounds, albeit at the cost of a significant increase in albumin binding. Use of a longer, linear alkyl group (like *n*-hexyl, **18h**) had a less pronounced effect. Quite interestingly, the pyridine *N*-oxide derivative **18i** also showed an excellent potency and

Table 3. SAR of Alkynylphenoxyacetic Acids



Cpd.	R	Ki (nM)	KHSA
17a		73	ND
17b		28	0.97
17c		41	ND
17d		72	ND
17e		22	0.98
18a		1120	0.94
18b		117	0.91
18c		601	ND
18d		143	ND
18e		25	0.95
18f		4.3	>0.99
18g		4.9	>0.99
18h		43	ND
18i		17	0.62

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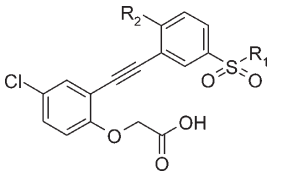
a markedly lower albumin binding. When these pyridine derivatives were tested in a dielectric impedance functional assay, we were surprised to find that they behaved as partial agonists.[24] In antagonist mode (i.e., in the presence of PGD2 at a concentration corresponding to the EC_{80}) they showed only a partial inhibition of the PGD2-induced signal (for example, **18e** gave inhibitions in the range 70−80%), and in agonist mode (i.e., in the absence of PGD2) they all showed a compound-induced response, with an efficacy lower than that of the natural agonist (for example, 20% E_{max} for **18e**). All analogues, independent of the gain of potency linked to the nature of the lipophilic substituent, presented this partial agonist behavior and were not further characterized.

In order to verify the effect of further increasing the polarity on the second aromatic ring on the protein binding, a series of derivatives bearing a sulfone derivative were prepared (Table 4). While the compound bearing the methylsulfone group in the 4-position was not found to be particularly active (**21a**, K_i = 86 nM), the derivatives bearing the sulfone in the 3-position showed an improved potency in the binding assay and a reduced protein binding. For example, **19b** showed a K_i of 9 nM and an improved fraction unbound (K_{HSA} of 0.96; F_u of 0.8%, 1.0%, and 2.4% in human, rat, and mouse, respectively), leading to a much improved activity in the whole blood assay (IC_{50} = 750 nM). The compound also did not show any agonistic activity in the cellular dielectric impedance assay and showed very good stability in human and rat liver microsomes (Cl_{int} of 11 and <10 μL min^{-1} (mg protein)$^{-1}$, respectively) and an excellent thermodynamic solubility in water buffer at pH 7.4 (0.76 mg/mL in 50 mM PBS buffer). In PK experiments, the compound was shown to be a low clearance compound in both mouse and rat (Cl of 0.3 L kg^{-1} h^{-1} and $t_{1/2}$ of 2.2 h in mouse; Cl of 0.4 L kg^{-1} h^{-1} and $t_{1/2}$ of 4.4 h in rat), with a moderate oral bioavailability (F_z of 37% and 28%, respectively) despite a good permeability in the Caco-2 assay (P_{app} = 11.8 × 10^{-6} cm/s).

Exploration of further sulfone derivatives confirmed their high potency. In particular, those compounds bearing an additional lipophilic substituent in the para position with respect to the sulfone group had an additional improvement of potency (see, for example, **19e** vs **19a** and **19k** vs **19b**). This improvement was found to be quite similar in the case of a methyl/alkyl group and a halogen (fluorine or chlorine) and so was attributed to the lipophilic character rather than to an electronic effect on the aromatic ring. Contrary to what had been seen in the case of the pyridine compounds, increasing the length or branching of the alkyl group in this position did not result in a significant increase in potency (see, for example, **19h** vs **19e**). The SAR of the sulfone substituent was found to be relatively flat; the length and branching of an aliphatic sulfone substituent, the presence of an additional aromatic group with or without an alkyl linker, or the presence of alcoholic groups on the chain had very limited effect on the potency of the compounds in the binding competition assay. In contrast, the nature of this substituent had some effect on the behavior of the compounds in the functional assays: two of the methylsulfone compounds (**19f** and **19a** but not **19e**) showed a weak partial agonist behavior (E_{max} of 10−12%) in the cellular dielectric impedance assay, whereas none of the other (i.e., different from methyl) alkyl- or aryl-substituted sulfone compounds showed any agonist behavior. In fact, some compounds (for example, **19k** or **19s**) even showed an inverse agonist behavior in the GPTγS binding assay, even if they normally were not able to inhibit the background activity of the receptor as much as other known CRTH2 antagonists (E_{max} of around −5% to −7% or E_{max} of −12% to −15% for **4** or **5**).

Table 4. SAR of Sulfone−Sulfonamide Compounds



compd	R_1	R_2	K_i (nM)
19a	Me	H	15
19b	Pr	H	9
19c	$CH_2CH_2CH_2OH$	H	28
19d	CH_2CH_2OH	H	10
19e	Me	Me	5
19f	Me	F	3.3
19g	Me	Cl	3.5
19h	Me	nPr	8.6
19i	Me	iPr	4.6
19j	Et	Me	8.6
19k	*n*-Pr	Me	2.0
19l	*i*-Pr	Me	6
19m	*i*-Bu	Me	3.0
19n	CH_2Ph	Me	5
19o	CH_2CH_2Ph	Me	7
19p	Ph	Me	6
19q	CH_2CH_2OH	Me	19
19r	$CH_2CH_2CH_2OH$	Me	8
19s	*n*-Pr	F	3.4
19t	*i*-Pr	F	4.0
19u	*n*-Pr	Cl	2.1
19v	*i*-Pr	Cl	2.9
20a	NMe_2	H	14
20b	NMe_2	Me	5
20c	NMe_2	F	3.1
20d	NEt_2	Me	3.4
20e	NH-*t*-Bu	Me	5
20f	NHMe	Me	10
20g	NHEt	Me	4.4
20h	NH-*i*-Pr	Me	3.4
20i	NMe-*i*-Pr	Me	3.8
20j	NMe-*i*-Bu	Me	4.2
20k	piperidine	Me	5
20l	morpholine	Me	3.6
20m	2-methylpiperidine	Me	1.9
20n	$NMeCH_2CH_2OMe$	Me	4.1
20o	$NHCH_2CH_2OMe$	Me	3.6
20p	*N*-methylpiperazine	Me	21
20q	$NMeCH_2CH_2CH_2NMe_2$	Me	287
20r	$NMeCH_2CH_2NMe_2$	Me	369

Despite the fact that most of these compounds had very close affinities in the binding assay, a more complicated SAR was found when looking at the results of the whole blood assay (Table 5); the presence of the substituent in position 5 had a positive effect when a methyl group or especially a fluorine or chlorine atom

Table 5. Whole Blood Assay Data and Comparison with Affinity and Protein Binding

compd	K_i (nM)	PPB F_u (%), h/r/m	KHSA	whole blood IC_{50} (nM)
19b	9	0.8/1.0/2.4	0.96	750
19d	10	4.7/8.1/30	0.89	990
19e	5	1.7/1.4/2.7	0.96	64
19f	3.3	1.3/1.1/5.4	0.92	95
19g	3.5	0.7/0.3/2.5	0.96	26
19h	8.6	0.2/0.2/0.6	0.98	1200
19i	4.6	0.3/0.4/1.3	0.97	360
19k	2.0	0.4/0.4/0.8	0.98	81
19l	6	ND	0.97	190
19m	3.0	ND	0.98	120
19p	6	ND	>0.99	260
19r	8	1.5/1.8/4.8	0.92	310
19s	3.4	1.0/0.4/0.7	0.97	23
19t	4.0	ND	0.97	91
19u	2.1	0.3/0.2/0.6	0.99	23
19v	2.9	ND	0.98	67
20b	5	0.4/0.4/1	0.94	77
20c	3.1	0.9/0.7/2.9	0.96	23
20g	4.4	0.5/0.8/0.8	0.97	40
20h	3.4	0.4/0.4/1.0	0.97	250
20i	3.8	0.2/ND/0.5	0.99	140
20m	1.9	ND	>0.99	310
20n	4.1	0.6/0.7/0.7	0.98	77
20o	3.6	ND	0.96	88

were employed (see **19k**, **19s**, and **19u** vs **19b**), giving rise to several compounds with IC_{50} < 100 nM, whereas larger groups (e.g., isopropyl, **19i**) were less favorable. Similarly, bulkier groups on the sulfone substituent gave somewhat lower potencies (compare, for example, **19t** to **19s** or **19p** to **19k**). These results could be in part rationalized taking into account the protein binding of the compounds, with compounds like **19p** or **19i** having reduced unbound fraction compared to the most active analogues. However, some other aspects of the SAR could not be readily explained in such a way. For example, **19d**, despite having a good affinity in the binding assay and a larger unbound fraction, proved to be less active. In fact trying to compare the affinity data, corrected by the fraction unbound, of various compounds with the potency in the whole blood assay showed a fairly poor correlation. Using the GTPγS or cellular dielectric impedance data instead of the binding data did not improve the correlation, which was actually significantly worse in the latter case. This seems to point to the presence of a different factor. In our opinion, this could be due to kinetic factors, possibly relating to differences in the K_{off} of the compounds. K_{off} values for the compounds described in this publication have not been determined, but it is interesting to note that **6**, a compound described in the literature as an insurmountable antagonist and whose behavior has been explained as being derived from a very long K_{off},[25] has given in our assay IC_{50} values in the WBA very close to those obtained in the binding assay (IC_{50} = 3 nM in the WBA vs K_i = 5 nM), as if protein binding was not having any effect on it.

