Novel Immunosuppressive Butenamides

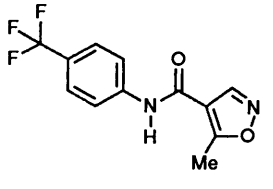
Christopher A. Axton, Michael E. J. Billingham, (the late) Paul M. Bishop, Peter T. Gallagher,* Terence A. Hicks, E. Ann Kitchen, Graham W. Mullier, W. Martin Owton, Mark G. Parry, Susan Scott and David J. Steggles
Lilly Research Centre Limited, Eli Lilly and Company, Windlesham, Surrey, GU20 6PH, UK

2-[4-(1,1-Dimethylethyl)phenyl]thiophene **12** was carboxylated using butyllithium and carbon dioxide to give 5-[4-(1,1-dimethylethyl)phenyl]thiophene-2-carboxylic acid **13**. Conversion of the acid **13** using diphenyl phosphazidate and triethylamine gave 5-[4-(1,1-dimethylethyl)phenyl]thiophene-2-carbonyl azide **14**, which was rearranged in toluene at 110 °C with loss of nitrogen to give the isocyanate **15**; this in turn was treated with sodium 1-cyanoprop-1-ene 2-oxide **16** in tetrahydrofuran to give 2-cyano-*N*-{5-[4-(1,1-dimethylethyl)phenyl]thiophen-2-yl}-3-hydroxybut-2-enamide **17**. Analogous chemistry has been utilised to synthesize both phenylheteroarylbutenamides and phenylbutenamides which display immunosuppressive activity towards proliferating concanavalin A-stimulated T-lymphocytes.

Leflunomide [5-methyl-*N*-(4-trifluoromethylphenyl)isoxazole-4-carboxamide] **1** has been shown to normalise the rate of proliferation of lymphocytes stimulated *in vitro* with mitogenic stimuli such as concanavalin A (Con A), phytohaemagglutinin (PHA) and lipopolysaccharide (LPS).[1]

The known improvement in the condition of rheumatoid arthritis patients when subjected to thoracic duct drainage, a procedure which temporarily alleviates the disease, due to depletion of circulating T-lymphocytes,[2] suggests, that should Leflunomide **1** be capable of reducing circulating T-lymphocytes *in vivo* it may be of value in the treatment of rheumatoid arthritis.

Cyclooxygenase enzyme inhibitors, *e.g.* Indomethacin **2**, are effective anti-inflammatory agents due to their ability to block the production of prostaglandins.[3,4] The biosynthesis of gastroprotective prostaglandins, *e.g.* PGE_2, is also inhibited by cyclooxygenase enzyme inhibitors and this can lead to gastric ulceration. Leflunomide **1** is not a cyclooxygenase enzyme inhibitor (see Table 6) and this, together with its immunosuppressive ability (Table 6), merited a structure–activity relationship study.



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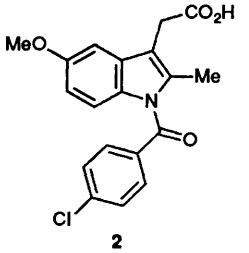

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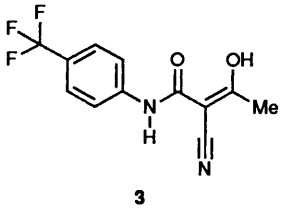

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Studies with Leflunomide have confirmed that, owing to metabolism,[5] Leflunomide **1** is not the active species. The metabolic product of Leflunomide **1** is the ring opened 2-cyano-3-hydroxy-*N*-(4-trifluoromethylphenyl)butenamide **3** which is a cyclooxygenase inhibitor (Table 6) although it does also possess immunosuppressive activity as evidenced by its ability to inhibit Con A-stimulated T-cell proliferation (see Table 6).

