Synthesis, Structure−Activity Relationships, and Pharmacokinetic Properties of Dihydroorotate Dehydrogenase Inhibitors: 2-Cyano-3-cyclopropyl-3-hydroxy-*N*-[3′-methyl-4′-(trifluoromethyl)phenyl]propenamide and Related Compounds

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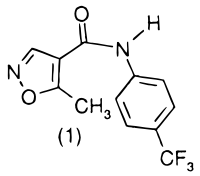
The active metabolite (**2**) of the novel immunosuppressive agent leflunomide (**1**) has been shown to inhibit the enzyme dihydroorotate dehydrogenase (DHODH). This enzyme catalyzes the fourth step in *de novo* pyrimidine biosynthesis. A series of analogues of the active metabolite **2** have been synthesized. Their *in vivo* biological activity determined in rat and mouse delayed type hypersensitivity has been found to correlate well with their *in vitro* DHODH potency. The most promising compound (**3**) has shown activity in rat and mouse collagen (II)-induced arthritis models (ED_{50} = 2 and 31 mg/kg, respectively) and has shown a shorter half-life in man when compared with leflunomide. Clinical studies in rheumatoid arthritis are in progress.

Introduction

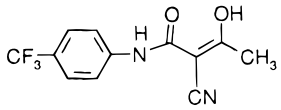
Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by painful and swollen joints with possible progression to irreversible joint damage. It is believed to have an underlying autoimmune pathology. Initial treatment is with nonsteroidal antiinflammatory drugs (NSAIDs) which provide symptomatic relief but do not modify the course of the disease. Except for the mildest cases, they are used in combination with disease-modifying antirheumatic drugs (DMARDs). Recent recommendations have focused on the early and aggressive use of these agents because of the serious long-term outcome of RA. This group of compounds includes gold salts, sulfasalazine, D-penicillamine, hydroxychloroquine, azathioprine, and methotrexate, but their modes of action remain largely unknown.[1] RA synovitis is currently regarded as an immunological process involving T-cells, antigen-presenting cells, macrophages, synoviocytes, and cytokines.[2,3] New and potentially disease-modifying therapies for RA are under investigation based on these components and include anti-cytokine treatments, such as tenidap[4] and neutralizing antibodies,[5] and immunomodulatory compounds, such as cyclosporine[6] and leflunomide.[7]

Leflunomide (**1**) was first reported in 1976,[8] and its pharmacological, kinetic, and clinical profile has been described in the literature.[9] The species responsible for the biological activity of leflunomide (**1**) has been reported to be the ring-opened metabolite (**2**).[9] Despite the abundance of data on leflunomide (**1**) itself, at the outset of this work the mode of action was not understood. Consequently, rationalization of the available structure−activity data which related largely to its *in vivo* activity was difficult.

We therefore established a program of further work, with the objective of determining the mode of action of a series of molecules related to **2** and the rationalization of their structure−activity relationships. As a consequence of this work we were able to identify analogues with equivalent activity to leflunomide (**1**) but which had differential pharmacokinetic properties and were therefore candidates for clinical development in areas where it is not appropriate to develop leflunomide itself. During these studies we reported the ability of the active metabolite **2** to inhibit the enzyme dihydroorotate dehydrogenase (DHODH),[10] the enzyme which catalyzes the fourth step in the synthesis of the pyrimidine bases necessary for cell proliferation.

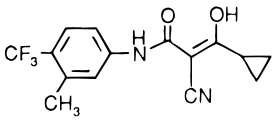


(1)



(2)

This paper reports the structure−activity relationships between a series of analogues of the leflunomide metabolite **2** with regard to their inhibition of DHODH and relates this activity to the observed *in vivo* pharmacological activity of this series. One compound from the series, **3**, has progressed into phase II clinical trials for RA.



(3)

Chemistry

Several general synthetic routes have been employed in the synthesis of this series of compounds (Tables 1 and 2). Two methods (Schemes 1 and 2) have been used for the preparation of the intermediate cyanoacetamides, which were then acylated to afford the β-hydroxy enamides using sodium hydride and the required acyl halide. The preparation of 3-acetylenic derivatives was carried out using acyl fluorides for the acylation,[11] while

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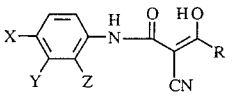
for the preparation of the 3-olefinic derivative **4**, the acylation using propenoyl chloride afforded only very poor yields of the required product. The acylation was best performed by method C[12] (Scheme 3) in which the acid chloride is substituted with a potential leaving group, phenylseleno, thus allowing the generation of the carbon−carbon double bond as a last step in the synthesis.

Table 1. Physical Properties of β-Hydroxy Enamides



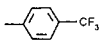
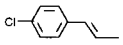
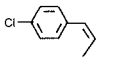
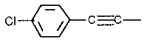
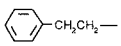
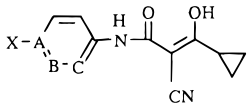
compd no.	R	X	Y	Z	method	mp (°C)	empirical formula	analysis
3		CF_3	CH_3	H	A	186-8	$C_{15}H_{13}F_3N_2O_2$	CHNF
17		CF_3	H	H	B	212-3	$C_{14}H_{11}F_3N_2O_2$	CHNF
18		CF_3	H	H	B	177-8	$C_{15}H_{13}F_3N_2O_2$	CHNF
19		CF_3	H	H	B	177-8	$C_{16}H_{15}F_3N_2O_3$	CHNF
20		CF_3	H	H	A[a]	147-8	$C_{15}H_{13}F_3N_2O_3$	CHNF
21		CF_3	H	H	A[a]	186-8	$C_{15}H_{13}F_3N_2O_3$	CHNF
22		CF_3	H	H	E	266-8	$C_{15}H_{14}F_3N_3O_2$	CHNF
23		CF_3	H	H	F	168-70	$C_{16}H_{16}F_3N_3O_2$	CHNF
24		CF_3	H	H	D	218-20	$C_{18}H_{10}F_6N_2O_2$	CHNF
25		CF_3	H	H	A	155-6	$C_{14}H_{13}F_3N_2O_2$	CHNF
26		CF_3	H	H	A[b]	191-3	$C_{14}H_{11}F_3N_2O_2$	CHNF
4		CF_3	H	H	C	206-8	$C_{13}H_9F_3N_2O_2$	CHNF
27		CF_3	H	H	A[c]	170	$C_{13}H_7F_3N_2O_2$	CHNF
28		CF_3	H	H	A[d]	210-20	$C_{14}H_{11}F_3N_2O_2$	CHN
29	$-CH_2CH=CH_2$	CF_3	H	H	A[e]	166-8	$C_{14}H_{11}F_3N_2O_2$	CHNF
30		H	H	H	A	117	$C_{13}H_{12}N_2O_2$	CHN
31		CH_3	H	H	A	103-4	$C_{14}H_{14}N_2O_2$	CHN
32		H	CF_3	H	A	165-6	$C_{14}H_{11}F_3N_2O_2$	CHNF
33		H	H	CF_3	B[f]	213-5	$C_{14}H_{11}F_3N_2O_2$	HN[g]
34		Cl	H	H	B	189-91	$C_{13}H_{11}ClN_2O_2$	CHNCl
35		H	Cl	H	B	137-9	$C_{13}H_{11}ClN_2O_2$	CHNCl
36		H	H	Cl	B[f]	99-100	$C_{13}H_{11}ClN_2O_2$	HNCl[h]
37		Br	H	H	B	190-2	$C_{13}H_{11}BrN_2O_2$	CHNBr
38		CN	H	H	A	254-6	$C_{14}H_{11}N_3O_2$	CHN
39		$-CH_2CN$	H	H	B[f]	182-4	$C_{15}H_{13}N_3O_2$	CHN
40		CF_3S	H	H	A	175-6	$C_{14}H_{11}F_3N_2O_2S$	CHNFS
41		CF_3SO	H	H	G	155-7	$C_{14}H_{11}F_3N_2O_3S$	CHNFS
42		CF_3SO_2	H	H	G	168-70	$C_{14}H_{11}F_3N_2O_4S$	CHNFS
43		CH_3S	H	H	A	152-4	$C_{14}H_{14}N_2O_2S$	CHNS
44		CH_3SO	H	H	D	177-9	$C_{14}H_{14}N_2O_3S$	CHNS
45		CH_3SO_2	H	H	H	199-201	$C_{14}H_{14}N_2O_4S$	CHNS
46		CF_3O	H	H	B	173-5	$C_{14}H_{11}F_3N_2O_3$	CHNF
47		CH_3O	H	H	A	135	$C_{14}H_{14}N_2O_3$	CHN
48		HO	H	H	D	172-4	$C_{13}H_{12}N_2O_3$	CHN
49		NO_2	H	H	B	244-6	$C_{13}H_{11}N_3O_4$	
50		HCl. H_2N-	H	H	I	>300	$C_{13}H_{14}ClN_3O_2$	CHN
51		CH_3CO	H	H	A	166-8	$C_{15}H_{14}N_2O_3$	CHN
52		H_2NCO-	H	H	D	>280	$C_{14}H_{13}N_3O_3$	CHN
53		HO_2C-	H	H	A[i]	decomp.240	$C_{14}H_{12}N_2O_4$	
54		CH_3O_2C-	H	H	A	188-9	$C_{15}H_{14}N_2O_4$	CHN
55			H	H	B	217-8	$C_{16}H_{13}N_3O_3$	
56			H	H	A	217-9	$C_{17}H_{12}F_3N_3O_2S$	CHNFS
57			H	H	B	192-3	$C_{17}H_{14}N_2O_2S$	CHNS
58			H	H	B	188-90	$C_{17}H_{14}N_2O_3$	CHN
59		CF_3	CH_3CH_2	H	A	172-4	$C_{16}H_{15}F_3N_2O_2$	CHNF
60		C_2F_5	CH_3	H	A	135-7	$C_{16}H_{13}F_5N_2O_2$	CHNF
61		Cl	CH_3	H	A	189-90	$C_{14}H_{13}ClN_2O_2$	CHNCl
62		Cl	H	CH_3	A	167	$C_{14}H_{13}ClN_2O_2$	CHNCl
63		CH_3	Cl	H	A	122-4	$C_{14}H_{13}ClN_2O_2$	
64		Br	CH_3	H	B[f]	158	$C_{14}H_{13}BrN_2O_2$	CHN
65		CN	CH_3	H	A	255-7	$C_{15}H_{13}N_3O_2$	CHN
66		CF_3S	CH_3	H	A	165-6	$C_{15}H_{13}F_3N_2O_2S$	CHNFS
67		CF_3O	CH_3	H	A	148-50	$C_{15}H_{13}F_3N_2O_3$	CHNF
68			H	H	A	142	$C_{19}H_{16}N_2O_3$	CHN
69			H	H	A	151-2	$C_{20}H_{15}F_3N_2O_3$	CHNF
70			H	H	A[k]	161-3	$C_{19}H_{15}ClN_2O_3$	CHNCl
71			H	H	A[k,l]	168-70	$C_{20}H_{15}ClN_2O_3$	CHNCl
72			H	H	A	204-5	$C_{21}H_{18}ClN_3O_3$	CHCl[m]