A number of these compounds were also profiled in PK experiments in mice (Table 6). Compounds generally showed medium

Table 6. Key Mouse PK Data

compd	Cl (L kg^{-1} h^{-1})	F_z (%)	AUC po (ng·h/mL)[a]
19b	0.3	37	5758
19e	1.4	49	1801
19f	0.4	24	3237
19g	0.7	141	1393[b]
19i	0.5	29	2652
19k	1.0	80	3685
19s	0.4	63	6143
19t	0.35	130	18691
19u	1.0	112	5795
19v	0.7	64	4889
20c	0.6	58	4880
20l	1.9	48	1225
20o	2.0	33	831
21o	0.1	57	30942
23k	1.1	24	1368
23l	8.7	26	151

[a] After a dose of 5 mg/kg po. [b] After a dose of 1 mg/kg po in a cocktail experiment.

Table 7. Stability of Selected Compounds in Human and Rat Liver Microsomes

compd	Cl_{int} (HLM) (μL min^{-1} (mg protein)$^{-1}$)	Cl_{int} (RLM) (μL min^{-1} (mg protein)$^{-1}$)
17b	<10	<10
19b	11	<10
19e	14	18
19k	<10	<10
19s	12	<10
19u	<10	<10
20g	<10	24
20l	<10	10
20b	<10	22
20i	<10	64
20h	<10	12
20c	<10	<10
20o	<10	<10
20n	32	49
20j	79	119
20m	89	418

to moderate clearance values, with a tendency for lower clearances with more hindered substituents on the sulfone (*i*-Pr < *n*-Pr < Me; compare, for example, **19l** Cl = 0.4 L kg^{-1} h^{-1}; **19k** Cl = 1.0 L kg^{-1} h^{-1}; **19e** Cl = 1.4 L kg^{-1} h^{-1}). Concerning the substituent in position 5, the presence of a larger group led in some instances to an increase in clearance compared to a smaller fluorine or hydrogen atom (compare, for example, **19k** Cl = 1.0 L kg^{-1} h^{-1} and **19u** Cl = 1.0 L kg^{-1} h^{-1} with **19b** Cl = 0.3 L kg^{-1} h^{-1}, **19s** Cl = 0.4 L kg^{-1} h^{-1}). However, the trend was not always respected, so, for example, compound **19i**, bearing an isopropyl group, had a lower clearance (Cl = 0.5 L kg^{-1} h^{-1}) than the corresponding compound bearing a methyl group (**19e** Cl = 1.4 L kg^{-1} h^{-1}).

From continuation of the SAR study, moving the sulfone moiety away from the aromatic ring by inserting a methylene

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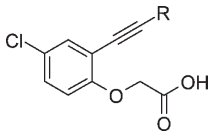
Table 8



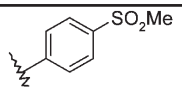
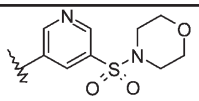
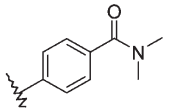
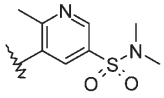
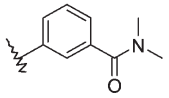
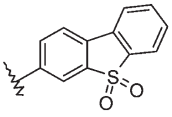
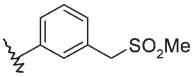
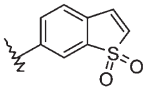
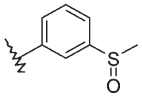
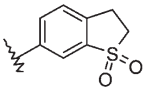
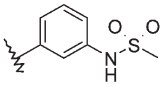
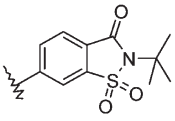
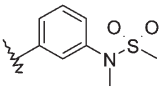
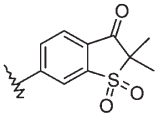
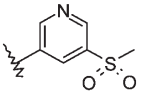
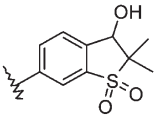
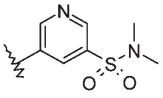
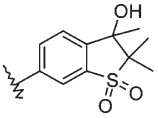
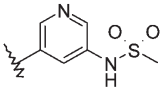
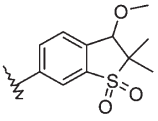
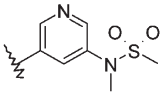
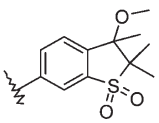
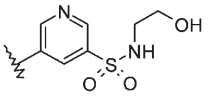
Cpd.	R	Ki (nM)	Cpd.	R	Ki (nM)
21a		86	21m		41
21b		55	21n		41
21c		52	21o		12
21d		48	21p		25
21e		3.2	21q		23
21f		54	21r		14
21g		122	21s		31
21h		60	21t		33
21i		37	21u		28
21j		19	21v		21
21k		378	21w		25
21l		301			

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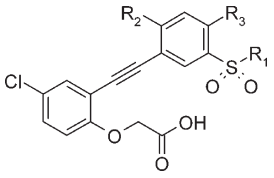
Table 9

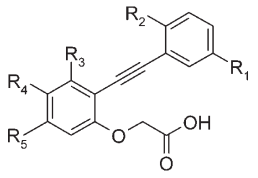


compd	R_3	R_1	R_2	K_i (nM)
22a	phenyl	Me	H	5
22b	4-methoxyphenyl	Me	H	7
22c	3-methoxyphenyl	Me	H	8
22d	4-trifluoromethylphenyl	Me	H	43
22e	4-chlorophenyl	Me	H	14
22f	3-chlorophenyl	Me	H	16
22g	2-chlorophenyl	Me	H	3.5
22h	NHCOMe	*n*-Pr	Me	1.3
22i	$CONMe_2$	*i*-Pr	H	65
22j	$CONEt_2$	*i*-Pr	H	46
22k	CO-morpholine	*i*-Pr	H	87

spacer led to a reduction in activity, with K_i values close to those of the unsubstituted analogue (**19d**, K_i = 48 nM). The sulfone group could be successfully replaced by a sulfoxide (e.g., **21e**) or sulfonamide group (e.g., **20a**), retaining an excellent level of potency. The positive effect of a lipophilic group para to the sulfonamide was confirmed (compare **20b** and **20c** vs **20a**), as well as the possibility of introducing very diverse substituents on the sulfonamide side chain. Only the presence of an ionizable nitrogen (**20p** and more significantly **20q** and **20r**) caused a significant decrease of potency, among all the substituents explored. Similar to what had been observed for the methylenesulfone compound **19d**, reversed sulfonamides were significantly less potent (**27f**, **21g**). Replacement of the sulfone/sulfonamide derivative with an amide group, in either the 3- or 4- position (**21b**,**c**) led to a significant reduction in potency, showing that the geometry of the SO_2 group (tetrahedral geometry vs the planar trigonal structure of the amide group) and possibly its electronic properties are crucial to the potency increase compared to the nonsubstituted structure. All these data together seem to indicate that one of the oxygen atoms of the sulfone/sulfoxide/sulfonamide group is involved in a direct interaction with one of the residues of the receptor.

While being relatively unimportant for the affinity, the nature of the substituents on the sulfonamide was found to be important in relation to oxidative metabolism. Whereas all the sulfone derivatives tested in the microsome stability studies had proved to be very stable, independent of the substitution, for the sulfonamide derivatives the situation was more diverse (Table 7). The compounds derived from a primary amine were all found to be stable, but among the compounds derived from a secondary amine some were found to be significantly less stable (for example, **20m** and **20j**), whereas derivative **20c** was found to be stable, despite the *N*,*N*-dimethylamino substitution. Compound **20c** was further profiled in a PK study in mice and was found to have moderate clearance (Cl = 0.6 L kg^{-1} h^{-1}) and good oral bioavailability (F_z = 58%), resulting in a satisfactory exposure after oral dosing (AUC_z = 4880 h·ng/mL for a dose of 5 mg/kg). In contrast, compounds **20o** and **20l** showed a higher clearance in vivo (Cl of 2.0 and 1.9 L kg^{-1} h^{-1}, respectively) despite being highly stable in the microsomes. **20c** was also found to be the most active

Table 10. SAR of First Aromatic Ring



compd	R_3	R_4	R_5	R_1	R_2	K_i (nM)
23a	H	H	H	H	Cl	747
23b	H	Me	H	H	Cl	283
23c	H	F	H	SO_2Pr	H	65
23d	H	H	F	H	Cl	4780
23e	Cl	H	H	SO_2Pr	H	55
23f	naphthyl		H	SO_2Pr	H	44
23g	H	CF_3	H	H	Cl	12
23h	H	CF_3	H	SO_2Pr	H	10
23i	H	CN	H	H	Cl	24
23j	H	CN	H	SO_2Pr	H	21
23k	H	CN	H	SO_2Pr	Me	7
23l	H	CN	H	SO_2Me	*i*Pr	12
27a	phenyl	H	H	SO_2Pr	H	19
27b	2,4-dimethyl-thiazol-5-yl	H	H	SO_2Pr	H	54
27c	3-thienyl	H	H	SO_2Pr	H	18
27d	2-thienyl	H	H	SO_2Pr	H	12

of the sulfonamide compounds in the WBA, with an IC_{50} of 23 nM.