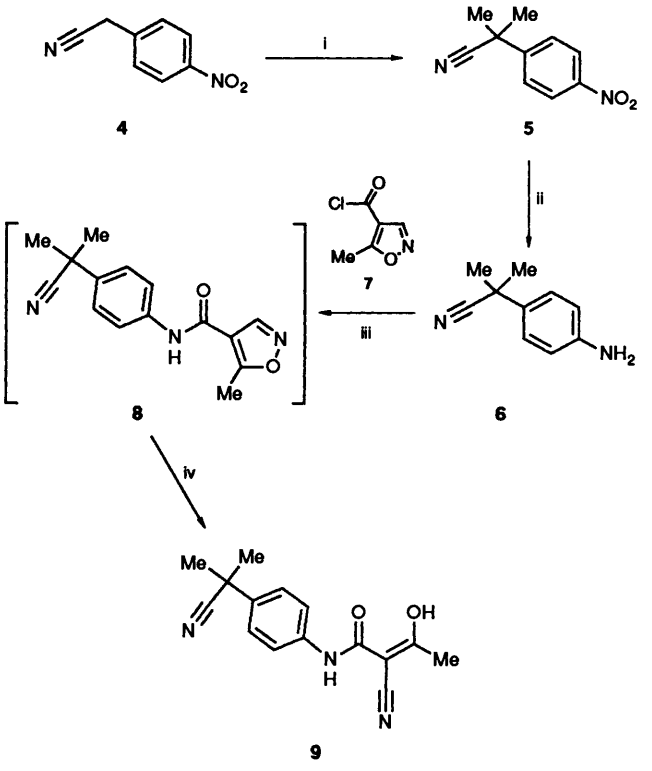
With the aim of minimising cyclooxygenase enzyme inhibition as measured in an *in vitro* assay of cyclooxygenase metabolites of arachidonic acid,[6] (see Experimental section) together with effectiveness in inhibiting the proliferation *in vitro* of Con A-stimulated T-cells,[7,8] (see Experimental section) a structure–activity relationship based on structure **3** has been established. In order to develop a structure–activity relationship based on structure **3**, the syntheses of arylheterocycles and related carboxylic acids and amines have been studied. This paper presents the synthetic chemistry of the intermediates leading to the butenamides, together with the chemistry and relevant biology of the more active analogues.

Results and Discussion

Chemistry.—The synthesis of the butenamide series is facilitated by two good routes from amines and carboxylic acids respectively.[9] The syntheses in Schemes 1 (amine route) and 2 (carboxylic acid route) exemplify these options.

Dialkylation of 4-nitrophenylacetonitrile **4** using phase-transfer conditions[10] gave 2-methyl-2-(4-nitrophenyl)propionitrile **5**, which was reduced using tin(II) chloride dihydrate in ethyl acetate[11] to give 2-(4-aminophenyl)-2-methylpropionitrile **6** in 83% yield. Treatment of the amino nitrile **6** in dichloromethane containing pyridine with 5-methylisoxazole-4-carbonyl chloride[12] **7** (method A) produced a 97% yield of *N*-[4-(1-cyano-1-methylethyl)phenyl]-5-methylisoxazole-4-carboxamide **8**, which without purification was deprotonated at the 3 position of the isoxazole and ring opened using sodium hydroxide in methanol to give 2-cyano-*N*-[4-(1-cyano-1-methylethyl)phenyl]-3-hydroxybut-2-enamide **9** (Scheme 1).

The alternative (carboxylic acid) route is typified by the synthesis in Scheme 2. Nickel-catalysed[13] coupling of 1-bromo-4-(1,1-dimethyl)benzene **10** with 2-bromothiophene **11** as in method B produced 2-[4-(1,1-dimethylethyl)phenyl]thiophene **12**. Compound **12** was then treated with butyllithium in diethyl ether and then with solid carbon dioxide[14] (method C) and subsequent acidification gave 5-[4-(1,1-dimethylethyl)phenyl]-thiophene-2-carboxylic acid **13**. The acid **13** was converted (method D) into 5-[4-(1,1-dimethylethyl)phenyl]thiophene-2-carbonyl azide **14** by using diphenyl phosphorazidate[15] in the presence of triethylamine. Curtius rearrangement of inter-



Scheme 1 *Reagents:* i, MeI, NaOH, Bu_4NBr, CH_2Cl_2, water; ii, $SnCl_2 \cdot 2H_2O$, EtOAc; iii, pyridine, CH_2Cl_2; iv, NaOH, MeOH

mediate **14** in toluene at 110 °C with loss of nitrogen produced a quantitative yield of 2-[4-(1,1-dimethylethyl)phenyl]-5-isocyanatothiophene **15**. Isocyanate **15** was then treated immediately with sodium 1-cyanoprop-1-ene-2-oxide[9] **16** (available from sodium ethoxide treatment of 5-methylisoxazole) in tetrahydrofuran (THF) to give, as in method D, 2-cyano-*N*-{5-[4-(1,1-dimethylethyl)phenyl]-2-thienyl}-3-hydroxybut-2-enamide **17** (Scheme 2).