Table 1 (Continued)

compd no.	R	X	Y	Z	method	mp (°C)	empirical formula	analysis
73			H	H	A	213-7	$C_{21}H_{17}ClN_2O_2$	CHNCl
74			H	H	A	182-3	$C_{21}H_{17}ClN_2O_2$	CHNCl
75			H	H	A	205-7	$C_{21}H_{15}ClN_2O_2$	CHNCl
76			H	H	A	154-5	$C_{21}H_{20}ClN_3O_2$	CHN
77			H	H	A	222-3	$C_{19}H_{15}FN_2O_2$	CHNF
78			H	H	A	202-3	$C_{19}H_{16}N_2O_2$	CHN

[a] Acid imidazolide instead of acid chloride (method E, step 1, for preparation of imidazolide). Added at −30 °C to cyanoacetamide. [b] Sodium hydride (80% dispersion in oil) and catalytic imidazole. Methacryloyl chloride added to reaction mixture at −78 °C. Stirred for 30 min at −78 °C and quenched with glacial acetic acid. Stirred for 30 min and poured onto aqueous HCl at 0 °C. Product filtered, washed with water and ether, and then dried *in vacuo*. [c] Propargoyl fluoride[11] distilled directly into cold (−70 °C) reaction mixture *via* an air condenser. Reaction mixture stirred at −78 °C for 1 h. Product purified by trituration with ether. [d] Crotonyl chloride added at −78 °C. Reaction mixture stirred at −78 °C for 2 h. Reaction quenched with iced aqueous HCl. [e] Acid chloride added to reaction mixture at −50 °C. [f] Dichloromethane as solvent with dicyclohexylcarbodiimide. Temperature increased to 40 °C during addition of DCC. [g] Calcd: C, 56.76; F, 19.24. Found: C, 57.27; F, 18.76. [h] Calcd: C, 59.44. Found: C, 59.93. [j] Prepared from **54** by standard literature procedures. [k] Product extracted with ethyl acetate and the organic phase washed with brine, dried $MgSO_4$, and concentrated *in vacuo*. [l] Product purified by trituration with ether. [m] Calcd: N, 10.62. Found: N, 10.09.

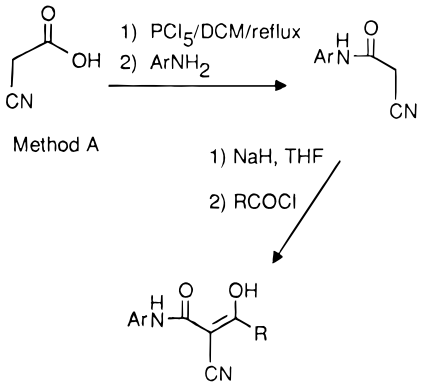
Table 2. Physical Properties of Pyridyl Amides



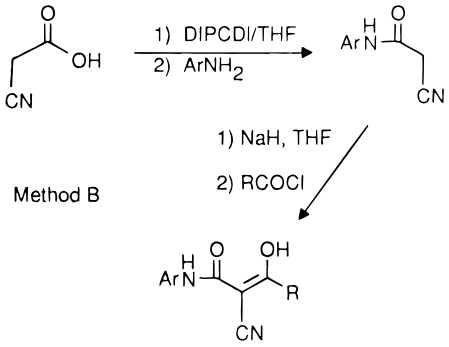
compd no.	A	B	C	X	method	mp (°C)	empirical formula	anal.
79	C	CH	N	Cl	Br[a]	223−5	$C_{12}H_{10}ClN_3O_2$	CHN
80	C	CH	N	Br	A	202−3	$C_{12}H_{10}BrN_3O_2$	CHBr[b]
81	C	CH	N	CF_3	B[a]	202−3 dec	$C_{13}H_{10}N_3F_3O_2$	CHNF
82	C	N	CH	Cl	B[a]	196−8	$C_{12}H_{10}N_3ClO_2$	CHNCl
83	N	CH	CH		D[c,d]	>300	$C_{12}H_{11}N_3O_2$	CHN

[a] Dichloromethane as solvent with dicyclohexylcarbodiimide. Temperature increased to 40 °C during addition of DCC. [b] Calcd: N, 13.64. Found: N, 13.19. [c] Pyridine (0.72 g, 1 equiv) was added during the reaction of the isoxazole acid chloride with 4-aminopyridine (1.1 equiv). The product was filtered and used without further purification. [d] Ring opening of isoxazole using triethylamine in methanol at reflux for 1 h. Reaction mixture treated with concentrated HCl to pH 1 and product crystallized at 4 °C for 16 h.

Scheme 1



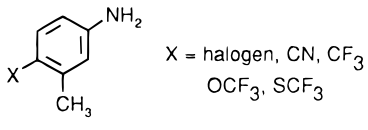
Scheme 2[a]



[a] DIPCDI, diisopropylcarbodiimide.

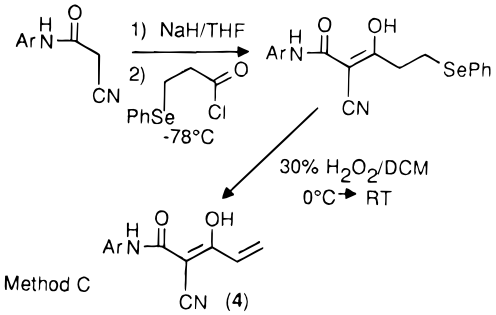
Occasionally it was necessary to prepare the β-hydroxy enamides by ring opening of the corresponding isoxazoles (method D in Scheme 4). This was particularly useful where the aryl substituents were groups such as hydroxyl, amino, or pyridyl.

Most anilines required for the synthesis of the cyanoacetamides were either commercially available or prepared by standard methods. However, new synthetic routes were developed for the synthesis of some of the anilines, in particular a variety of 3-methyl 4-substituted anilines (**5**) (Scheme 5). For example, diazotization of 2-methyl-4-nitroaniline followed by treatment with the appropriate potassium halide[13] or potassium cyanide afforded the halo- or cyano-substituted nitrobenzenes in good yield which could then be reduced to the corresponding anilines. The 4-iodo-3-methylni-

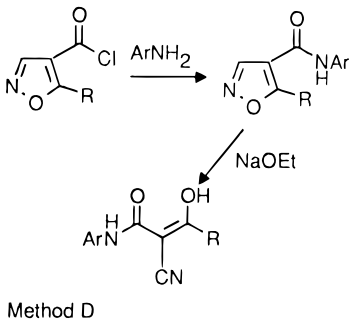


(5)

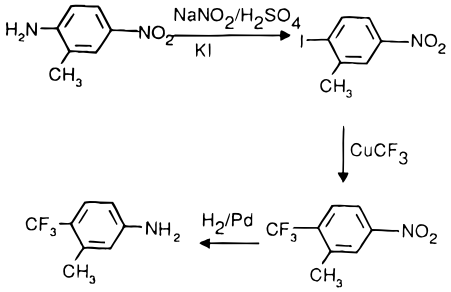
Scheme 3



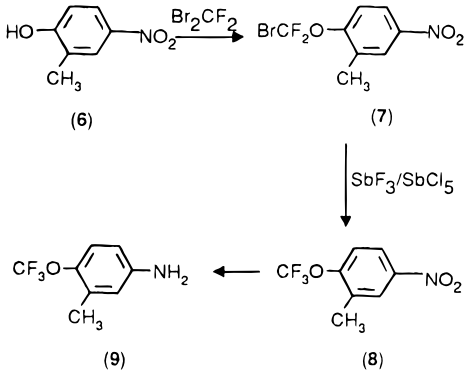
Scheme 4



Scheme 5



Scheme 6



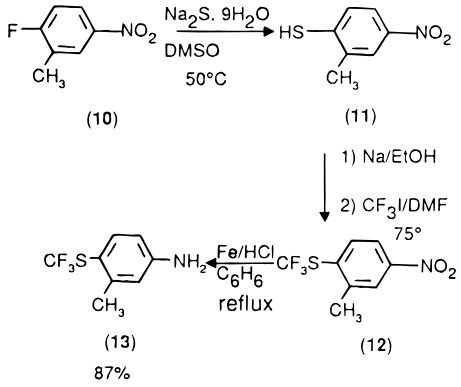
trobenzene was further reacted with (trifluoromethyl)-copper[14,15] to afford, after reduction, 3-methyl-4-(trifluoromethyl)aniline in good yield. This method provides a far superior route to that available in the literature.[16]

The synthesis of 3-methyl-4-(trifluoromethoxy)aniline was developed as shown (Scheme 6). Conversion of the phenol **6** to the 1-(bromodifluoromethoxy)-2-methyl-4-nitrobenzene (**7**) with dibromodifluoromethane[17] proceeded in 29% yield. Reaction with antimony trifluoride/antimony pentachloride[18] afforded the 2-methyl-4-nitro-1-(trifluoromethoxy)benzene (**8**) in 83% yield. This was readily reduced to the required aniline **9**, which was used without purification due to its volatile nature.

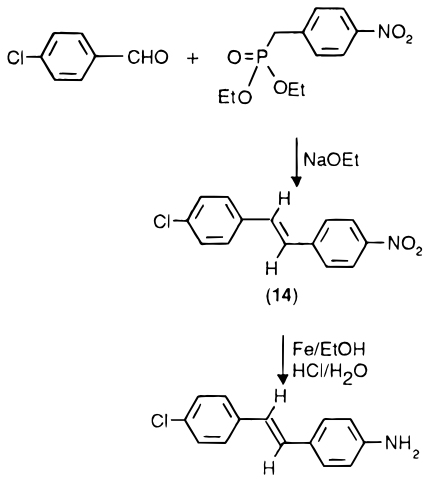
The synthesis of 3-methyl-4-[(trifluoromethyl)thio]-aniline (**13**) was carried out as shown (Scheme 7). The thiol **11** was prepared in 71% yield from the fluoride **10** using sodium sulfide nonahydrate. Trifluoromethylation was carried out using trifluoromethyl iodide. This gave a poor yield (17%) of **12**, which was readily reduced using iron/HCl in refluxing benzene to the required aniline **13** (87% yield).