A number of compounds combining the pyridine and sulfonamide/sulfone moieties were also prepared (Table 8). Some of the compounds showed interesting activities (see, for example, **21i**, K_i = 37 nM and **21h**, K_i = 60 nM). In this case, not only the compounds with the sulfone/sulfonamide groups were active but also some compounds bearing a reverse sulfonamide moiety (for example, **21j**, K_i = 19 nM but not **21k**, K_i = 378 nM). In particular, **21i** was further characterized. The compound showed a good in vitro and in vivo ADME profile, with low clearance in human and rat liver microsomes, good thermodynamic solubility in aqueous solution at pH 7.4 (0.193 mg/mL), and a relatively high unbound fraction (F_u of 3.3%, 3.4%, and 5.7% in human, mouse, and rat plasma, respectively). In mice, the compound was shown to have a moderate clearance (Cl of 0.8 L kg^{-1} h^{-1} and $t_{1/2}$ of 4.4 h) and a surprisingly high oral bioavailability (F_z of 95%), considering its low log $D_{7.4}$ of −1.4 and correspondingly low to medium permeability in Caco-2 cells (P_{app} = 1.8 × 10^{-6} cm/s). However, the compound had a moderate potency in the whole blood assay (IC_{50} = 289 nM) because of its moderate affinity for the receptor, and the analogues prepared with the aim of increasing this affinity (e.g., **21n**) failed to achieve a level of affinity similar to those of the phenylsulfone derivatives. Interestingly, the compound was found to be one of the most effective inverse agonists in the GTPγS assay (agonist mode), giving an E_{max} comparable to that of **4** or **5**.

Continuing to explore possible substitution patterns around the sulfone moiety, we discovered that the tricyclic compounds **21o**, in which the sulfone was part of a cycle, retained a high affinity for the receptor (K_i = 12 nM). This compound also showed excellent behavior in a mouse PK experiment, with low clearance (Cl = 0.1 L kg^{-1} h^{-1}) and a good oral bioavailability (F_z = 57%). Unfortunately, the compound also had poor potency

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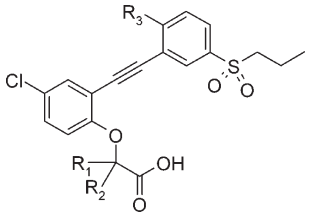
Table 11. Effect of Substitution at Postion α to the Carboxylic Acid



compd	R_1	R_2	R_3	K_i (nM)
29a	Me	H	F	3.0
29b	Me	Me	F	260
32a	Me	H	Me	1.2
32b	Et	H	Me	30
32c	*n*-Pr	H	Me	57
32d	*i*-Bu	H	Me	515

in the WBA (IC_{50} = 4.3 μM) due to its very high protein binding (F_u < 0.1% in human plasma, K_{HSA} > 0.99). We explored several other cyclic derivatives (Table 8) lacking the third aromatic ring but with different steric hindrances and the presence or absence of additional HBD/HBA groups (**21p**−**w**), but all the compounds could not improve over **21o** in terms of affinity and all gave K_i values within a fairly narrow range (from 14 nM for **21r** to 33 nM for **21t**). Another strategy for improving the potency of **21o** was attempted, disconnecting the bond between the sulfone group and the extra aromatic ring, giving rise to compounds **22a**−**g** (Table 9). In this case, the variation of affinity among the different compounds was more noticeable (from 3.5 nM for **22g** to 43 nM for **22d**) and several single-digit nanomolar antagonists could be discovered, depending probably on both the electronic properties of the second ring and the relative geometry of the two rings (electron-donating substituents seem to be favored (see, for example **22b** vs **22d**), but the good activity of **22g** despite the chlorine substituent could be linked to a difference in geometry induced by the ortho substitution), even if not enough compounds of this type were prepared to draw a definitive conclusion.

Replacing the extra aromatic ring with an amide group (Table 9) led to a moderate loss of potency when the amide was of the type $ArCONR_1R_2$ (**22i**−**k**) but led to an increase of potency when the amide was of the type $ArNHCOR_1$ (**22h**, K_i = 1.3 nM), although some of the affinity is probably given by the methyl group in the 5- position.

In the meantime, the SAR of the first aromatic ring had also been explored (Table 10). Removing the chloro substituent (**23a**) resulted in a marked decrease of potency, similarly observed by replacing it with a methyl group (**23b**). Replacement with a fluorine atom was better tolerated (**23c**) but still with loss of almost an order of magnitude of affinity. We also verified that the 4- position was indeed the optimal position for the halogen substituent and found that moving the halogen to the 3- or 5-position led to a decrease of potency (**23d** and **23e**). Replacing the chloroaryl group by a naphthyl group also led to a reduction in potency, although less marked (**23f**).

Better results were obtained with a trifluoromethyl or nitrile groups, which gave compounds with K_i values very close to those of their chloro-substituted analogues. However, several of these these compounds showed a partial agonist behavior

Table 12. Pharmacological and ADME Profile of 19k and 19s

parameter	19k	19s
K_i (hCRTH2) (nM)	2.0	3.4
MW	406.9	410.8
K_i (mCRTH2) (nM)	2.4	0.37
IC_{50} GTPγS (nM)	7	8
IC_{50} CDI (nM)	32	28
IC_{50} WBA (nM)	80	23
Cl (HLM/RLM) (μL min^{-1} (mg protein)$^{-1}$)	<10/<10	12/<10
solubility (pH 7.4/pH 1.2) (μg/mL)	242/< 2	not determined
Caco-2 P_{app} (10^{-6} cm/s)	5.2	10.8
PPB F_u, h/r/m (%)	0.4/0.4/0.8	1.0/0.4/0.7
selected PK parameter (mouse)	**19k**	**19s**
Cl (L kg^{-1} h^{-1})	1.0	0.4
F_z (%)	80	63
$t_{1/2}$, iv/po (h)	1.7/3.9	2.0/1.9
AUC, po (ng·h/mL)	3730	6143

(for example, **23g** with an E_{max} of 60% and **23j** with an E_{max} of 30% but not **23k**). In addition, some of these compounds were tested in PK experiments in mice and found to have an inferior pharmacokinetic profile compared with the corresponding chloro compounds (Table 5). For example, **23l** showed a very high clearance (Cl = 8.7 L kg^{-1} h^{-1} compared to Cl = 0.5 L kg^{-1} h^{-1} for analogue **19i**). The best of the nitrile-subsituted compounds was **23k**, which showed a clearance of 1.1 L kg^{-1} h^{-1} but a lower oral bioavailability compared to **19k** (F_z of 24% vs 80%)

The halogen atom could also be replaced by an additional aryl or heteroaryl group (**27a**−**d**) with, in some cases, a good level of potency (Table 10). Here again, however, the compounds were found to be partial agonists, for example, with an E_{max} of 68% (cellular dielectric impedance assay) for **27a**. While the relationship between structure and agonistic activity within this series of compounds has proven to be quite complex, the available body of data clearly indicates a correlation with the size of the group in the 4-position of the first aromatic ring. The presence of a lipophilic substituent in the 5-position of the second aromatic ring and possibly the presence of a group larger than a methyl on the sulfone in position 3 seem to have a negative effect on the agonistic behavior. However, the interplay between these factors is quite complex and it has not been possible to consistently predict the partial agonist behavior of compounds. We have not performed receptor modeling studies, but the available data on partial agonism in this series, which seems to depend on specific substitution patterns, could be of high interest to studies on the mechanism of GPCR activation.