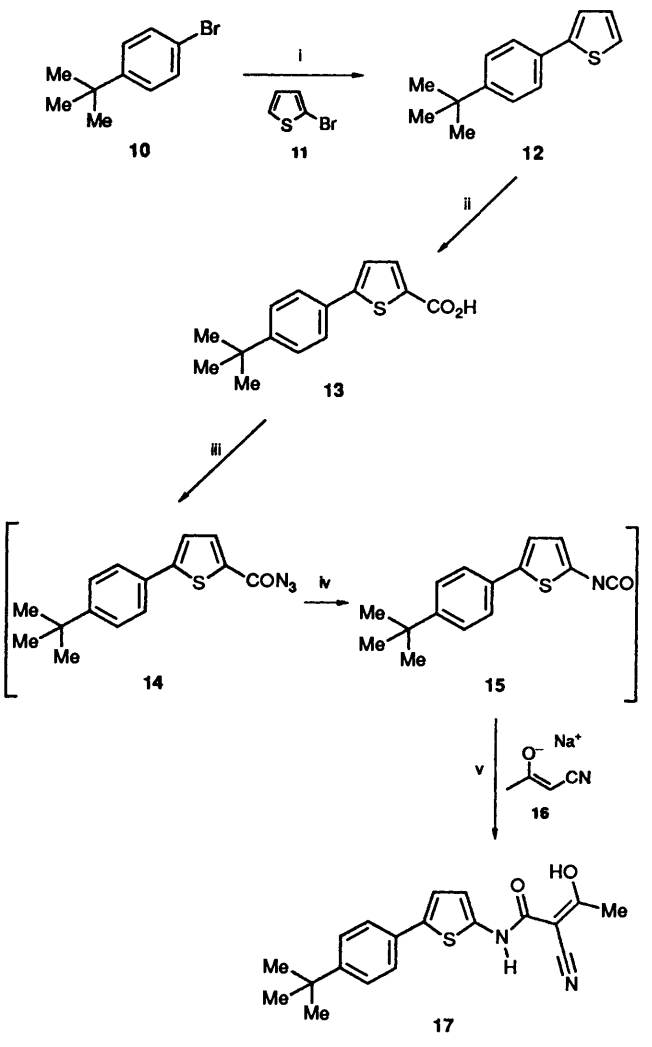
Commercially available isocyanates (method E) were used where possible to synthesize butenamides and were treated as for isocyanates generated in the carboxylic acid route.

The structural assignments of all the butenamides (Fig. 1) were based on their analytical (Table 1) and their ^1H NMR spectroscopic data (Tables 2 and 3). Typically the methyl singlet of a butenamide resonated in the range δ 2.1–2.4, and in solutions of $CDCl_3$ the enolised OH proton was usually seen at δ 15–16.

Compounds structurally related to compound **3** were already known in the literature prior to the commencement of this work; these included compound **18**,[16] a cyano hydroxy ketone where the nitrogen in the amide linkage had been removed and the trifluoromethyl group had been exchanged for chlorine.

Replacement of the amide nitrogen by oxygen and carbon gave the compounds **19**[17] and **20**[18], respectively, which were also known. All of these modifications resulted in loss of activity against Con A-stimulated T-cell proliferation. Immunosuppressive activity was also lost when the cyano group of compound **3** was exchanged for an ethoxycarbonyl group (ester **22**).

Minor alterations to the aromatic ring, *e.g.* the replacement of the 4-trifluoromethyl group by chlorine resulting in compound **21**,[19] gave marginally reduced activity, though alkylation of the hydroxy group of compound **21** to give compound **23**[20] also caused activity to be lost. Compounds **18**[16] **19**,[17] **20**,[18] **21**,[19] and **23**[20] were prepared by literature methods. Compound **22** was synthesized by addition of ethyl acetoacetate under basic conditions to commercially available 4-trifluoromethylphenyl isocyanate in moderate yield. Amide **21** was the only active analogue (see Table 6).