The olefinic and acetylenic linked biphenyl amines were prepared by the routes shown (Schemes 8−10). The trans olefin **14** was prepared in 65% yield by Wittig reaction (Scheme 8) and converted to the acetylene **15**

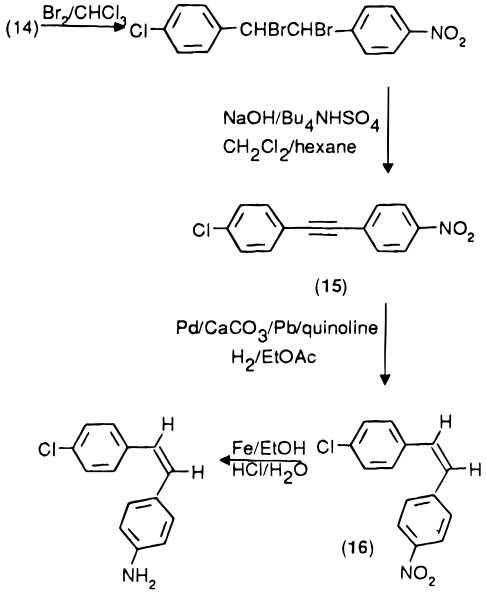
Scheme 7



Scheme 8



Scheme 9

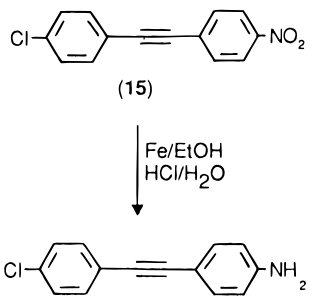


by bromination/dehydrobromination in 94% yield. Hydrogenation of the acetylene **15** using Lindlar's catalyst gave the cis olefin **16** in 88% yield (Scheme 9).

Biological Results and Discussion

Dihydroorotate dehydrogenase catalyzes the fourth step in *de novo* pyrimidine biosynthesis, the conversion of dihydroorotate to orotic acid. Its inhibition would lead to pyrimidine nucleotide depletion and conse-

Scheme 10



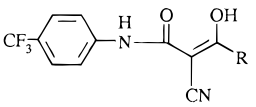
quently inhibition of DNA and RNA synthesis and cell proliferation. Pyrimidines are also required for glycosylation of lipids and proteins. Cell proliferation is a critical component of the immune response, during which the immune cells have a high nucleotide requirement. A DHODH inhibitor would thus be a potential immunosuppressive agent in, e.g., delayed type hypersensitivity (DTH) reponses and in addition would have potential therapeutic benefits in disorders involving aberrant cell proliferation such as arthritis. The biological activities of this series of compounds were measured after oral administration in both rat and mouse DTH. For compounds showing DTH activity in both species, an approximate half-life in mouse and rat was also determined. Compounds were later tested in the inhibition of DHODH, and the correlation between their *in vivo* DTH activity and *in vitro* DHODH potency was studied.

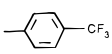
An investigation of different alkyl groups on the 3-position of the molecule showed that increasing the size of the substituent reduced DTH activity, e.g., **17** vs **19** (Table 3). The most successful replacement for methyl in **2** was found to be cyclopropyl (**17**), which showed an identical pharmacological profile with **1**, including kinetic properties. In the admitted absence of a full metabolic study, this result was taken as indicating that there was no major metabolism occurring through the cyclopropyl group. Olefin-containing substituents were also investigated, with the hope that this might introduce a metabolic site into the molecule. Introduction of the 3-isopropenyl group gave a molecule (**26**) showing reduced potency in both rat and mouse DTH but which showed improved kinetic properties. Other compounds containing olefin groups such as allyl (**29**) showed DTH activity but only slightly modified kinetic properties (Table 3).

Aromatic substituents were investigated for structure−activity relationships (Table 4) and also to investigate the metabolism of various functional groups. The most potent compounds contain a 4-substituent, with 3-substituted being less active and 2-substituted the least active, e.g., **17** vs **32** vs **33** (Table 4). The nature of the substituent was also important with polar, hydrogen bond donor groups (**48**, **50**, **53**) and larger heterocyclic 4-substituents (**55**−**58**) being inactive or very weakly active compounds in DTH. The compounds showing the best overall pharmacological profile of combined rat and mouse DTH activity and modified kinetic properties when compared with **1** were **38**, **40**, and **49**. In the case of **38** and **49** no metabolites could be observed in either rate or mouse plasma by HPLC. However, **40** showed two metabolites in HPLC which were identified by chemical synthesis as **41** and **42**, with **42** itself displaying potent DTH activity and long kinetics. Many of the most potent DTH active molecules showed similar long mouse kinetics to leflunomide (**1**) (**17**, **34**, **37**, **46**). By extending the arylamine group we hoped to investigate the binding site for the molecule, which was an unknown protein at the start of this work. For example, phenyl ethers (**68**, **69**, **70**) showed weak DTH activity as did cis and trans olefin-linked phenyl groups (**73**, **74**) and the acetylene-linked compound **75**. These compounds also showed long pharmacokinetic properties.

The introduction of chemical functional groups expected to undergo metabolism was investigated for the molecules containing potent 4-substituents but displaying long kinetics. An alkyl group was considered for this, and the approach proved to be very successful. The introduction of a 3-methyl group into **17** to give **3** afforded a molecule with identical DTH activity and greatly modified kinetic profile in both rat and mouse (Table 5). From an investigation of the chloro,methyl-substituted series (**61**−**63**) the best substitution pattern

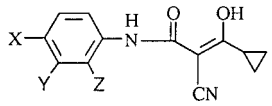
Table 3. Investigation of 3-Substituents



Cmpd.No.	R	DTH (mouse)[a]	DTH (rat)[a]	t½(hours) mouse	t½(hours) rat	DHODH[b] mouse	DHODH[b] rat
1	-	84** (30)	78** (10)	30	9	-	-
2	CH_3	-	-	-	-	69	13
17		85** (30)	53**(10)	28	8	47	21
18		4 (100)	1 (50)			209	282
19		58** (100)	19 (50)			28200	31600
20		29* (100)	14 (50)			11700	17800
21		29* (100)	11 (50)			7940	44700
22		-4[c] (100)	-16[c] (50)			>100000	>100000
23		10 (100)	23 (50)			>100000	>100000
24		17 (10)	16 (10)			>10000	>10000
25		64* (100)	51* (50)			92	295
26		54** (100)	78** (50)	13	7	195	251
4		7 (100)	74** (50)			178	23
27		45* (100)	4 (50)			2090	500
28		54* (100)	16 (50)			4270	1410
29	$-CH_2CH=CH_2$	75** (30)	79** (50)	23	4	63	107

[a] Percent inhibition (mg/kg). [b] IC_{50} (nM). [c] Negative value indicates potentiation of response. $*P < 0.05$; $**P < 0.01$ (Student's *t*-test).

Table 4. Investigation of Aromatic Substituents



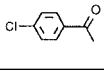
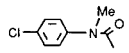
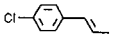
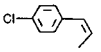
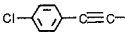
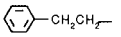
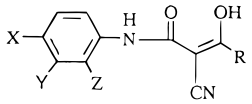
Cmpd. No.	X	Y	Z	DTH (mouse)a	DTH (rat)a	t½(hours) mouse	t½(hours) rat	DHODH[b] mouse	DHODH[b] rat
30	H	H	H	25 (30)	43** (50)			1580	2000
31	CH_3	H	H	47* (100)	28* (50)			288	234
17	CF_3	H	H	85** (30)	53** (10)	28	8	47	21
32	H	CF_3	H	41* (30)	49** (50)			525	479
33	H	H	CF_3	-10 (100)	-4 (50)			>100000	>100000
34	Cl	H	H	Toxic (30)	74** (3)	21	22	36	63
35	H	Cl	H	55* (100)	25 (50)	2	3	3000	343
36	H	H	Cl	43* (100)	-12 (50)			16600	13300
37	Br	H	H	64** (30)	58** (10)	33	20	36	79
38	CN	H	H	73** (30)	47** (10)	6	5	42	53
39	$-CH_2CN$	H	H	36* (100)	3 (50)			37200	4570
40	CF_3S	H	H	39 (30)	58** (1)	6	2	100	5
41	CF_3SO	H	H	25 (30)	75** (3)	3	3	417	16
42	CF_3SO_2	H	H	43* (30)	66** (3)	14	14	89	3
43	CH_3S	H	H	46* (100)	60** (50)	<2	<1	445	13
44	CH_3SO	H	H	1 (100)	71** (50)			22400	832
45	CH_3SO_2	H	H	36 (100)	50** (50)			15100	158
46	CF_3O	H	H	85** (30)	78** (1)	42	22	173	5
47	CH_3O	H	H	9 (100)	57* (50)			3720	186
48	HO	H	H	-11 (100)	37* (50)			>100000	7940
49	NO_2	H	H	64** (30)	90** (50)	8	4	50	21
50	HCl. H_2N-	H	H	32* (100)	1 (50)			31600	21900
51	CH_3CO	H	H	32 (100)	59** (10)	4	6	3800	68
52	H_2NCO-	H	H	-18 (100)	20 (50)			>100000	14100
53	HO_2C-	H	H	-6 (100)	39* (50)			3720	1480
54	CH_3O_2C-	H	H	34* (100)	59** (50)			1120	158
55		H	H	17 (100)	42* (50)			20000	4270
56		H	H	Toxic (100)	30** (10)			50000	3000
57		H	H	28 (30) Toxic(100)	34* (10)			2510	631
58		H	H	-13 (100)	58** (50)			5900	1780
68		H	H	9 (100)	6 (50)			630	295
69		H	H	-17 (30)	39* (10)			1510	3550
70		H	H	63** (30)	31 (10)	21	39	832	1510
71		H	H	4 (30)	-19 (10)			562	590

Table 4 (Continued)

Cmpd. No.	X	Y	Z	DTH (mouse)a	DTH (rat)a	t½(hours) mouse	t½(hours) rat	DHODH[b] mouse	DHODH[b] rat
72		H	H	-8 (100)	22 (50)			2240	19100
73		H	H	69** (100)	46** (50)	43	>30	12600	11700
74		H	H	19 (100)	52** (50)			22	40
75		H	H	61** (30)	61** (10)	68	84	5000	17800
76		H	H	5 (100)	51** (50)			630	178
77		H	H	53* (30)	79** (10)			8510	2140
78		H	H	33* (100)	70** (50)			1580	1480

[a] Percent inhibition (mg/kg). [b] IC_{50} (nM). *$P < 0.05$; **$P < 0.01$ (Student's *t*-test).