Finally, the position α to the carbocylic acid moiety was explored (Table 11). Contrary to what we had observed for another chemical series,[17c] in this case some level of substitution in this position is allowed. Indeed, inserting a methyl group actually has a neutral or even positive impact on potency (**29a** vs **19s**, **32a** vs **19k**). However, even small increases in the size of this substituent (such as ethyl, **32b**) or adding a second methyl group (**29b**) rapidly led to the loss of at least 1 order of magnitude in affinity. However, the possibility of including a methyl group in this position could potentially have a significant effect on the in vivo behavior of the compound, as the steric hindrance at the position α to a carboxylic acid moiety is well-known to have a

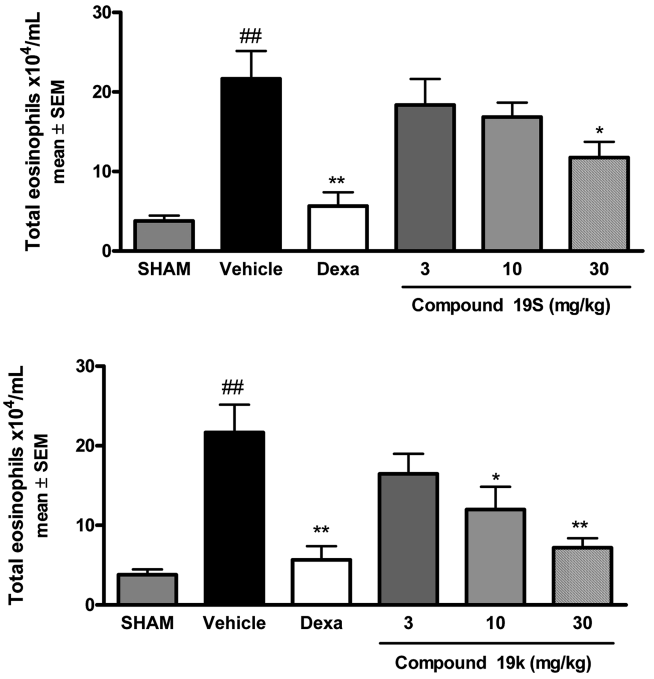


Figure 2. Ovalbumin induced airway eosinophilia in mouse. The compounds were dosed orally 1 h prior to and 7 h after each aerosol challenge. Eosinophils in BALF were enumerated 8 h after the last challenge. (a, top) Compound **19k** significantly inhibited eosinophilia by 54% at 10 mg/kg and 81% at 30 mg/kg. (b, bottom) Compound **19s** significantly inhibited eosinophilia by 55% at 30 mg/kg. Shown are the results from one way ANOVA with Dunnett's multiple comparison post test: (##) $P < 0.01$ compared with sham; (*) $P < 0.05$ compared with vehicle; (**) $P < 0.01$ compared with vehicle.

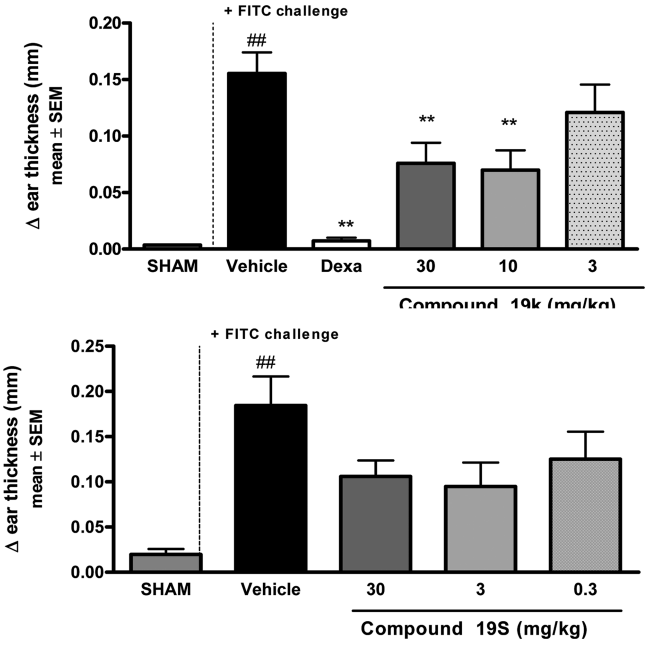


Figure 3. FITC induced contact hypersensitivity response. Ear swelling 24 h after FITC challenge. The reference compound dexamethasone, dosed ip at 5 mg/kg 1 h before and 7 h after the challenge, completely inhibits the ear swelling response. (a, top) Compound **19k** dosed at the same time points po significantly inhibited the ear swelling by 52% at 30 mg/kg and 56% at 10 mg/kg. (b) Compound **19s** dosed at the same time points po inhibited the ear swelling by 48% at 30 mg/kg and 54% at 3 mg/kg. Statistical test is one-way ANOVA with Dunnett's post test: (##) $P < 0.01$ compared with sham group; (**) $P < 0.01$ compared with vehicle treated group.

profound impact on the rate of acid glucuronidation and on the stability of the resulting acylglucuronide adducts.

Pharmacology. On the basis of the SAR information available, compounds **19k** and **19s** were selected for in vivo testing, based on the potency in the whole blood assay and on favorable PK properties (see Table 12 for a summary of data on these two compounds). Prior to testing these compounds in vivo, it was decided to verify if the excellent selectivity profile of the hit compound **8c** had been maintained during the optimization. To this end, **19k** was profiled on a panel of 50 receptors and ion channels, and an inhibition of >50% (at 10 μM) was only observed for EP2 (54% inhibition of receptor binding). For the closely associated DP1 receptor, some affinity was also observed, with a K_i of 1.58 μM, giving a selectivity factor of over 500. In a functional assay (cAMP) **19k** was found to be an antagonist of the DP1 receptor, with an IC_{50} of around 10 μM. Finally, **19k** was also tested on aldose reductase (AR), an enzyme that is known to have similar pharmacophoric characteristics. Indeed, **19k** was found to be active on AR, with an IC_{50} of 172 nM. On the basis of the 50- to 100-fold selectivity ratio, the intracellular nature of the target, and the benign nature of the AR antagonists tested in several clinical trials (for various diabetes indications), this was not considered a critical issue for the compound. Finally, both compounds were tested for affinity on the mouse orthologue of the CRTH2 receptor and found to have high affinity.

As CRTH2 antagonism could be of benefit for both asthma and atopic dermatitis, it was decided to test both compounds in two different proof-of-principle (PoP) models, each representative for one of the two diseases. The first model used was an ovalbumin-induced lung eosinophilia model in mice, which was selected based on the known importance of CRTH2 activation for the migration of eosinophils as well as the marked airway eosinophilia observed in asthma patients.[2,3,8] Briefly, BALB/c mice were immunized with ovalbumin (OVA) ip on days 0 and 7, then challenged with aerosolized OVA for 30 min on days 15, 16, and 17. The compounds were dosed orally 1 h prior to and 7 h after each challenge. The mice were sacrificed 8 h after the last challenge, and the number of eosinophils as well as total cells in the bronchoalveolar lavage fluid (BALF) was measured. Both compounds were found to be able to significantly decrease the number of eosinophils infiltrating the BALF in a dose-proportional manner, with a dose of 30 mg/kg po of **19k** inhibiting lung eosinophilia at the same level as dexamethasone (1 mg/kg ip) (Figure 2).

As a PoP more representative for AD, the acute FITC (fluorescein isothiocyanate) mediated contact hypersensitivity (CHS) model was selected.[12c] After sensitizing BALB/c mice on days 0 and 1 by painting the abdomen with FITC in acetone/dibutylphthalate (A/DBP), the mice were challenged on day 6 by applying the FITC in A/DBP on one ear. The compounds were administered 1 h before and 7 h after challenge. The ear swelling of the treated ear vs the untreated ear was measured before and 24 h after the challenge. Both compounds showed a statistically significant inhibition of the ear swelling at doses of 10 and 30 mg/kg (not at 3 mg/kg) (Figure 3), but the establishment of a dose response was more complicated, as the doses at 30 mg/kg were not found to be more active than those at 10 mg/kg, with both giving inhibitions in the range of 45−60%.

In fact, all CRTH2 antagonists that were tested in this assay, including those from different chemical classes, were found to have the same behavior, with a plateau of activity indicating that several mechanisms are involved in the FITC-induced ear swelling response, some of which are not impacted by CRTH2 antagonism.

CONCLUSION

A novel series of phenoxyacetic acid CRTH2 antagonists have been identified. Optimization had given several compounds with excellent potencies (41 compounds with K_i < 10 nM), including several with excellent potencies in a whole blood assay. Optimization of the PK characteristics led to the identification of several compounds suitable for in vivo testing. Of these, **19k** and **19s** were tested in two different pharmacological models and found to be active after oral dosing (10 and 30 mg/kg).

GENERAL EXPERIMENTAL METHODS: PROCEDURES

Melting points were measured with a Büchi melting point B-545 apparatus and were uncorrected. NMR spectra were recorded on a Bruker DPX-300 MHz spectrometer. Data were reported as follows: chemical shift in ppm using residual DMSO (2.49 ppm), $CHCl_3$ (7.24 ppm), or MeOH (3.35 ppm) as internal standards on the δ scale; multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet); coupling constant (J) in hertz; and integration. MS data provided were obtained using a Waters ZMD mass spectrometer operated in positive or negative electrospray. Purity for all final compounds was determined by HPLC and was generally >95%. Analytical HPLC method A parameters were the following: Xbridge C_8 50 mm × 4.6 mm at a flow of 2 mL/min; 8 min gradient from 0.1% TFA in H_2O to 0.07% TFA in CH_3CN. Analytical HPLC method B parameters were the following: column Waters ACQUITY UPLC BEH C_{18} 50 mm × 2.1 mm, 1.7 μm, at a flow of 1 mL/min; 3 min gradient from 95% (10 mM NH_4OAc in H_2O)/5% CH_3CN to 100% CH_3CN; UV detection max plot at 230−400 nm for all conditions. Reactions were not optimized with respect to maximizing the yields.