Scheme 2 *Reagents and conditions:* i, Mg, Ni(dppp)Cl_2, THF; ii, BuLi, Et_2O, CO_2; iii, Et_3N, $(PhO)_2PON_3$, DMF; iv, toluene, 110 °C; v, THF

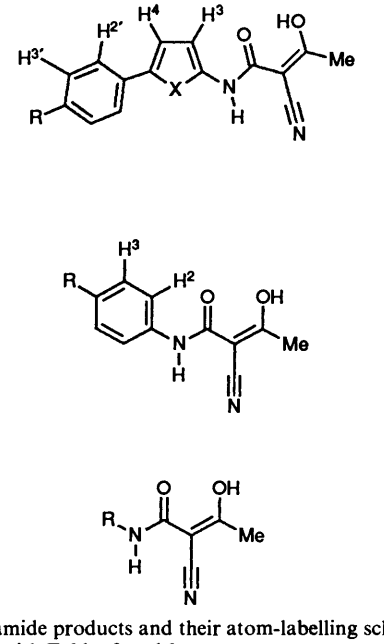
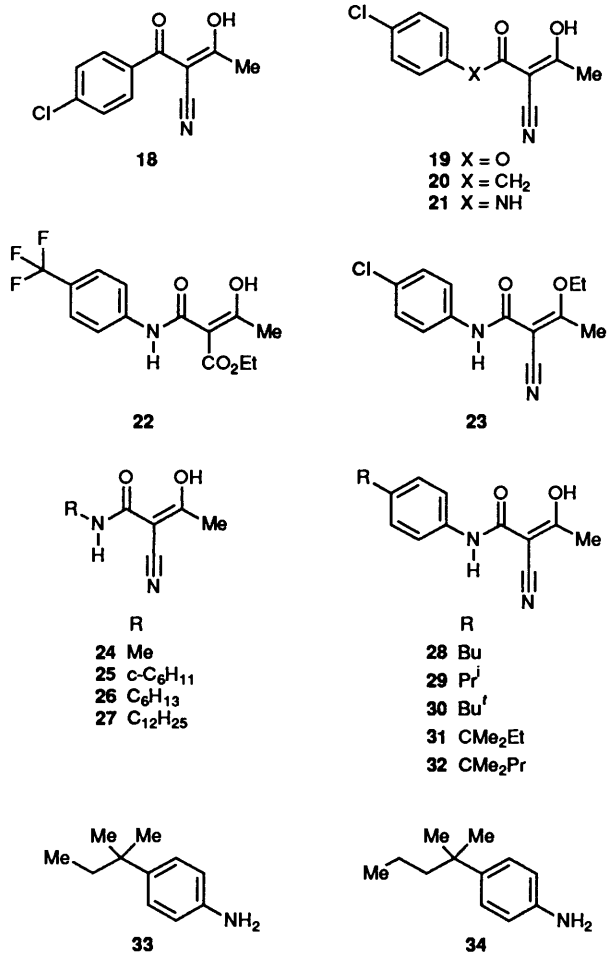
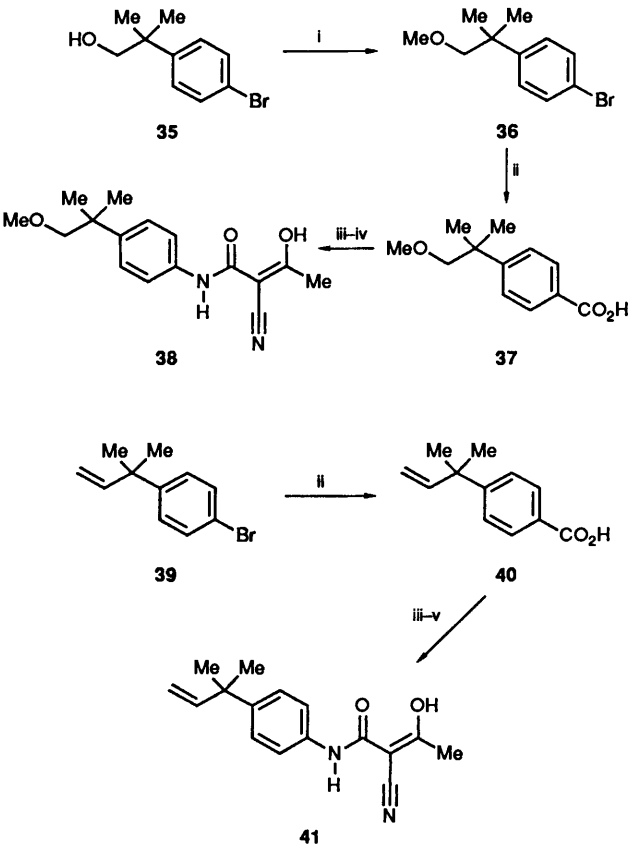


Fig. 1 Butenamide products and their atom-labelling schemes for use in conjunction with Tables 2 and 3.