Table 5. Investigation of Alkyl Substituents as Potential Metabolic Sites



Cmpd. No.	R	X	Y	Z	DTH (mouse)[a]	DTH (rat)[a]	t½(hours) mouse	t½(hours) rat	DHODH[b] mouse	DHODH[b] rat
1					84** (30)	78** (10)	30	9	-	-
2	CH_3	CF_3	H	H	-	-	-	-	69	13
17		CF_3	H	H	85** (30)	53** (10)	28	8	47	21
3		CF_3	CH_3	H	81** (30)	59** (10)	8	4	55	14
59		CF_3	CH_3CH_2	H	61** (30)	74** (10)	6	6	71	40
60		C_2F_5	CH_3	H	65** (30)	70** (3)	11	10	282	11
34		Cl	H	H	Toxic (30)	74** (3)	21	22	36	63
61		Cl	CH_3	H	41** (30)	42** (10)	8	2	40	33
62		Cl	H	CH_3	54* (30)	30 (10)			3720	1250
63		CH_3	Cl	H	43** (100)	31 (50)			282	79
64		Br	CH_3	H	86** (100)	66** (50)	2	4	63	50
65		CN	CH_3	H	66** (30)	50* (10)	7	6	36	28
66		CF_3S	CH_3	H	55** (30)	68** (1)	10	13	178	11
67		CF_3O	CH_3	H	61** (30)	46** (3)	14	4	178	18

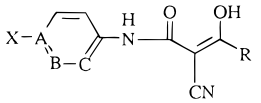
[a] Percent Inhibition (mg/kg). [b] IC_{50} (nM). *$P < 0.05$; **$P < 0.01$ (Student's *t*-test).

was shown to be with the 4-chloro-3-alkyl analogue, e.g., **61** (Table 5). All compounds with potent DTH activity and long kinetics, e.g., **34** and **46** (Table 4), also showed good DTH and reduced kinetics in both rat and mouse following the introduction of a 3-methyl group, e.g., **61** and **67** (Table 5). The minimum energy conformation for **3** contains the aryl ring coplanar with the enolic amide system.[19] The introduction of a 2-methyl group gave less potent compounds as did the introduction of other 2-substituents, e.g., **33** vs **17** (Table 4). This may be due to the steric effects of the 2-alkyl group which cause the conformation of the aromatic ring to be less coplanar (~40° out of plane) (Figure 1, **61** vs **62**).

The aryl amide group was investigated for its effects on both DTH and kinetic properties. Use of pyridyl-substituted molecules (e.g., **81**) (Table 2) gave good DTH and kinetic properties (Table 6).

More recently the target protein for this series of drugs has been identified as DHODH.[10] The structure–activity for this series has now been investigated by the *in vitro* determination of inhibition of both rat and mouse DHODH. In general, potent DHODH inhibitors also show good *in vivo* DTH activity, and poor *in vitro* enzyme inhibitors also show poor *in vivo* pharmacology. For example, **40** is one of the most potent compounds in rat DTH (58% inhibition at 1 mg/kg) and is also one of the most potent *in vitro* rat DHODH enzyme inhibitors (IC_{50} = 5 nM, **2** IC_{50} = 13 nM). The excellent correlation of *in vivo* to *in vitro* potency gives support to the inhibition of DHODH being largely responsible

Table 6. Investigation of Amides



Cmpd. No.	R	A	B	C	X	DTH (mouse)[a]	DTH (rat)[a]	t½(hours) mouse	t½(hours) rat	DHODH[b] mouse	DHODH[b] rat
1						84 (30)	78** (10)	30	9	-	-
2	CH_3	C	CH	CH	CF_3	-	-	-	-	69	13
17		C	CH	CH	CF_3	85** (30)	53** (10)	28	8	47	21
79		C	CH	N	Cl	48 (100)	64* (50)			200	76
80		C	CH	N	Br	4 (30)	66** (10)	7	13	158	37
81		C	CH	N	CF_3	67** (100)	40* (3)	5	7	158	17
82		C	N	CH	Cl	9 (30)	71** (50)			1780	630
83		N	CH	CH	-	22 (100)	17 (50)			8910	11700

[a] Percent inhibition (mg/kg). [b] IC_{50} (nM). *P < 0.05; **P < 0.01 (Student's t-test).

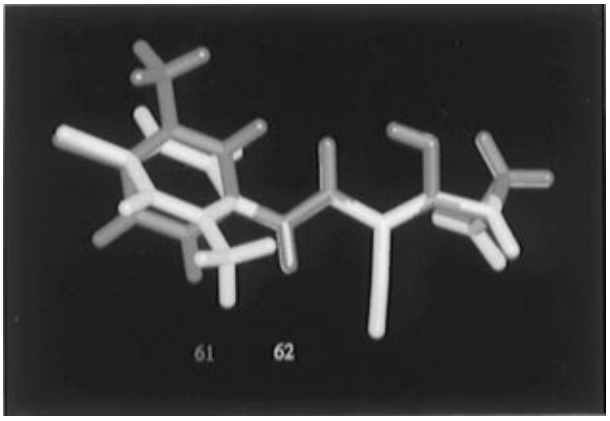


Figure 1. Overlay of **61** and **62**, showing the conformational difference between 2-alkyl-substituted (**62**) and 3-alkyl-substituted (**61**) aryl rings.

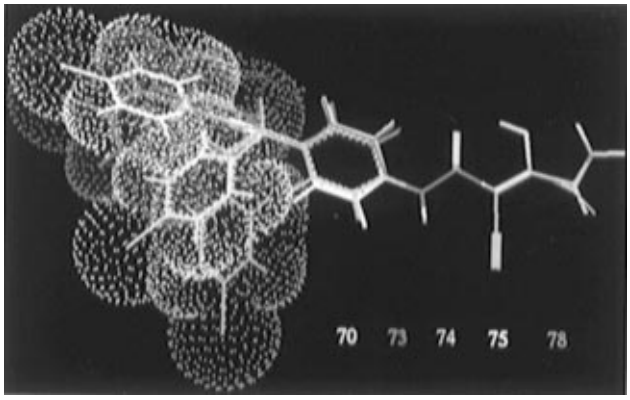


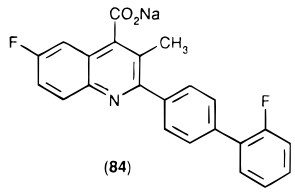
Figure 2. Overlay of aryl amides to show space available in drug binding site. Biological activity (DHODH inhibition) decreases in the order: **74** > **70** > **78** > **75** > **73**.

for the *in vivo* pharmacology for this series of compounds. Some examples which do not correlate well are the cis olefin **74**, which is weaker in mouse DTH than would be expected from its mouse DHODH *in vitro* potency. This may be due to short kinetics or poor oral bioavailability (not determined). Also the biphenyl compounds **77** and **78** are more potent *in vivo* than would be expected based on DHODH inhibition.

A number of compounds in this series are potent inhibitors of rat DHODH (IC_{50} < 10 nM). In general this series provides more potent inhibitors of the rat DHODH enzyme than of the mouse enzyme. The best inhibitors of the rat enzyme (**40**, **42**, **46**) all contain the small cyclopropyl ketone group. In addition, all contain small substituents on the aromatic ring with the best being 4-substituted. The best substituents are lipophilic and, also, weak hydrogen bond acceptor groups, e.g., **46**. The most potent inhibitors of the mouse DHODH enzyme again predominantly contain 3-cyclopropyl groups, an indication of restricted space in this binding region. The best substituents on the aromatic ring for the mouse enzyme are lipophilic in nature (e.g., **34**, **37**, **74**). For both rat and mouse, polar, hydrogen bond donor groups afford poor inhibitors in both DTH and DHODH assays (e.g., **48**, **50**, **53**).

The larger aryl amides give more information about the space available in the drug binding site. Thus the cis olefin-linked phenyl groups in **74**, which showed good DHODH inhibition, can be compared with the phenyl ether **70**, the biphenyl **78**, the acetylene **75**, and the trans olefin **73**, all of which show decreasing activity. Molecules such as the cis olefin **74** and the trans olefin **73**, with their obviously different spatial requirements, help to define the space available in the rat and mouse enzyme binding site for these compounds (Figure 2).

Brequinar sodium (**84**) inhibits DHODH noncompetitively with respect to either the substrate (dihydroorotate) or the cofactor ubiquinone.[20] Studies to determine whether **3** and **84** bind to the same site on DHODH are in progress and will be reported later.[21] The structure activity for **84** on the mouse and human enzyme has



(**84**)

been investigated.[22,23] Figure 3 shows an overlay of **84** and the structure of **3**.[19] The critical regions which have been defined in the literature[22,23] for **84** are (1) the carboxylic acid group which we have overlapped with the acidic enolic hydroxyl group, indicating that there is probably an electrostatic interaction between the acidic groups of the drugs and their binding site, (2) bulky hydrophobic substituents (biphenyl) which can overlay the 4-substituted aryl ring, and (3) the benzo

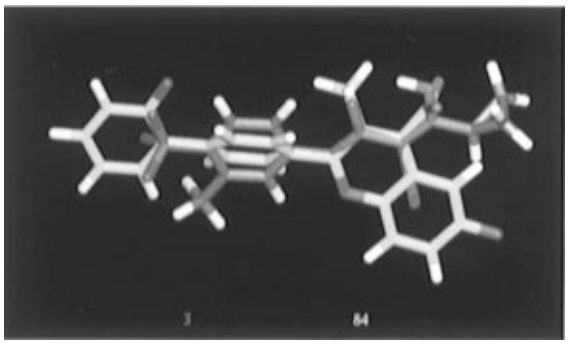


Figure 3. Overlay of brequinar sodium (**84**) and HR 325 (**3**).

portion of the quinoline ring which overlays the **3** nitrile group. Our structure−activity data for the mouse DHODH enzyme fits with the literature data for **84**,[22,23] which shows a hydrophobic group requirement such as the biphenyl or 4-*tert*-butylphenyl group, and our most potent mouse DHODH inhibitors, which contain hydrophobic 4-substituents on the aryl amide group e.g., **34**, **37**, and **74**.

The compound with the best overall pharmacological profile (**3**) was further tested in animal models of arthritis. Good inhibition was achieved in mouse collagen II arthritis[24] (ED_{50} = 31 mg/kg) and in both rat developing and established collagen II arthritis[25] [ED_{50} = 2 mg/kg for developing arthritis (leflunomide (**1**) 102% ($p < 0.01$) at 10 mg/kg) and 65% ($p < 0.01$) inhibition at 25 mg/kg or 32% ($p < 0.05$) at 10 mg/kg for established arthritis (leflunomide (**1**) 29% ($p < 0.05$) at 10 mg/kg)].

Single-dose (20 mg) human kinetics have also been determined for **3** ($t_{1/2}$ = 3.5 h), indicating that the objective to produce a derivative with reduced human kinetics over leflunomide has been achieved. Clinical studies are in progress with this molecule.

Conclusion

The structure−activity relationship of a series of β-hydroxy enamides on the dihydroorotate dehydrogenase enzyme shows good correlation with the *in vivo* DTH activity for this series. The pharmacokinetic properties of the series have been studied and modified. The most successful change in pharmacokinetics was achieved with the introduction of a 3-methyl substituent onto the aryl amide group. The compound **3** was selected for development.