***tert*-Butyl (2-Bromo-4-chlorophenoxy)acetate (10a).** A solution of 2-bromo-4-chlorophenol (13.1 g, 63.2 mmol) in acetone (100 mL) was treated with potassium carbonate (9.61 g, 69.5 mmol), stirred for 10 min, then treated with *tert*-butyl bromoacetate (9.34 mL, 63.2 mmol). The reaction mixture was stirred at 65 °C for 18 h. Then the mixture was filtered, the solid was washed with acetone, and the filtrate was concentrated to dryness under vacuum to give the title compound as a yellow sticky solid (19.4 g, 95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 7.68 (1H, d, J = 2.6 Hz), 7.39 (1H, dd, J = 9.0 Hz, J = 2.6 Hz), 7.37 (1H, d, J = 9.0 Hz), 4.80 (2H, s), 1.41 (9H, s). MS (ESI$^+$): 340.1 (M + NH_4^+). HPLC (condition A): t_R = 5.06 min (HPLC purity 96.8%).

Sonogashira General Method 1. A solution of *tert*-butyl (2-bromo-4-chlorophenoxy)acetate (**10a**, 350 mg, 1.09 mmol) and arylacetylene (1.20 mmol) in degassed, anhydrous CH_3CN (2.80 mL) was treated with $Pd(PPh_3)Cl_2$ (38 mg, 0.05 mmol), CuI (10 mg, 0.05 mmol), and TEA (0.45 mL, 3.26 mmol). The reaction mixture was heated at 50 °C under stirring for 16 h. The intermediate *tert*-butyl ester compounds were isolated after aqueous workup and silica chromatography (eluting with cyclohexane/EtOAc gradients), or the solvent was evaporated and the crude residue used directly for the deprotection. The crude products were normally purified either by preparative HPLC or by precipitation/recrystallization from appropriate solvents.

Sonogashira General Method 2. A mixture of aryl halide (0.82 mmol), *tert*-butyl (4-chloro-2-ethynylphenoxy)acetate (**16a**, 200 mg, 0.75 mmol), $Pd(PPh_3)Cl_2$ (33 mg, 0.04 mmol), and CuI (8.6 mg, 0.04 mmol) was degassed for 2 min under nitrogen. Then THF (3 mL) and TEA (208 μL, 1.50 mmol) were added and reaction mixture was stirred at 60 °C for 16 h. The intermediate *tert*-butyl ester compounds were isolated after aqueous workup and silica chromatography (eluting with cyclohexane/EtOAc gradients), or the solvent was evaporated and the crude residue used directly for the deprotection. The crude products were normally purified either by preparative HPLC or by precipitation/recrystallization from appropriate solvents.

Sonogashira General Methods 3 and 4. A mixture of aryl halide (1.87 mmol), *tert*-butyl (4-chloro-2-ethynylphenoxy)acetate (**16a**, 500 mg, 1.87 mmol), $Pd(PPh_3)Cl_2$ (52 mg, 0.07 mmol), and piperidine (550 μL, 5.6 mmol, method 3) or TEA (780 μL, 5.6 mmol, method 4) was heated at 70 °C (method 3) or 60 °C (method 4) for 18 h. The reaction mixture was taken up in EtOAc, washed twice with saturated ammonium acetate and once with brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated to dryness affording a crude, which was purified by flash column chromatography (silica), eluting with a cyclohexane/EtOAc gradient. The purified intermediate *tert*-butyl ester was dissolved in a minimum amount of DCM and treated with HCl (4 N) in dioxane for 16−18 h at room temperature. After evaporation of the solvents, the crude products were normally purified either by preparative HPLC or by precipitation/recrystallization from appropriate solvents.

Sonogashira General Method 5. A suspension of (4-chloro-2-ethynylphenoxy)acetic acid *tert*-butyl ester (**16a**, 200 mg, 0.78 mmol), aryl halide (0.78 mmol), $Pd(dppf)Cl_2$ (34 mg, 0.05 mmol), and CuI (9 mg, 0.05 mmol) was degassed for 2 min under nitrogen. Then anhydrous THF (3 mL) and TEA (215 μL, 1.55 mmol) were added and the reaction mixture was stirred at 60 °C for 16 h. The solvents were removed under reduced pressure, and the residue was was purified by flash column chromatography (silica), eluting with a cyclohexane/EtOAc gradient. The purified intermediate *tert*-butyl ester was dissolved in a minimum amount of DCM and treated with HCl (4 N) in dioxane for 16−18 h at room temperature. After evaporation of the solvents, the crude products were normally purified either by preparative HPLC or by precipitation/recrystallization from appropriate solvents.

{4-Chloro-2-[(3-chlorophenyl)ethynyl]phenoxy}acetic Acid (8c). **8c** was prepared according to Sonogashira general method 1, starting from *tert*-butyl (2-bromo-4-chlorophenoxy)acetate (**10a**) and 3′-chlorophenyl acetylene, without purification of the intermediate ester, and deprotecting using TFA (20%) in DCM. After purification by preparative HPLC the title compound was obtained as a brown solid in 11% overall yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 13.19 (1H, s), 7.62 (1H, m), 7.58 (1H, d, J = 2.7 Hz), 7.45−7.54 (3H, m), 7.42 (1H, dd, J = 9.1, J = 2.7 Hz), 7.01 (1H, d, J = 9.1 Hz), 4.83 (2H, s). MS (ESI$^-$): 319.0. HPLC (condition A): t_R = 4.91 min (HPLC purity 100%).

***tert*-Butyl {4-Chloro-2-[(trimethylsilyl)ethynyl]phenoxy}acetate (15a).** A solution of *tert*-butyl (2-bromo-4-chlorophenoxy)acetate (**10a**, 19.40 g, 60.3 mmol) and $Pd(dppf)Cl_2$ (2.65 g, 3.62 mmol) in THF (290 mL) was degassed for 2 min under nitrogen. Then triethylamine (12.5 mL, 91 mmol) and (trimethylsilyl)acetylene (10.2 mL, 72.4 mmol) were added. The reaction mixture was stirred under nitrogen for 10 min before being treated with CuI (689 mg, 3.62 mmol) and triethylamine (12.5 mL, 90.5 mmol) and stirred at 60 °C for 24 h. The reaction mixture was filtered through Celite, and the cake of Celite was washed with EtOAc. The resulting filtrate was washed with HCl (1 N) and brine, dried over $MgSO_4$, filtered, and concentrated to dryness affording a dark brown sticky solid, which was suspended in petroleum ether (350 mL). The resulting precipitate was filtered, washed with petroleum ether (2 × 150 mL) and the filtrated was concentrated to dryness affording the crude product (17.7 g, 87%) as a brown oil . ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 7.43 (1H, d, J = 2.7 Hz), 7.38 (1H, dd, J = 8.9, J = 2.7 Hz), 6.92 (1H, d, J = 8.9 Hz), 4.74 (2H, s), 1.43 (9H, s), 0.23 (9H, s). MS (ESI$^+$): 356.2 (M + NH_4^+). HPLC (condition A): t_R = 6.32 min.

***tert*-Butyl (4-Chloro-2-ethynylphenoxy)acetate (16a).** A solution of *tert*-butyl {4-chloro-2-[(trimethylsilyl)ethynyl]phenoxy}

acetate (**15a**, 17.70 g, 52.2 mmol) in THF (180 mL) was treated with TBAF·$3H_2O$ (16.48 g, 52.2 mmol). The reaction mixture was stirred for 4 h. Then ethyl acetate (450 mL) was added and the organic phase was washed with water (750 mL) and then with brine (750 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum to give a brown oily residue which was purified by flash chromatography (silica), eluting with cyclohexane containing increasing amounts of ethyl acetate. The title compound was obtained as a brown oil (5.15 g, 37%) which solidified after prolonged standing. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 7.47 (1H, d, J = 2.7 Hz), 7.39 (1H, dd, J = 9.0, J = 2.7 Hz), 6.93 (1H, d, J = 9.0 Hz), 4.77 (2H, s), 4.39 (1H, s), 1.42 (9H, s). MS (ESI$^+$): 267.1. HPLC (condition A): t_R = 4.79 min (HPLC purity 95.5%).

[4-Chloro-2-(pyridin-3-ylethynyl)phenoxy]acetic Acid (18b). **18b** was prepared according to Sonogashira general method 2, starting from *tert*-butyl (4-chloro-2-ethynylphenoxy)acetate (**16a**) and 3-bromopyridine, without purification of the intermediate ester, and deprotecting using HCl (4 N) in dioxane. After purification by preparative HPLC the title compound was obtained as an off-white solid in 21% overall yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 13.17 (1H, s), 8.75 (1H, d, J = 2.1 Hz), 8.61 (1H, dd, J = 4.9, J = 1.8 Hz), 7.97 (1H, dt, J = 7.9, J = 1.8 Hz), 7.61 (1H, d, J = 2.7 Hz), 7.52−7.42 (2H, m), 7.02 (1H, d, J = 9.0 Hz), 4.85 (2H, s). MS (ESI$^+$): 288.0. HPLC (condition A): t_R = 2.56 min (HPLC purity 98.1%).