Scheme 3 *Reagents and conditions:* i, NaH, MeI, THF; ii, BuLi, THF, CO_2; iii, Et_3N, $(PhO)_2PON_3$, DMF; iv, toluene, 110 °C; v, **16**, THF

Compounds **24–27** were all synthesized from commercially available isocyanates in one step by reaction of the salt **16**. Removal of the aromatic ring of amide **3** and replacement with methyl (**24**), cyclohexyl (**25**), hexyl (**26**) or dodecyl (**27**) residues resulted in a loss of immunosuppressive activity.

Noting that the immunosuppressive activity *in vitro* of compound **3** was greater than that of chloro amide **21** we reasoned that this might in part be due to the enhanced lipophilicity of the trifluoromethyl group over the chloro group. This prompted us to synthesize the 4-butylphenyl (**28**) and the 4-(1-methylethyl)phenyl (**29**) analogues, both of which were active at 1 μmol in the immunosuppressive assay (see Table 6).

The 4-(1,1-dimethylethyl)phenyl analogue **30** was active and had a reduced ability to block the cyclooxygenase enzyme (see Table 6). Compounds **28–30** were synthesized from commercially available aryl isocyanates by reaction with compound **16**.

Other tertiary alkyl analogues included compounds **31** and **32** which were synthesized from the known[21,22] amines **33** and **34**, respectively. Methoxyalkyl and alkene analogues were also synthesized: alkylation of 2-(4-bromophenyl)-2-methylpropanol **35**[23] with sodium hydride and iodomethane gave the methyl ether **36**. Low-temperature lithiation of bromide **36** with butyllithium, followed by treatment with carbon dioxide, gave the benzoic acid **37** in an overall yield for the two steps of 66%. This was transformed into the butenamide **38** (Scheme 3).

The alkene analogue was synthesized similarly by carboxylation of 3-(4-bromophenyl)-3-methylbut-1-ene **39**[24] with butyllithium and carbon dioxide to give 4-(1,1-dimethylprop-2-enyl)benzoic acid **40**, which was converted using the carboxylic acid route into the butenamide **41** (Scheme 3).

In our search for alternative groupings with which to substitute the 4 position on the phenyl ring, heterocyclic analogues were synthesized. Thus, following a literature procedure,[25] methyl 4-aminobenzoate was diazotised with sodium nitrite and hydrochloric acid in the presence of sodium tetrafluoroborate. The resulting diazonium tetrafluoroborate **42** was treated with thiophene to give methyl 4-(2-thienyl)-benzoate **43** in 10% yield. Subsequent hydrolysis with aq. sodium carbonate gave 4-(2-thienyl)benzoic acid **44**. Similarly methyl 4-(5-bromo-2-thienyl)benzoate **46** was synthesized *via* a diazonium tetrafluoroborate route, and hydrolysis gave the acid **47**. Both acids were converted *via* the acid route into their respective butenamides, **45** and **48**. As both compounds **45** and **48** were cyclooxygenase enzyme inhibitors it was decided to synthesize, by analogy with the monocyclic series (*cf.* **30**), the 5-substituted tertiary butyl analogue. Hence 2-(1,1-dimethylethyl)-thiophene **49*** was treated with butyllithium at −30 °C and converted into the organozinc bromide by the addition of anhydrous zinc bromide. The organozinc bromide was then coupled[26,27] with a catalytic quantity of bis(triphenylphosphine)palladium(II) chloride to 1-iodo-4-nitrobenzene **50** in 21% yield to give 2-(1,1-dimethylethyl)-5-(4-nitrophenyl)thiophene **51**. The nitrobenzene was hydrogenated over 10% palladium on charcoal in 95% yield to give the amine **52**, which was converted into the corresponding butenamide **53** *via* the amine route (Scheme 4). Compound **53** was a cyclooxygenase enzyme inhibitor (see Table 6).