Experimental Section

Instrumentation and Methods. Proton NMR spectra were recorded on a Bruker WP200SY spectrometer, and the data are reported with the chemical shifts in parts per million (ppm, δ) relative to tetramethylsilane as an internal standard. Mass spectra were obtained on a Fisons VG Autospec E mass spectrometer. Infrared spectra were recorded using a Pye Unicam SP3-200S instrument. Melting points were obtained using a Kofler hot stage apparatus and are uncorrected. Evaporations were done using a Buchi rotary evaporator. Elemental microanalyses were performed by CHN Analysis Ltd. of Leicester. Analytical thin layer chromatography (TLC) was performed on E Merck silica gel 60 F_{254} commercial plates to follow the course of reactions. Column chromatography was performed with Sorbsil C60 silica gel (mesh 40−60 μm). Analytical HPLC was performed using Hypersil HSAS (25 cm × 4.6 mm i.d. column). THF was freshly distilled from potassium benzophenone ketyl. Reagents were obtained commercially and used without further purification.

Method A. 2-Cyano-3-cyclopropyl-3-hydroxy-*N*-[3′-methyl-4′-(trifluoromethyl)phenyl]propenamide (3). Step 1.[26] Cyanoacetic acid (65.5 g, 0.77 mmol) was added to a stirred suspension of phosphorus pentachloride (159.5 g, 0.77 mmol) in dichloromethane (2.3 L) at ambient temperature. The reaction mixture was heated at reflux for 30 min. After cooling 3-methyl-4-(trifluoromethyl)aniline (89.4 g, 0.51 mmol) was added over 10 min and the reaction mixture heated for 2 h at reflux. The reaction mixture was cooled in an ice/water bath, and water (1 L) added. After stirring for 30 min, the reaction mixture was neutralized by the addition of sodium carbonate solution. The precipitated solid was filtered, washed with water, and dried to afford 110 g (89%) of 2-cyano-*N*-[3′-methyl-4′-(trifluoromethyl)phenyl]acetamide. This product was used without further purification: mp 142−3 °C; IR (KBr) 3270, 2274, 1677, 1620, 1552; ^{1}H NMR (DMSO-d_6) δ 10.54 (1H, s), 7.56 (3H, m), 4.00 (2H, s), 2.46 (3H, s).

Step 2. The product from step 1 (107 g, 0.44 mmol) was suspended in THF (4 L) and cooled to <5 °C. Sodium hydride (80% sodium hydride in oil) (29.2 g, 0.97 mmol) was added while the temperature was maintained at <10 °C. The mixture was allowed to warm to ambient temperature and stirred for 30 min. Cyclopropanecarbonyl chloride (55.4 g, 0.53 mmol) was added over 10 min, and the reaction mixture stirred at ambient temperature for 1 h. The reaction was quenched by careful addition of acetic acid (100 mL) and then poured onto ice/water (4 L) containing hydrochloric acid (100 mL). The product was filtered off, washed with water, and dissolved in dichloromethane (5 L). The solution was washed with water, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to a thick slurry. This was cooled and filtered to afford the title compound (116.2 g, 85%) as colorless crystals: mp 186−8 °C; IR 3395 (NH), 2204 (CN), 1630, 1600, 1580; ^{1}H NMR ($CDCl_3$) δ 15.63 (1H, s), 7.59 (2H, d), 7.43 (2H, d), 2.49 (3H, s), 2.15 (1H, m), 1.35 (2H, m), 1.19 (2H, m). Anal. C, H, N, F.

Method B. 2-Cyano-3-cyclopropyl-3-hydroxy-*N*-[4′-(trifluoromethyl)phenyl]propenamide (17). Step 1. Cyanoacetic acid (8.6 g, 0.10 mmol) and 4-(trifluoromethyl)aniline (13.5 mL, 0.11 mmol) were dissolved in THF (100 mL) and stirred at room temperature. Diisopropylcarbodiimide (DIPCDI) (16.4 mL, 0.10 mmol) was added over 10 min. The reaction temperature varied between 20 and 60 °C during the addition. The reaction was stirred at room temperature for 16 h and filtered and the solvent evaporated. The residue was washed with ethanol, dichloromethane, and then hexane and dried under reduced pressure at 60 °C. This afforded the product (18.85 g, 83%) which was used without further purification: mp 195−196 °C; IR (KBr) 3280, 1672, 1608, 1551; ^{1}H NMR (DMSO-d_6) δ 10.72 (1H, s), 7.81 (2H, d, J = 9 Hz), 7.74 (2H, d, J = 9 Hz), 4.03 (2H, s).

Step 2. The title compound was obtained in 70% yield by the method described for method A, step 2: mp 212−3 °C; IR (KBr) 3280 (NH), 2202 (CN), 1620, 1602, 1575; ^{1}H NMR ($CDCl_3$) δ 15.64 (1H, s), 7.77 (1H, s), 7.64 (4H, s), 2.16 (1H, m), 1.37 (2H, m), 1.18 (2H, m). Anal. C, H, N, F.

Method C. 2-Cyano-3-hydroxy-*N*-[4′-(trifluoromethyl)-phenyl]penta-2,4-dienamide (4). Step 1. As for method A or B.

Step 2. 2-Cyano-*N*-[4′-(trifluoromethyl)phenyl]acetamide (7.0 g, 0.0307 mmol) in dry THF (200 mL) was stirred under nitrogen during the addition of imidazole (0.02 g; catalyst) and sodium hydride (80% oil dispersion) (2.3 g, 0.077 mmol), and the suspension stirred for 2 h at room temperature. The mixture was cooled to −78 °C and treated dropwise with 3-(phenylseleno)propionyl chloride (9.11 g, 0.037 mmol).[12] The mixture was stirred for 90 min at −78 °C, poured onto dilute hydrochloric acid/ice, and filtered. The collected solid was dissolved in dichloromethane, washed with water, and dried ($MgSO_4$) and the solvent removed *in vacuo*. Trituration with diethyl ether gave 2-cyano-3-hydroxy-5-(phenylseleno)-*N*-[4′-(trifluoromethyl)phenyl]penta-2,4-dienamide as colorless crystals (13.40 g, 99%). This was used without further purification for step 3: mp 121−3 °C; IR (KBr) 3293, 2222, 1637, 1609, 1589; ^{1}H NMR ($CDCl_3$) δ 15.53 (1H, s), 7.75 (1H, s), 7.58 (6H, m), 7.29 (3H, m), 3.16 (2H, m), 3.00 (2H, m).

Step 3. 2-Cyano-3-hydroxy-5-(phenylseleno)-*N*-[4′-(trifluoromethyl)phenyl]penta-2,4-dienamide (8.0 g, 0.018 mmol) in dichloromethane (200 mL) was cooled to 0 °C and treated with 30% hydrogen peroxide (4.0 mL), and the mixture stirred vigorously for 30 min giving a colorless suspension of the intermediate selenoxide. The mixture was diluted with methanol (40 mL) and dichloromethane (200 mL), stirred at room temperature for 1 h, and passed through a column of silica gel. The eluent was concentrated under reduced pressure and diluted with diethyl ether. Colorless crystals of the title compound were collected (2.8 g, 54%): mp 206−8 °C; IR (KBr) 3300 (NH), 2215 (CN), 1632, 1604; ^{1}H NMR (DMSO-d_6) δ 12.17 (1H, s), 7.81 (2H, d), 6.88 (1H, dd), 6.20 (1H, dd), 5.69 (1H, dd). Anal. C, H, N, F.

Method D. Step 1. 5-[4′-(Trifluoromethyl)phenyl]-*N*-[4′-(trifluoromethyl)phenyl]isoxazole-4-carboxamide (24). 5-[4′-(Trifluoromethyl)phenyl]isoxazole-4-carboxylic acid[27] (2.6 g, 10 mmol) was warmed with thionyl chloride (10 mL) at 50 °C for 1 h.[27] The excess thionyl chloride was removed *in vacuo* by azeotroping with toluene. The residue was taken up with dichloromethane and 4-aminobenzotrifluoride (2 g, 1.24 equiv, 1.6 mL) added. The reaction mixture was stirred at room temperature for 30 min, washed with dilute HCl, water, aqueous sodium carbonate, and water, dried ($MgSO_4$), and evaporated to dryness. The product was purified by flash chromatography (dichloromethane) and recrystallized from diethyl ether by addition of petroleum ether to afford 2.9 g, 72%, of the title compound. This was used without further purification: mp 211−3 °C; IR (KBr) 3300, 1655, 1605, 1525, 1475, 1410; ^{1}H NMR ($CDCl_3$/MeOH-d_4) δ 8.85 (1H, s), 8.2 (2H, d), 7.8 (4H, m), 7.60 (2H, d).

Step 2.[27] 2-Cyano-2-[4′-(trifluoromethyl)benzoyl]-*N*-[4′-(trifluoromethyl)phenyl]ethanamide. The isoxazole from step 1 (2.5 g, 6.3 mmol) was added to a solution of Na (1 g, 43 mmol) in ethanol (50 mL) and stirred until all the solids had dissolved (15 min). The reaction mixture was poured onto aqueous HCl (400 mL, 2 N) and stirred for 20 min. The product was filtered and washed with dilute hydrochloric acid and then water. Recrystallization from hot DMF by the dropwise addition of water afforded the title compound (2.42 g, 97%): mp 218−20 °C; IR (KBr) 3300, 2200, 1635, 1625, 1605, 1595; ^{1}H NMR (DMSO-d_6) δ 12.35 (1H, brs), 7.85−7.55 (8H, m). Anal. C, H, N, F.

Method E. 3-[*N*-(*tert*-Butyloxycarbonyl)pyrrolidin-2-yl]-2-cyano-3-hydroxy-*N*-[4′-(trifluoromethyl)phenyl]propenamide. Step 1. Carbonyldiimidazole (4.21 g, 1.3 equiv, 25.96 mmol) was added at −30 °C to a solution of *N*-(*tert*-butyloxycarbonyl)proline (5.16 g, 1.2 equiv, 23.97 mmol) in THF (20 mL), and the reaction mixture was warmed to room temperature for 2 h. The resulting mixture was then used to acylate the required cyanoacetamide at room temperature (method A, step 2). Final product was obtained by addition of water and extraction of the aqueous phase with ethyl acetate. The organic phase was washed with saturated sodium chloride with the addition of dilute hydrochloric acid until pH $\simeq$ 5. The extracts were then washed with saturated $NaHCO_3$, dried $MgSO_4$, and evaporated. The product was purified by trituration with diethyl ether to give (6.3 g, 62%) of a colorless solid: mp 205−10 °C; IR (KBr) 3480, 3387, 2188, 1650, 1601, 1556; ^{1}H NMR (DMSO-d_6) δ 12.40, 12.25 (1H, 2 × s, coalescence at 373 K), 7.76 (2H, d, J = 8.6 Hz), 7.59 (2H, d, J = 8.6 Hz), 4.64 (1H, m), 3.50 (2H, m), 2.17 (1H, m), 1.83 (3H, m), 1.43, 1.34 (9H, 2 × s, coalescence at 373 K).