1-Bromo-3-(propylsulfonyl)benzene (12a). A solution of 3-bromobenzenethiol (5.00 g, 26.4 mmol) in anhydrous DMF (30 mL) was treated with K_2CO_3 (7.30 g, 52.8 mmol) followed by 1-bromopropane (3.90 g, 31.7 mmol), and the mixture was heated to about 50 °C under nitrogen for 12 h. The solvent was distilled out completely, and the residue was dissolved in DCM and washed with water and brine. The organic layer was dried over sodium sulfate and evaporated to afford 5.50 g (90%) of 1-bromo-3-(propylthio) benzene as pale yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] 7.44 (1H, s), 7.29−7.27 (1H, m), 7.24−7.21 (1H, m), 7.15−7.11 (1H, m), 2.90 (2H, t) 1.73−1.64 (2H, m), 1.04 (3H, t).

A solution of 1-bromo-3-(propylthio)benzene (5.50 g, 23.7 mmol) in DCM (75 mL) was treated with *m*-chloroperbenzoic acid (12.3 g, 71.3 mmol) and stirred at room temperature for 5 h. The solid formed was filtered off, and the filtrate was washed with 10% solution of sodium bicarbonate, water, and brine. The organic layer was dried over Na_2SO_4 and evaporated to afford 5.7 g (91%) of the title compound as a yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] 8.05 (1H, s), 7.85−7.83 (1H, m), 7.80−7.77 (1H, m), 7.45 (1H, t), 3.10−3.06 (2H, m), 1.80−1.71 (2H, m), 1.02 (3H, t). HPLC (condition D): t_R = 3.54 min (HPLC purity 97.4%).

(4-Chloro-2-{[3-(propylsulfonyl)phenyl]ethynyl}phenoxy)-acetic Acid (19b). Prepared according to Sonogashira general method 3, starting from *tert*-butyl (4-chloro-2-ethynylphenoxy)acetate (**16a**) and 1-bromo-3-(propylsulfonyl)benzene (**12a**), the title compound was obtained as a beige solid in 34% overall yield after purification by preparative HPLC. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 13.17 (1H, s), 8.01 (1H, t, J = 1.6 Hz), 7.94−7.88 (2H, m), 7.73 (1H, t, J = 7.6 Hz), 7.64 (1H, d, J = 2.7 Hz), 7.44 (1H, dd, J = 9.0, J = 2.7 Hz), 7.02 (1H, d, J = 9.0 Hz), 4.83 (2H, s), 3.39−3.34 (2H, m), 1.57 (2H, sext, J = 7.6 Hz), 0.93 (3H, t, J = 7.6 Hz). MS (ESI$^-$): 391.3. HPLC (condition A): t_R = 4.27 min (HPLC purity 99.8%).

1-Methyl-4-(propylsulfonyl)benzene (46a). A cooled (0 °C) solution of 4-methylthiophenol (20.0 g, 161 mmol) in MeOH (400 mL) was treated with a 5 N solution of NaOH in water (40 mL) and with 1-iodopropane (18.0 mL, 185 mmol). The mixture was stirred at 0 °C for 1 h and then concentrated under reduced pressure. The concentrated solution was diluted with EtOAc and then washed with brine. The organic phase was dried on $MgSO_4$, filtered, and concentrated under reduced pressure to give a residue, which was dissolved in DCM (200 mL) and cooled to 0 °C. This solution was treated over 20 min with a suspension of 3-chloroperbenzoic acid (83.12 g, 337.2 mmol) in DCM (600 mL). The reaction suspension was stirred at 0 °C for 3 h and then treated with a further portion of 3-chloroperbenzoic acid (18.86 g, 76.52 mmol) in DCM (150 mL). The mixture was warmed to room temperature and stirred for 16 h. The reaction solution was filtered and the filtrate reduced in volume under reduced pressure and diluted with EtOAc, then washed twice with a 1 N solution of NaOH in water and then brine. The organic phase was dried on $MgSO_4$, filtered, and concentrated under reduced pressure to give the title compound (24.80 g, 78%) as an oil which solidified upon standing. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 7.76 (2H, d, J = 8.1 Hz), 7.45 (2H, d, J = 8.1 Hz), 3.31−3.15 (2H, m), 2.41 (3H, s), 1.63−1.42 (2H, m), 0.89 (3H, t, J = 7.4 Hz). HPLC (condition A) purity 95.4%, t_R = 1.4 min.

2-Bromo-1-methyl-4-(propylsulfonyl)benzene (12k). A finely ground mixture of 1-methyl-4-(propylsulfonyl)benzene (**46a**, 24.80 g, 0.13 mol) and *N*-bromosuccinimide (26.8 g, 0.15 mol) was treated with concentrated sulfuric acid (115 mL, 2.15 mol). The reaction mixture was stirred for 16 h and then treated with a further portion of *N*-bromosuccinimide (1.33 g, 0.01 mol). The reaction solution was stirred for 1 h and then carefully poured into 800 mL of crushed ice. The aqueous solution was extracted with 400 mL of AcOEt. The layers were separated, and the organic phase was washed first with ∼300 mL of brine, then twice with a 1 N solution of NaOH in water and twice with brine. The organic phase was dried on $MgSO_4$, filtered, and concentrated under reduced pressure to give the title compound (29.3 g, 85%) as a brown oil which solidified upon standing. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 8.03 (1H, d, J = 1.9 Hz), 7.80 (1H, dd, J = 8.0, 1.9 Hz), 7.64 (1H, d, J = 8.0 Hz), 3.39−3.28 (2H, m), 2.45 (3H, s), 1.63−1.47 (2H, m), 0.92 (3H, t, J = 7.4 Hz). HPLC (condition A) t_R = 3.8 min.

(4-Chloro-2-{[2-methyl-5-(propylsulfonyl)phenyl]ethynyl}-phenoxy)acetic Acid (19k). A mixture of 2-bromo-1-methyl-4-(propylsulfonyl)benzene (**12k**, 14.86 g, 53.6 mmol), *tert*-butyl (4-chloro-2-ethynylphenoxy)acetate (**16a**, 13.00 g, 48.7 mmol), $Pd(PPh_3)Cl_2$ (1.37 g, 1.95 mmol), and piperidine (14.5 mL) was heated at 70 °C for 18 h. The reaction mixture was taken up in EtOAc, washed twice with ammonium chloride, and once with brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated to dryness affording a crude, which was purified by flash column chromatography (cyclohexane/EtOAc gradient). The compound thus obtained as a brown sticky solid was recrystallized twice from EtOAc/petroleum ether to afford *tert*-butyl (4-chloro-2-{[2-methyl-5-(propylsulfonyl)phenyl]ethynyl}phenoxy)-acetate as a beige solid (10.16 g, 45%). ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 7.95 (1H, d, J = 1.9 Hz), 7.80 (1H, dd, J = 8.0 Hz, J = 1.9 Hz), 7.65 (1H, d, J = 2.7 Hz), 7.62 (1H, d, J = 8.0 Hz), 7.45 (1H, dd, J = 9.0 Hz, J = 2.7 Hz), 7.04 (1H, d, J = 9.0 Hz), 4.82 (2H, s), 3.31 (2H, m), 2.58 (3H, s), 1.55 (2H, sext, J = 7.5 Hz), 1.43 (9H, s), 0.92 (3H, t, J = 7.5 Hz). HPLC (condition A) purity 98.5%, t_R = 5.8 min.

A solution of *tert*-butyl (4-chloro-2-{[2-methyl-5-(propylsulfonyl)-phenyl]ethynyl}phenoxy)acetate (10.16 g, 21.94 mmol) in DCM (70 mL) was treated with a 4 N solution of HCl in dioxane (165 mL, 660 mmol), and the mixture was stirred at room temperature for 2 days. The reaction mixture was concentrated to dryness and the crude product recrystallized from ACN, affording the title compound as a white solid (7.32 g, 82%). ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 13.17 (1H, bs), 7.95 (1H, t, J = 2.0 Hz), 7.80 (1H, dd, J = 8.0 Hz, J = 2.0 Hz), 7.65 (1H, d, J = 2.7 Hz), 7.62 (1H, d, J = 8.0 Hz), 7.45 (1H, dd, J = 9.0 Hz, J = 2.7 Hz), 7.04 (1H, d, J = 9.0 Hz), 4.84 (2H, s), 3.32 (2H, m), 2.58 (3H, s), 1.55 (2H, sext, J = 7.5 Hz), 0.92 (3H, t, J = 7.5 Hz). MS (ESI$^-$): 405.2. HPLC (condition A): t_R = 4.61 min (HPLC purity 98.6%).

(4-Chloro-2-{[2-fluoro-5-(propylsulfonyl)phenyl]ethynyl}-phenoxy)acetic Acid (19s). Prepared according to Sonogashira general method 4, starting from *tert*-butyl (4-chloro-2-ethynylphenoxy)-acetate (**16a**) and 2-bromo-1-fluoro-4-(propylsulfonyl)benzene (**12l**), the title compound was obtained as a beige solid in 25% overall yield. ^{1}H

NMR (300 MHz, DMSO-d_6) δ [ppm] 13.18 (1H, bs), 8.13 (1H, dd, J = 6.5 Hz, J = 2.4 Hz), 7.95 (1H, ddd, J = 8.7 Hz, J = 4.6 Hz, J = 2.4 Hz), 7.68−7.62 (2H, m), 7.47 (1H, dd, J = 9.0 Hz, J = 2.7 Hz), 7.04 (1H, d, J = 9.0 Hz), 4.86 (2H, s), 3.38 (2H, m), 1.58 (2H, m), 0.93 (3H, t, J = 7.5 Hz). MS (ESI$^-$): 409.1. HPLC (condition A): t_R = 4.48 min (HPLC purity 96.1%).