Step 2. 2-Cyano-3-hydroxy-3-(pyrrolidin-2-yl)-*N*-[4′-(trifluoromethyl)phenyl]propenamide (22). The protected amine (425 mg, 1 mmol) in anisole (2 mL) and dichloromethane (5 mL) was cooled to 0 °C and trifluoroacetic acid (5 mL) added. The reaction mixture was stirred at room temperature for 4 h and evaporated to dryness. The residue was triturated with diethyl ether and filtered to afford the trifluoroacetic acid salt of the title compound (330 mg, 75%). The salt was dissolved in methanol/dichloromethane and basified with aqueous sodium bicarbonate. The precipitate was filtered to give the free base: mp 266−8 °C; IR (KBr) 3380, 1660, 1640, 1600, 1579; ^{1}H NMR (DMSO-d_6) δ 10.64 (1H, s), 8.10 (1H, s), 7.81 (2H, d, J = 8.6 Hz), 7.67 (2H, d, J = 8.6 Hz), 4.04 (1H, m), 3.32 (2H, m), 2.12 (3H, m), 1.44 (1H, m). Anal. C, H, N, F.

Method F. 2-Cyano-3-hydroxy-3-(*N*-methylpyrrolidin-2-yl)-*N*-[4′-(trifluoromethyl)phenyl]propenamide (23). The trifluoroacetic acid salt of **22** (7.5 g, 0.0243 mmol) in THF (300 mL) was treated with sodium hydride (80% dispersion in oil) (1.5 g, 2 equiv) and stirred at room temperature for 40 min. Methyl iodide (4.92 mL, 0.078 mmol) was added, and the reaction mixture stirred for 24 h. The reaction mixture was acidified (dilute HCl), extracted with ethyl acetate, dried ($MgSO_4$), and concentrated. Trituration with dichloromethane/methanol followed by chromatography of the residues (0−1% methanol in dichloromethane) afforded the required product (4.16 g, 50%): mp 168−70 °C; IR (KBr) 3260, 1650, 1590; ^{1}H NMR ($CDCl_3$) δ 10.44 (1H, s), 8.82 (1H, brs), 7.71 (2H, d, J = 8.6 Hz), 7.53 (2H, d, J = 8.6 Hz), 4.08 (1H, m), 3.64 (1H, m), 3.23 (1H, m), 3.16 (3H, d, J = 5.4 Hz) (singlet when decoupled with OH at 8.8 ppm), 2.22 (3H, m), 1.64 (1H, m). Anal. C, H, N, F.

Method G. Preparation of *N*-[4′-[(Trifluoromethyl)-sulfinyl]phenyl]cyanoacetamide and *N*-[4′-[(Trifluoromethyl)sulfonyl]phenyl]cyanoacetamide. Step 1. *N*-[4′-[(Trifluoromethyl)thio]phenyl]cyanoacetamide (4.35 g, 0.0167 mol) in acetic acid (15 mL) was stirred at room temperature overnight with peracetic acid (8.0 mL of 32%, w/v, 0.035 mol). The mixture was poured onto ice/water and extracted into ethyl acetate. The extracts were washed with aqueous sodium bicarbonate solution, dried ($MgSO_4$), and evaporated to give a colorless solid (4.6 g). Chromatography on silica gel (1−10% acetone in dichloromethane) gave *N*-[4′-[(trifluoromethyl)-sulfinyl]phenyl]cyanoacetamide (2.7 g, 59%) as colorless crystals: mp 164−5 °C; IR (KBr) 3280, 1678, 1615, 1592; ^{1}H NMR (DMSO-d_6) δ 10.80 (1H, brs), 7.91 (4H, s), 4.03 (2H, s). This product was used without further purification.

The residual fractions (1.7 g) from the above chromatography were combined, dissolved in acetic acid (6 mL), and treated with peracetic acid (6 mL). The mixture was heated to 50 °C overnight, poured onto water, and extracted into ethyl acetate. The extracts were washed with aqueous sodium bicarbonate, dried ($MgSO_4$), and evaporated. Chromatography on silica gel (100 g) (2−3% acetone in dichloromethane) gave *N*-[4′-[(trifluoromethyl)sulfonyl]phenyl]cyanoacetamide as colorless crystals (1.06 g, 22%): mp 135−7 °C; IR (KBr) 3343, 3316, 1724, 1608, 1592; ^{1}H NMR (DMSO-d_6) δ 11.09 (1H, s), 8.15 (2H, d, J = 9 Hz), 8.00 (2H, d, J = 9 Hz), 4.08 (2H, s). This product was used without further purification.

Step 2. Preparation of 2-Cyano-3-cyclopropyl-3-hydroxy-*N*-[4′-[(trifluoromethyl)sulfinyl]phenyl]prop-2-enamide (41). *N*-[4′-[(Trifluoromethyl)sulfinyl]phenyl]cyanoacetamide (3.1 g, 0.0112 mol) in dry THF (100 mL) was treated with imidazole (10 mg; catalytic) and sodium hydride (80% oil dispersion) (0.75 g, 0.0246 mol) and stirred at room temperature for 90 min. The solution was treated dropwise with cyclopropylcarbonyl chloride (1.32 mL, 0.014 mmol), stirred at room temperature for 10 min, poured onto ice/1 M HCl, and filtered. The precipitated product was collected, dissolved in dichloromethane, and passed through a short silica gel column with dichloromethane to give 2-cyano-3-cyclopropyl-3-hydroxy-*N*-[4′-[(trifluoromethyl)sulfinyl]phenyl]prop-2-enamide (2.54 g, 66%): mp 155−7 °C; IR (KBr) 3280, 2219, 1603, 1575; ^{1}H NMR ($CDCl_3$) δ 15.49 (1H, s), 7.89 (1H, s), 7.82 (4H, s), 2.18 (1H, m), 1.37 (2H, m), 1.22 (2H, m). Anal. C, H, N, F, S.

Preparation of 2-Cyano-3-cyclopropyl-3-hydroxy-*N*-[4′-[(trifluoromethyl)sulfonyl]phenyl]prop-2-enamide (42). Treatment of *N*-[4′-[(trifluoromethyl)sulfonyl]phenyl]cyanoacetamide by the above method gave 2-cyano-3-cyclopropyl-3-hydroxy-*N*-[4′-[(trifluoromethyl)sulfonyl]phenyl]prop-2-enamide (65%): mp 168−70 °C; IR (KBr) 3300, 2218, 1638, 1579; ^{1}H NMR ($CDCl_3$) δ 15.30 (1H, s), 8.03 (2H, d, J = 8.8 Hz), 7.92 (1H, s), 7.85 (2H, d, J = 8.8 Hz), 2.18 (1H, m), 1.41 (2H, m), 1.25 (2H, m). Anal. C, H, N, F, S.

Method H. 2-Cyano-3-cyclopropyl-3-hydroxy-*N*-[4′-(methylsulfonyl)phenyl]propenamide (45). 2-Cyano-3-cyclopropyl-3-hydroxy-*N*-[4′-(methylthio)phenyl]propenamide (**43**) (8.0 g, 29.16 mmol) was dissolved in acetic acid (500 mL), and

peracetic acid (25 mL of 32% solution in acetic acid, 105 mmol) was added. The reaction mixture was stirred overnight at room temperature, and water (250 mL) was added. The precipitate was filtered, washed with water, and dried *in vacuo*. Crystallization from ethyl acetate/hexane afforded (4.6 g, 51%) of a white solid: mp 199−201 °C; IR (KBr) 3250, 2930, 2220, 1630, 1570; ^{1}H NMR (DMSO-d_6) δ 11.42 (1H, s), 7.84 (4H, m), 3.21 (3H, s), 2.23 (1H, m), 1.02 (4H, m). Anal. C, H, N, S.

Method I. *N*-(4′-Aminophenyl)-2-cyano-3-cyclopropyl-3-hydroxypropenamide Hydrochloride (50). 2-Cyano-3-cyclopropyl-3-hydroxy-*N*-(4′-nitrophenyl)propenamide (**49**) (4.3 g, 15.7 mmol) was dissolved in ethanol/0.5 N sodium hydroxide (1:1, 150 mL) and treated with hydrogen (1 atm) for 6 h in the presence of 10% palladium/carbon (150 mg). The reaction mixture was filtered through Celite, and the solution was acidified to pH 1 with concentrated hydrochloric acid. The product was filtered, washed with water, and dried *in vacuo* to afford the title compound (3.7 g, 84%): mp >300 °C; IR (KBr) 3300, 2840, 2590, 2200 (CN), 1630, 1570. ^{1}H NMR (DMSO-d_6) δ 11.16 (1H, s), 7.66 (2H, m), 7.35 (2H, m), 2.21 (1H, m), 1.04 (4H, m). Anal. C, H, N.

Synthesis of Anilines. Preparation of 1-Iodo-2-methyl-4-nitrobenzene. 2-Methyl-4-nitroaniline (230 g, 1.51 mmol) was suspended in dilute sulfuric acid (concentrated H_2SO_4 (189 mL)/H_2O (1.38 L)) and cooled to below 5° using an ice/salt bath. A solution of sodium nitrite (109.6 g, 1.59 mmol in 345 mL of H_2O) was added at a rate to maintain a temperature of 0−5 °C. This diazonium salt solution was added to a solution of potassium iodide (422.1 g, 2.54 mmol in 1.27 L of H_2O) maintaining a temperature of 0−5 °C during the addition. The mixture was stirred at ambient temperature for 2 h and then heated to 50−60 °C for 1 h. The mixture was cooled and filtered and the solid washed with water. Solid was dissolved in dichloromethane (4 L) and washed with sodium metabisulfite solution (2 × 2 L). The solution was passed down a column of silica gel to remove the majority of the dark brown coloration. The solvent was concentrated *in vacuo* and the orange crystalline solid triturated with ether, filtered, and dried to afford the title compound (327 g, 82%): ^{1}H NMR ($CDCl_3$) δ 8.07 (2H, m), 7.72 (1H, dd, J = 8.7, 2.8 Hz), 2.55 (3H, s).

2-Ethyl-1-iodo-4-nitrobenzene: prepared by the above method starting from 4-amino-3-ethyl-1-nitrobenzene,[28] 60% yield; mp 32−3 °C; ^{1}H NMR ($CDCl_3$) δ 8.05 (1H, d, J = 2.7 Hz, H-3), 8.01 (1H, d, J = 8.6 Hz, H-6), 7.73 (1H, dd, J = 2.7, 8.6 Hz, H-5), 2.83 (2H, q, J = 7.5 Hz, $-CH_2$), 1.28 (3H, t, J = 7.5 Hz, $-CH_3$); MS (70 eV) *m/e* 277 (M^+, 100), 262, 204, 133, 104, 103, 78, 77.

Preparation of 2-(Trifluoromethyl)-5-nitrotoluene. A 450 mL Parr pressure reactor was charged with 2-iodo-5-nitrotoluene (81.0 g, 0.308 mmol), active copper powder[14,15] (62.3 g, 0.98 mol), and dimethylformamide (200 mL). The reactor was purged with argon and cooled to ca. −25 °C, and trifluoromethyl iodide (93 g, 0.475 mmol) was introduced via the dip-tube inlet valve. The reactor was then heated gradually to 150 °C, with stirring, and maintained at 150 °C for 5 h. The cooled reaction mixture was poured onto ice/water (1.0 L), washing out the reactor with ethyl acetate. The copper salt was filtered off and washed with ethyl acetate. The aqueous layer was separated and extracted with ethyl acetate. All extracts were combined, washed with water, and dried over magnesium sulfate. The solvent was concentrated *in vacuo* and the crude product purified by distillation at ca. 10 mbar, bp 89−92 °C. The title product was obtained (54.5 g, 95%): IR (film) 1536, 1355, 1314, 1273, 1178, 1127, 1047, 925, 899, 843, 807; ^{1}H NMR ($CDCl_3$) δ 8.13 (2H, m), 7.81 (1H, d, J = 8.3 Hz), 2.61 (3 H, m). Anal. C, H, N.

3-Methyl-1-nitro-4-(pentafluoroethyl)benzene: prepared by the same method using pentafluoroethyl iodide, 68% yield; IR (film) 1536, 1350, 1334, 1304; ^{1}H NMR ($CDCl_3$) δ 8.14 (2H, m), 7.75 (1H, d, J = 8.4 Hz), 2.65 (3H, t, J = 3.1 Hz).

3-Ethyl-1-nitro-4-(trifluoromethyl)benzene: prepared by the same method, 71% yield; IR (film) 2977, 2942, 1535, 1352, 1310; ^{1}H NMR ($CDCl_3$) δ 8.22 (1H, d, J = 1.5 Hz), 8.14 (1H, dd, J = 8.6, 1.5 Hz), 7.81 (1H, d, J = 8.6 Hz); ^{13}C NMR ($CDCl_3$) δ 150.05, 145.54, 133.71 (q, J = 30.5 Hz, C-1), 127.32 (q, J = 5.5 Hz, C-6), 125.05, 123.48 (q, J = 273.0 Hz, CF_3), 120.72, 25.58, 15.08. Anal. C, H, N. F.

Preparation of 1-(Bromodifluoromethoxy)-2-methyl-4-nitrobenzene. 2-Methyl-4-nitrophenol (6 g, 43.13 mmol) was added to sodium (0.92 g, 40 mmol) dissolved in ethanol (30 mL). The solvent was evaporated *in vacuo*, and benzene (20 mL) was added and reevaporated. The sodium salt was added to DMF (24 mL), dibromodifluoromethane (30 mL), and ethanethiol (3 drops; catalyst). The reaction mixture was heated at 70 °C for 10 h, poured onto ice/water, and extracted with ethyl acetate. The organic phase was washed with aqueous sodium hydroxide (0.5 M) and water, dried ($MgSO_4$), and concentrated *in vacuo*. The product was purified by flash chromatography (4% ethyl acetate in hexane) to afford 1-(bromodifluoromethoxy)-2-methyl-4-nitrobenzene (3.35 g, 29%) which was used without further purification: IR (film) 1591, 1529, 1490, 1351, 1310, 1237; ^{1}H NMR ($CDCl_3$) δ 8.15 (2 H, m), 7.46 (1H, d, J = 8.9 Hz), 2.43 (3H, s).

Preparation of 2-Methyl-4-nitro-1-(trifluoromethoxy)-benzene. 1-(Bromodifluoromethoxy)-2-methyl-4-nitrobenzene (0.3 g, 1.06 mmol), antimony trifluoride (0.12 g, 0.67 mmol), and antimony pentachloride (0.02 g; catalyst) were heated in a sealed flask at 175 °C for 4 h. The reaction mixture was cooled, diluted with ether, washed with water, dried ($MgSO_4$), and concentrated *in vacuo*. This afforded the title compound (0.13 g, 83%) which was used without further purification: IR (film) 3413, 2928, 1593, 1529, 1494, 1350, 1339; ^{1}H NMR ($CDCl_3$) δ 8.15 (2H, m), 7.44 (1H, d, J = 8.9 Hz), 2.43 (3H, s); ^{13}C NMR ($CDCl_3$) δ 120.34 (CF_3, J_{CF} = 260 Hz).

Preparation of 4-(Trifluoromethyl)-3-toluidine. 2-(Trifluoromethyl)-5-nitrotoluene (145 g, 0.759 mmol) in methanol (1.7 L) was hydrogenated at 1250 mbar using 5% palladium on charcoal (14.5 g) as catalyst. When hydrogen uptake had ceased, the catalyst was filtered off and the solvent removed under reduced pressure to leave the product as a pale yellow oil (123 g, 93%): IR (film) 3500, 3400, 3240, 1630, 1590, 1520, 1465, 1445, 1320, 1280, 1195, 1163, 1115, 1045, 865, 825; ^{1}H NMR ($CDCl_3$) δ 7.36 (1H, d, J = 8.2 Hz), 6.50 (2H, m), 3.86 (2H, brs), 2.37 (3H, m). Anal. C, H, N, F.

1-Amino-3-methyl-4-(pentafluoroethyl)benzene: prepared by the same method, 94% yield; IR (KBr) 3391, 3331, 3227, 1676, 1650, 1599, 1554, 1342; ^{1}H NMR ($CDCl_3$) δ 7.30 (1H, s), 6.53 (2H, m), 3.80 (2H, brs), 2.37 (3H, t, J = 3 Hz).

4-Amino-2-ethyl-1-(trifluoromethyl)benzene: prepared by the same method, 93% yield, colorless liquid; ^{1}H NMR ($CDCl_3$) δ 7.37 (1H, d, J = 8.4 Hz), 6.58 (1H, d, J = 2.0 Hz), 6.50 (1H, dd, J = 8.4, 2.0 Hz), 3.88 (2H, brs), 2.72 (2H, q, J = 7.5 Hz), 1.22 (3H, t, J = 7.5 Hz).

Preparation of 4-Amino-2-methyl-1-(trifluoromethoxy)-benzene. 5-Nitro-2-(trifluoromethoxy)toluene (2.5 g, 0.0113 mol) in toluene (100 mL) was treated with iron powder (13 g) and concentrated HCl (3 drops) and stirred vigorously under reflux. Water (2.0 mL) was added over 2 h; the mixture was filtered and concentrated to a low volume. The product was used in the next step without further purification.

Preparation of 4-Amino-2-methyl-1-[(trifluoromethyl)-thio]benzene. 2-Fluoro-5-nitrotoluene (15.5 g, 0.1 mmol) and sodium sulfide nonahydrate (30 g, 0.125 mmol) in DMSO (50 mL) were heated at 50 °C under a nitrogen atmosphere for 2 h. The reaction mixture was poured onto ice/water and extracted with dichloromethane. The organic phase was washed with water, dried ($MgSO_4$), and concentrated *in vacuo* to afford 4-mercapto-3-methyl-1-nitrobenzene (12 g, 71%) which was used without further purification. The product (9 g, 0.053 mmol) was added to sodium (1.22 g, 0.053 mmol) in ethanol (120 mL) and evaporated to dryness. The residue was azeotroped with benzene and evaporated to dryness. The residue was then dissolved in DMF (30 mL) in a Fischer Porter apparatus, flushed with argon, and cooled to −78 °C and the flask charged with trifluoromethyl iodide (21 g). The reaction mixture was stirred for 48 h at room temperature followed by heating at 75 °C for 24 h. The reaction mixture was cooled, poured onto ice/water/ether, and filtered. The aqueous phase was separated and reextracted with ether. The combined organic phases were dried ($MgSO_4$), evaporated, and purified

by flash chromatography (5% dichloromethane in hexane) to afford 2-methyl-4-nitro-1-[(trifluoromethyl)thio]benzene (2.15 g, 17%): ^{1}H NMR ($CDCl_3$) δ 8.18 (1H, d, J = 2.5 Hz), 8.07 (1H, dd, J = 8.5, 2.5 Hz), 7.85 (1H, d, J = 8.5 Hz), 2.66 (3H, s).

The product (2.15 g, 9.14 mmol) in benzene (50 mL) was treated with iron powder (11 g) and stirred with concentrated hydrochloric acid (3 drops). The reaction mixture was stirred vigorously and heated at reflux during the addition of water (1.5 mL) over 2 h. The mixture was heated at reflux for a further 2 h, cooled, filtered, and evaporated *in vacuo* to give the title compound (1.65 g, 87%) which was used without further purification: IR (film) 3487, 3399, 1626, 1598, 1573, 1485, 1321; ^{1}H NMR ($CDCl_3$) δ 7.40 (1H, d, J = 8.3 Hz), 6.60 (1H, m), 6.50 (1H, m), 3.87 (2H, brs), 2.43 (3H, s).

Preparation of (*E*)-(4-Chlorophenyl)(4′-nitrophenyl)-ethene. Sodium (2.21 g, 96.1 mmol, 1.05 equiv) was added in lumps over 1.75 h to ethanol (100 mL). The solution was stirred for 20 min following dissolution of sodium and then added dropwise over 50 min to a solution of diethyl 4-nitrobenzyl phosphonate (25 g, 91.5 mmol) and 4-chlorobenzaldehyde (12.9 g, 91.5 mmol) in ethanol (150 mL). The mixture was stirred for 16 h and then filtered. The yellow solid was washed with petroleum ether (2 × 50 mL) and dried *in vacuo* to afford the required product (15.35 g, 65%): IR (KBr) 1583, 1498, 1336, 1096, 1084, 962; ^{1}H NMR ($CDCl_3$) δ 8.23 (2H, d, J = 8.9 Hz), 7.63 (2H, d, J = 8.9 Hz), 7.49 (2H, d, J = 8.6 Hz), 7.37 (2H, d, J = 8.6 Hz), 7.23 (1H, d, J = 16 Hz), 7.11 (1H, d, J = 16 Hz).