3-Bromo-4-fluoro-*N*,*N*-dimethylbenzenesulfonamide (12p). A cooled (0 °C) solution of 4-fluorobenzenesulfonyl chloride (2.00 g, 10.3 mmol) in THF (40 mL) is treated with a 2 M solution of dimethylamine in THF (11.3 mL, 22.6 mmol) and stirred at 0 °C for 30 min. The solvents were removed under reduced pressure. The residue was taken up in EtOAc. The organic phase was washed with a saturated solution of NH_4Cl twice and with water. The organic phase was dried on $MgSO_4$, filtered and the solvent removed under reduced pressure to afford 4-fluoro-*N*,*N*-dimethylbenzenesulfonamide (1.80 g, 86%). A solution of 4-fluoro-*N*,*N*-dimethylbenzenesulfonamide (1.80 g, 8.86 mmol) in concentrated sulfuric acid (7 mL, 130 mmol) was treated with *N*-bromosuccinimide (1 730 mg, 9.74 mmol) and stirred at room temperature for 3 h. The reaction mixture was carefully poured on crushed ice, extracted with AcOEt, and the organic phase was washed with a 0.1 N solution of NaOH in water twice, then with brine twice. The organic phase was dried on $MgSO_4$, filtered, and concentrated under reduced pressure to give the title compound as a white solid (2.04 g, 82%). ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 8.04 (1H, dd, J = 6.5 Hz, J = 2.2 Hz), 7.83 (1H, ddd, J = 8.7 Hz, J = 4.6 Hz, J = 2.3 Hz), 7.66 (1H, t, J = 8.7 Hz), 2.65 (6H, s).

[4-Chloro-2-({5-[(dimethylamino)sulfonyl]-2-fluorophenyl}ethynyl)phenoxy]acetic Acid (20c). Prepared according to Sonogashira general method 4, starting from *tert*-butyl (4-chloro-2-ethynylphenoxy)acetate (**16a**) and 3-bromo-4-fluoro-*N*,*N*-dimethylbenzenesulfonamide (**12p**), the title compound was obtained as a white solid in 16% overall yield after purification by preparative HPLC. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 13.18 (1H, bs), 7.97 (1H, dd, J = 6.5 Hz, J = 2.4 Hz), 7.86 (1H, ddd, J = 8.7 Hz, J = 4.6 Hz, J = 2.4 Hz), 7.67−7.61 (2H, m), 7.47 (1H, dd, J = 9.0 Hz, J = 2.7 Hz), 7.04 (1H, d, J = 9.0 Hz), 4.85 (2H, s), 2.66 (6H, s). MS (ESI$^-$): 410.0. HPLC (condition A): t_R = 4.27 min (HPLC purity 99.7%).

{4-Chloro-2-[(5,5-dioxidodibenzo[*b*,*d*]thien-3-yl)ethynyl]phenoxy}acetic Acid (21o). Prepared according to Sonogashira general method 3, starting from *tert*-butyl (4-chloro-2-ethynylphenoxy)-acetate (**16a**) and 3-iododibenzo[b,d]thiophene 5,5-dioxide (**38d**), the title compound was obtained as a beige solid in 65% overall yield after purification by preparative HPLC. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 13.21 (1H, bs), 8.27 (2H, m), 8.17 (1H, d, J = 1.4 Hz), 8.03 (1H, d, J = 7.6 Hz), 7.95 (1H, dd, J = 8.0 Hz, J = 1.4 Hz), 7.84 (1H, dt, J = 7.6 Hz, J = 1.0 Hz), 7.69 (1H, dt, J = 7.6 Hz, J = 1.0 Hz), 7.65 (1H, d, J = 2.7 Hz), 7.46 (1H, dd, J = 9.0 Hz, J = 2.7 Hz), 7.04 (1H, d, J = 9.0 Hz), 4.86 (2H, s). MS (ESI$^-$): 423.0. HPLC (condition A): t_R = 4.56 min (HPLC purity 100%).

Methyl 2-(2-Bromo-4-chlorophenoxy)propanoate (28a). A mixture of 2-bromo-4-chlorophenol (250 mg, 1.21 mmol) and methyl 2-bromopropionate (135 μL, 1.21 mmol) in DME (5 mL) was treated with K_2CO_3 (250 mg, 1.81 mmol), and the mixture was refluxed for 18 h. The reaction mixture was filtered. The filtrate was concentrated and purified by flash column chromatography (silica), eluting with heptane containing increasing amounts of EtOAc. The title compound was obtained as a yellow liquid (306 mg, 87%). ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 7.71 (1H, d, J = 2.6 Hz), 7.38 (1H, dd, J = 9.0 Hz, J = 2.6 Hz), 6.99 (1H, d, J = 9.0 Hz), 5.10 (1H, q, J = 6.8 Hz), 3.68 (3H, s), 1.54 (3H, d, J = 6.8 Hz). HPLC (condition A) purity 98.8%, t_R = 4.6 min.

2-(4-Chloro-2-{[2-fluoro-5-(propylsulfonyl)phenyl]ethynyl}phenoxy)propanoic Acid (29a). A solution of methyl 2-(2-bromo-4-chlorophenoxy)propanoate (**28a**, 130 mg, 0.44 mmol), 2-ethynyl-1-fluoro-4-(propane-1-sulfonyl)benzene (**14c**, 110 mg, 0.49 mmol), Pd-$(PPh_3)_2Cl_2$ (9.3 mg, 0.01 mmol), PPh_3 (23.2 mg, 0.09 mmol), and CuI (2.5 mg, 0.01 mmol) in TEA (985 μL) was degassed with nitrogen. The reaction mixture was heated overnight at 80 °C, diluted with EtOAc, and washed with saturated ammonium chloride solution and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated to dryness affording a dark brown sticky solid, which was purified by flash column chromatography (cyclohexane/EtOAc gradient). The intermediate thus obtained was dissolved in a mixture of dioxane (2.6 mL) and water (2.6 mL) and treated with a 4 N solution of HCl in dioxane (1.33 mL). After the mixture was heated at 100 °C for 16 h, water was added and the reaction mixture was extrated 3 times with EtOAC. The organic phase was dried over $MgSO_4$, filtered, and concentrated to dryness affording a yellow sticky solid, which was triturated in diethyl ether/pentane to afford the title compound (22 mg, 12%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 13.27 (1H, bs), 8.10 (1H, dd, J = 6.5 Hz, J = 2.3 Hz), 7.98 (1H, m), 7.69−7.63 (2H, m), 7.47 (1H, dd, J = 9.0 Hz, J = 2.7 Hz), 6.95 (1H, d, J = 9.0 Hz), 4.96 (1H, q, J = 6.8 Hz), 3.38 (2H, m), 1.63−1.50 (5H, m), 0.93 (3H, t, J = 7.5 Hz). MS (ESI$^-$): 423.1. HPLC (condition A): t_R = 4.66 min (HPLC purity 91.9%).

Biological Assays. Construction of pCEP4-hCRTH2 Mammalian Expression Vector. Mouse CRTH2 cDNA was amplified by PCR using a mouse cDNA library and cloned into the pCEP4 vector (Invitrogen Life Technologies).

Stably Transfected Cell Lines. Chinese hamster ovary (CHO) cell line expressing hCRTH2 was purchased from Euroscreen (Belgium) and cultured in HAM's F12 containing 10% fetal calf serum (Cancerra, Australia), 50 μg/mL streptomycin, 50 U/mL penicillin (Invitrogen, U.S.) and 400 μg/mL Geneticin (G418) (Calbiochem, San Diego, CA), according to the manufacturer.

Human embryonic kidney (HEK) 293(EBNA) cells (Invitrogen Life Technologies, catalog no. R620-07) were cultured in Dulbecco's modified Eagle's F-12 medium (DMEM-F-12, Invitrogen Life Technologies) containing 10% heat inactivated fetal calf serum, 2 mM glutamine, 50 units mL^{-1} penicillin, 50 μg mL^{-1} streptomycin, and 250 μg mL^{-1} G418 (for EBNA selection). Using the GeneJammer transfection reagent (Stratagene, La Jolla, CA), HEK 293 (EBNA) cells were transfected with the mouse CRTH2 pCEP4 vector (containing the gene for the hygromycin resistance). Cells were maintained in culture for 48 h after transfection and then grown in the presence of 300 μg mL^{-1} of hygromycin B (Calbiochem) for 4 weeks to select for resistant cells expressing the mCRTH2.

Membrane Preparation. Cells expressing the human and mouse CRTH2 were disrupted by nitrogen cavitation (Parr Instruments, U.S.) at 4 °C, 800 psi for 30 min. Membranes were prepared by differential centrifugation (1000*g* for 10 min, then 100000*g* for 60 min). Membranes pellets were resuspended in 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, and 250 mM sucrose and stored at −80 °C.

Radioligand Binding Assay. A scintillation proximity assay was used for radioligand competition and saturation binding assays. For each assay point, an amount of 2−5 μg of human or mouse CRTH2 cell membranes was incubated in a final volume of 100 μL in 96 well plates (Corning, U.S.) for 90 min with shaking at room temperature in the presence of 100 μg of wheat germ agglutinin-coated scintillation proximity assay beads (WGA-SPA, RPNQ0001, GE Healthcare), 1.5 nM [^{3}H]PGD_2 (Amersham, 156 Ci/mmol) in binding buffer (10 mM HEPES/KOH, pH 7.4, 10 mM $MnCl_2$, with protease inhibitor cocktail tablets). Nonspecific binding was determined in the presence of 1 μM PGD_2 (Cayman, U.S.). Assay was performed in the presence of 1% DMSO. Binding activity was determined using a 1450 Microbeta scintillation counter (Wallac, U.K.). K_i values were calculated using the Cheng−Prusoff equation (Cheng and Prusoff, 1973) and represent the average of at least three independent dose response experiments.

[^{35}S]GTPγS Binding Assay. The [^{35}S]GTPγS binding assay was performed at 30 °C with gentle agitation in 96-well scintillating white polystyrene plates (Perkin-Elmer, U.S.), in a final volume of 200 μL containing 2% of DMSO. Briefly, an amount of 10 μg of membrane expressing human CRTH2 was incubated in 20 mM HEPES/KOH, pH 7.4, 10 mM $MgCl_2$, 10 μg/mL saponin, 3 μM GDP, 150 mM NaCl for 10 min, with various concentrations of PGD2. Nonspecific binding was determined in the presence of 10 μM GTPγS. Antagonist activity of compounds was measured in the presence of 80 nM PGD_2. [^{35}S]GTPγS (0.15 nM, reference) was subsequently added to each sample, and after incubation of 30 min, reactions were stopped by centrifugation at 700*g* for 10 min. Supernatant was removed, and [^{35}S]GTPγS binding was determined using a 1450 Microbeta scintillation counter. Data were analyzed using "Prism" (GraphPad Software, Inc. San Diego, CA, U.S.).

Cellular Dielectric Spectroscopy. CHO-CRTH2 cells were cultured in HAM's F12 (Lonza, Switzerland) supplemented with 10% fetal calf serum (PAA, Australia) and 400 μg/mL Geneticin. 100000 cells/well were seeded in standard 96W microplates (MDS Analytical Technologies) and incubated at 37 °C in 5% CO_2 for 24 h. Cells were washed twice with 135 μL of Hank's balanced salt solution 1× (HBSS) (Invitrogen) supplemented with 10 mM HEPES, pH 7.4, in the presence of 1% DMSO. For EC_{50} determination, an amount of 15 μL of increasing concentration of the test compounds diluted in 20 mM HEPES with 1× HBSS was added to the cells, and agonist activity was then recorded for 25 min. For IC_{50} determination, 16.6 μL of a fixed concentration of PGD2 (EC_{80}) diluted in 20 mM HEPES with 1× HBSS was added to the cell-compound mixture, and antagonist activity was measured during a 25 min period. Results are expressed as the amplitude between the highest and the lowest signal produced (max−min). Basal and maximum activities were respectively measured in the absence and presence of PGD2 (EC_{80}).

Human Eosinophil Chemotaxis. Eosinophils were purified from buffy coats of normal donors obtained from the regional hospital according to institutional guidelines of the ethical committee. Eosinophil purity was assessed by determining surface antigen expression or appropriate isotypic controls using flow cytometric analysis. The purity of eosinophil preparations was >95% ($CD16^-$, $CD11b^+$, VLA-4^+, $CCR3^+$, and $CRTH2^+$) with >98% viability. Eosinophil chemotaxis assay was performed in Boyden chambers with 5 μm pore size polycarbonate filter. The plates were incubated at 37 °C, 5% CO_2 for 90 min. Migrated cells were transferred to 96-well plates via centrifugation using a funnel adaptator and quantified by measuring fluorescence with a fluorescence counter. DK-PGD2, a metabolite of PGD2 that is selective for CRTH2 (100 nM), and CCL11/eotaxin (10 nM) were used as chemoattractants. Eosinophils were incubated with varying concentrations of CRTH2 antagonists for 30 min at 37 °C, 5% CO_2 before being applied to the chemotaxis chamber and migration toward DK-PGD2 or eotaxin.

PGD2-Induced Eosinophil Cell Shape Change Assay in Human Whole Blood. The test compounds were diluted in DMSO so that the total volume was kept constant at 2% DMSO. Serial dilutions of 200−0.09 μM were prepared. Samples of 90 μL of human blood from healthy volunteers (Centre de Transfusion Sanguine de Genève) were preincubated in polypropylene Falcon tubes (BD 352063) for 20 min in a water bath at 37 °C with 10 μL of diluted compounds. For CRTH2 activation, 100 μL of PGD2 (Cayman 12010) at 20 nM was added (10 nM final) to each tube, and cells were maintained at 37 °C. Cells treated with PBS were used as negative control. After 10 min, cell activation was stopped with 120 μL of 10% formaldehyde (4% final, Fluka 41650), and cells were rested for 10 min at room temperature. Fixed cells were transferred into polypropylene tubes and then treated for 1 h in a water bath at 37 °C with 2 mL of Triton−Surfact-Amps X-100 (Pierce 28314) at 0.166% (0.13% Triton final). After several washes with PBS (red cells lysed progressively during washes; two washes are necessary), cells were analyzed by flow cytometry on a FACSCalibur.

Table 13

compd	dose (mg/kg)	% inhibition total cells (mean ± SEM)	% inhibition eosinophils (mean ± SEM)
dexamethasone	1	88 ± 11	90 ± 10
19k	3	21 ± 13	29 ± 14
	10	42 ± 14	54 ± 16
	30	67 ± 8	81 ± 7
19s	3	12 ± 15	19 ± 18
	10	19 ± 8	27 ± 10
	30	44 ± 10	55 ± 11

Table 14

compd	dose (mg/kg)	% inhibition (mean ± SEM)
19k	30	52 ± 12
	10	56 ± 12
	3	23 ± 16
19s	30	48 ± 11
	10	54 ± 16
	3	36 ± 19

OVA-Induced Lung Eosinophilia in Mice. BALB/c mice (6−8 weeks old) were immunized with ovalbumin (10 μg ip) on days 0 and 7. In order to elicit a local inflammatory response in the lung, mice were challenged between days 15 and 17 with a nebulized solution of ovalbumin (10 μg/mL, De Vilibiss Ultraneb 2000, once daily for 30 min during the 3 days). On each separate day between days 15 and 17 each animal received via oral gavage the test compound, at $t - 1$ h and $t + 7$ h with respect to OVA exposure at $t = 0$ h. Eight hours after the final OVA challenge, bronchoalveolar lavage (BAL) was then carried out. Total cell numbers in the BAL fluid samples were measured using a hemocytometer. Cytospin smears of the BAL fluid samples were prepared by centrifugation at 1200 rpm for 2 min at room temperature and stained using a DiffQuik stain system (Dade Behring) for differential cell counts. Results are shown in Table 13.

FITC Model. Female 9-week-old BALB/c mice were sensitized on days 0 and 1 with 0.5% FITC in aectone/dibutyl phthalate (A/DBP). One group (sham) was sensitized with A/DBP alone. On day 6 all the mice including the sham group were challenged on the right ear (inner and outer surfaces) with 0.5% FITC in A/DBP. The mice were treated with the compounds via oral gavage 1 h before and 7 h after the challenge. The baseline ear thickness was measured before the challenge and 24 h after the challenge. At the end of the experiment (24 h after the challenge) the mice were sacrificed. Serum and plasma samples were taken. The challenged ear was excised and stored at −80 °C. Results are shown in Table 14.

■ ASSOCIATED CONTENT

Supporting Information. Synthesis and spectroscopic data of all final compounds and intermediates not described in the manuscript and protocols for the ADME experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: +41224149618. Fax: +41227312179. E-mail: stefano.crosignani@merckserono.net.

ABBREVIATIONS USED

A/DBP, acetone/dibutyl phthalate; AD, atopic dermatitis; ADME, absorption, distribution, metabolism, excretion; ANOVA, analysis of variance; AR, aldose reductase; AUC, area under the curve; BALF, bronchoalveolar lavage fluid; BSA, bovine serum albumin; cAMP, cylic adenosine monophosphate; CHO, Chinese hamster ovary; CHS, contact hypersensitivity; Cl, clearance; Cl_{int}, intrinsic clearance; CRTH2, chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes; FITC, fluorescein isothiocyanate; F_u, fraction unbound; F_z, absolute oral bioavailability; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; HEK, human embryonic kidney; K_{off}, constant of dissociation; mCPBA, *m*-chloroperbenzoic acid; NBS, *N*-bromosuccinimide; OVA, ovalbumin; po, per os (by oral administration); P_{app}, apparent permeability; PBS, phosphate buffered saline; PGD2, prostaglandin D2; PK, pharmacokinetic; PoP, proof of principle; SAR, structure−activity relationship; TBAF, tetrabutylammonium fluoride; V_{ss}, volume of distribution at steady state; WBA, whole blood assay

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