Preparation of 1-(4-Chlorophenyl)-1,2-dibromo-2-(4′-nitrophenyl)ethane. A solution of bromine (14.17 g, 4.57 mL, 88.9 mmol, 1.5 equiv) in chloroform (50 mL) was added dropwise over 100 min to a solution of (*E*)-(4-chlorophenyl)-(4′-nitrophenyl)ethene (15.4 g, 59.1 mmol) in $CHCl_3$ (700 mL). The suspension was stirred for 22 h and then evaporated. Dichloromethane (2 × 100 mL) was evaporated off the solid, to remove any remaining bromine, to give 23.4 g (95%) of product as a cream-colored solid: ^{1}H NMR ($CDCl_3$/CD_3OD) δ 8.29 (2H, d, J = 8.8 Hz), 7.69 (2H, d, J = 8.8 Hz), 7.49−7.37 (4H, m), 5.48 (1H, d, J = 11.4 Hz), 5.40 (1H, d, J = 11.4 Hz).

Preparation of (4-Chlorophenyl)(4′-nitrophenyl)ethyne. Aqueous sodium hydroxide (50%, 150 mL) was added dropwise over 1.25 h to a suspension of Bu_4NHSO_4 (68.3 g, 201 mmol, 3 equiv) and 1-(4-chlorophenyl-1,2-dibromo-2-(4′-nitrophenyl)-ethene (28.1 g, 67.0 mmol) in CH_2Cl_2 (200 mL) and hexane (200 mL). After 30 min water (500 mL) and then CH_2Cl_2 (200 mL) were added. The mixture was filtered, the layers were separated, and the aqueous fraction was extracted with CH_2Cl_2 (2 × 100 mL). The combined organics were washed with water (4 × 250 mL) and brine (100 mL) and then dried ($MgSO_4$), filtered, and evaporated to give 29.9 g of dark green solid. Flash column chromatography (5−100% ethyl acetate in 40−60 petroleum ether and then 60% acetone in ethyl acetate) gave 16.29 g (94%) of product: ^{1}H NMR ($CDCl_3$) δ 8.23 (2H, d), 7.67 (2H, d), 7.50 (2H, d), 7.37 (2H, d).

Preparation of (*Z*)-(4-Chlorophenyl)(4′-nitrophenyl)-ethene. A mixture of (4-chlorophenyl)(4′-nitrophenyl)ethyne (9.0 g, 34.9 mmol), palladium on calcium carbonate poisoned with lead (Lindlar catalyst; 900 mg), and quinoline (902 mg, 825 μL, 7.00 mmol, 0.2 equiv) was stirred under hydrogen in ethyl acetate (1 L) until hydrogen uptake ceased. The mixture was filtered and evaporated. ^{1}H NMR indicated a mixture of starting material and product. Thus the reaction mixture was dissolved in EtOAc (175 mL), quinoline (1 mL) and Lindlar catalyst (1.5 g) were added, and hydrogenation was restarted. After hydrogen uptake had finished the mixture was filtered, evaporated, and purified by flash column chromatography (5−20% ethyl acetate in 40−60 petroleum eluent) to give 7.98 g (88%) of product: ^{1}H NMR ($CDCl_3$) δ 8.09 (2H, d, J = 8.9 Hz), 7.36 (2H, d, J = 8.9 Hz), 7.23 (2H, d, J = 8.5 Hz), 7.12 (2H, d, J = 8.5 Hz), 6.75 (1H, d, J = 12.2 Hz), 6.63 (1H, d, J = 12.2 Hz).

Preparation of (*E*)-(4-Aminophenyl)(4′-chlorophenyl)-ethene. A solution of concentrated hydrochloric acid (5.4 mL, ca. 54 mmol) in ethanol (50 mL) and water (50 mL) was added dropwise over 1 h to a refluxing suspension of iron filings (9.03 g, 162 mmol, 3 equiv) and (*E*)-(4-chlorophenyl)(4′-nitrophenyl)-ethene (14.0 g, 53.9 mmol) in ethanol (100 mL) and water (100 mL). The mixture was refluxed for 4 h and allowed to cool to room temperature and then basified to ca. pH 11 with 10% aqueous sodium hydroxide. The suspension was filtered and the residue washed with hot ethyl acetate (3 × 200 mL). The solution was evaporated to remove the organic solvents, and the aqueous phase was extracted with ethyl acetate (3 × 200 mL). The combined organic phases were washed with water (2 × 200 mL) and brine (100 mL) and then dried ($MgSO_4$), filtered, and evaporated to give an orange solid. Flash column chromatography (10−100% ethyl acetate in petroleum ether) gave the required product (8.82 g, 71%): ^{1}H NMR ($CDCl_3$) δ 7.42−7.25 (6H, m), 7.00 (1H, d, J = 16.3 Hz), 6.85 (1H, d, J = 16.3 Hz), 6.68 (2H, d), 3.8 (2H, brs).

(4-Aminophenyl)(4′-chlorophenyl)ethyne: prepared by a similar method, 70% yield; ^{1}H NMR ($CDCl_3$) δ 7.44−7.26 (6H, m), 6.64 (2H, d, J = 8.64 Hz), 3.84 (2H, brs).

(*Z*)-(4-Aminophenyl)(4′-chlorophenyl)ethene: prepared by a similar method, 53% yield; ^{1}H NMR ($CDCl_3$) δ 7.21 (2H, d), 7.16 (2H, d), 7.03 (2H, d, J = 8.4 Hz), 6.50 (2H, d, J = 8.4 Hz), 6.48 (1H, d, J = 12.2 Hz), 6.33 (1H, d, J = 12.2 Hz), 3.61 (2H, brs).

Delayed Type Hypersensitivity Paw Edema in the Mouse (DTH-M).[29] On day 0 male CD-1 mice (25−30 g, n = 8−16) were sensitized by subcutaneous injection of 1 mg of methylated bovine serum albumin (MBSA) in saline/Freund's complete adjuvant (FCA) emulsion. Negative control mice received saline/FCA. On day 7 mice were challenged by injection into the right hind footpad with MBSA (0.1 mg in 0.05 mL of saline). The left paws received saline. DTH paw edema formation was measured after 24 h. Control vehicle or test compounds were administered orally once daily on days 4−6 and twice on day 7, 1 h before and 6 h after challenge.

Delayed Type Hypersensitivity Paw Edema in the Rat (DTH-R).[30] On day 0 male CFHB rats (160−180 g, n = 7−12) were sensitized by subcutaneous injection in the tail base of 0.1 mL of FCA (0.4 mg of *Mycobacterium tuberculosis* suspended in liquid paraffin). Negative control rats received Freund's incomplete adjuvant (liquid paraffin). On day 7 rats were challenged by injection into the right hind footpad with soluble *M. tuberculosis* extract (0.4 mg in 0.2 mL of saline). The left paws received saline. DTH paw edema formation was measured after 24 h. Control vehicle or test compounds were administered orally once daily on days 4−6 and twice on day 7, 1 h before and 6 h after challenge.

Collagen II Arthritis in the Rat (CA-R).[25] On day 0 female DA rats (130−160 g, n = 10−17) were sensitized by subcutaneous injection in the tail base with 0.4 mg of chick type II collagen in acetic acid/Freund's incomplete adjuvant (FIA) emulsion. Negative control rats received acetic acid/FIA. Arthritic hind paw edema formtion was measured on day 18. Control vehicle or test compound was administered orally once daily either throughout the experiment on days 0−4, 7−11, and 14−17, or to rats with established arthritis on days 14−17.

Collagen II Arthritis in the Mouse (CA-M).[24] On days 0 and 21 male DBA/1 mice (15−20 g, n = 12) were sensitized by intradermal injection in the tail base with 0.1 mg of chick type II collagen in acetic acid/FCA emulsion. Negative control mice received acetic acid/FCA. Arthritis severity was measured on day 32 based on a scoring system of 0−3/paw for edema and redness (maximum = 12/mouse). Control vehicle or test compound was administered orally once daily on days 21−25 and 28−31.

Statistical Analysis. Paw edema data were compared using the Student's t-test. Arthritis severity scores were compared using the Mann−Whitney U-test. The levels of statistical significance were as follows: $^*P < 0.05$ and $^{**}P < 0.01$.

Rat Kinetic Protocol. Male Wistar CFHB rats (190−220 g, n = 3/time point) were used. The drug was administered orally by gavage at a dose of 10 mg/kg in a dose volume of 10 mL/kg. Blood samples were obtained by cardiac puncture under ether anesthesia at 0, 1, 3, 6, 24, 48, and 72 h postdose. The samples were collected individually into heparinized tubes,

after which the plasma was separated and stored at −20 °C until assayed.

Mouse Kinetic Protocol. Male CD-1 mice (25−32 g, n = 5/time point) were used. The drug was administered orally by gavage at a dose of 30 mg/kg in a dose volume of 10 mL/kg. Blood samples were obtained by exsanguination under ether anesthesia at 0, 1, 3, 6, 24, 48, and 72 h postdose. Blood samples from all the animals at each time point were collected into a single heparinized tube, after which the plasma was separated and stored at −20 °C until assayed.

HPLC Assay. Calibration. Prior to analysis of the samples, calibration curves were constructed by spiking blank plasma samples with appropriate levels of drug and then subjecting them to the assay procedures described below. Chromatograms were recorded via a Trivector Trio data system with drug concentrations being calculated from the peak areas. Where samples were found to contain concentrations well outside the calibration range, the analysis was repeated using an appropriately modified injection volume.

HR 325 Analysis. An aliquot of plasma (0.5 mL) was spiked with internal standard (20 μL of a solution containing 200 μg/mL of **46** in methanol) and then diluted with acetonitrile (100 μL) with mixing by vortexing after each addition. A Bond-Elut extraction tube (3 mL, ODS) was conditioned with methanol (1 mL) and water (1 mL), and then the plasma sample was passed slowly through the tube. The tube was washed with 1 M saline (1 mL) and water (1 mL), after which the drug and internal standard were extracted with methanol (0.5 mL), and a 10 μL aliquot of extract was injected for HPLC analysis.

HPLC Conditions

column	125 × 4.6 mm 5 μm Nucleosil ODS	
mobile phase	0.1 M pH 7 phosphate buffer	65%
	acetonitrile	35%
flow rate	1.2 mL/min	
detection	UV at 300 nm	
retention times	HR 325 (**3**)	3.6 min
	46 (standard 1)	2.9 min
	metabolite	1.9 min

Dihydroorotate Dehydrogenase Assay. Dihydroorotate dehydrogenase assays were carried out as described in the literature[10] on mouse or rat spleen enzymes. For the rat enzyme assay, 0.13 mg of membrane proteins was used. Drug concentrations were generally increased in full log intervals with each concentration tested in duplicate.

Molecular Modeling. Geometry optimization and conformational search of **3** and **84** were done using the semiempirical method AM1 supplied by Spartan.[31] The lowest energy conformation of **3** was used as a template to construct other structures. Each molecule was first built using Quanta[32] and then subjected to minimization. All the minimizations were performed using the CHARMM[33] force field with the adopted-basis Newton Raphson method until the rms gradient was equal to or less than 0.05 kcal/mol Å. Overlaps (molecular similarity) of all the molecules were also done using Quanta.[32]

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