Other 4-substituted heterocyclic analogues of **3**, synthesized included the 4-[6-(1,1-dimethylethyl)pyridin-2-yl]- and the 4-(6-methylbenzothiazol-2-yl)-phenyl analogues. 4-(6-Methylbenzothiazol-2-yl)benzeneamine **54** was the commercially

* Alfred Bader Chemicals, Gillingham, Dorset, UK.

Table 1 Physical and analytical data for the butenamides

Compound	Yield (%) (Method)	M.p. T/°C	Solvent	Formula	Found (%) (required) C	H	N
9	76(A)	142–144	Et_2O–C_6H_{14}	$C_{15}H_{15}N_3O_2$	66.8 (66.90)	5.5 (5.61)	15.35 (15.60)
17	64(D)	225–226	PhMe	$C_{19}H_{20}N_2O_2S$	66.9 (67.03)	5.9 (5.92)	8.1 (8.23)
24	37(E)	96–97	Amorphous	$C_6H_8N_2O_2$	51.2 (51.42)	5.5 (5.75)	19.7 (19.99)
25	52(E)	99–100	aq. EtOH	$C_{11}H_{16}N_2O_2$	63.7 (63.44)	7.8 (7.74)	13.2 (13.45)
26	85(E)	56–57	aq. Me_2CO	$C_{11}H_{18}N_2O_2$	62.6 (62.83)	8.35 (8.63)	13.2 (13.32)
27	66(E)	77–78	EtOH	$C_{17}H_{30}N_2O_2$	69.6 (69.35)	10.1 (10.27)	9.3 (9.51)
28	87(E)	135–136	EtOH	$C_{15}H_{18}N_2O_2$	69.5 (69.74)	6.8 (7.02)	10.5 (10.84)
29	77(E)	132–133	aq. EtOH	$C_{14}H_{16}N_2O_2$	68.6 (68.83)	6.5 (6.60)	11.6 (11.47)
30	90(E)	134–135	EtOH	$C_{15}H_{18}N_2O_2$	69.7 (69.74)	7.0 (7.02)	11.0 (10.84)
31	95(A)	137–139	PhMe–C_6H_{14}	$C_{16}H_{20}N_2O_2$	70.4 (70.56)	7.5 (7.40)	10.45 (10.29)
32	87(A)	98–100	C_6H_{14}	$C_{17}H_{22}N_2O_2$	71.6 (71.30)	7.9 (7.74)	9.7 (9.78)
38	23(D)	121–123	Et_2O	$C_{16}H_{20}N_2O_3$	66.8 (66.65)	7.0 (6.99)	9.7 (9.72)
41	25(D)	115–117	C_6H_{14}	$C_{16}H_{18}N_2O_2$	70.9 (71.09)	6.9 (6.71)	10.4 (10.36)
45	95(D)	211–213	Amorphous	$C_{15}H_{12}N_2O_2S$	63.05 (63.36)	4.5 (4.25)	9.7 (9.85)
48	50(D)	235–236	PhMe	$C_{15}H_{11}BrN_2O_2S$	49.85 (49.60)	3.2 (3.05)	7.5 (7.71)
53	85(A)	185–186	PhMe–pet[a]	$C_{19}H_{20}N_2O_2S$	67.3 (67.03)	6.0 (5.92)	8.2 (8.23)
55	92(A)	>320	Amorphous	$C_{19}H_{15}N_3O_2S$	65.6 (65.31)	4.6 (4.33)	11.8 (12.03)
61	78(A)	162–164	Me_2CO–C_6H_{14}	$C_{20}H_{21}N_3O_2$	71.9 (71.62)	6.5 (6.31)	12.5 (12.53)
63	24(D)	240	EtOAc	$C_{15}H_{12}N_2O_2S$	63.3 (63.36)	4.2 (4.25)	9.6 (9.85)
66	40(D)	243–246	EtOAc	$C_{19}H_{20}N_2O_2S$	67.1 (67.03)	5.9 (5.92)	8.1 (8.23)
68	74(A)	114–115	MeOH	$C_{20}H_{22}N_2O_2S$	68.0 (67.77)	6.4 (6.26)	7.6 (7.90)

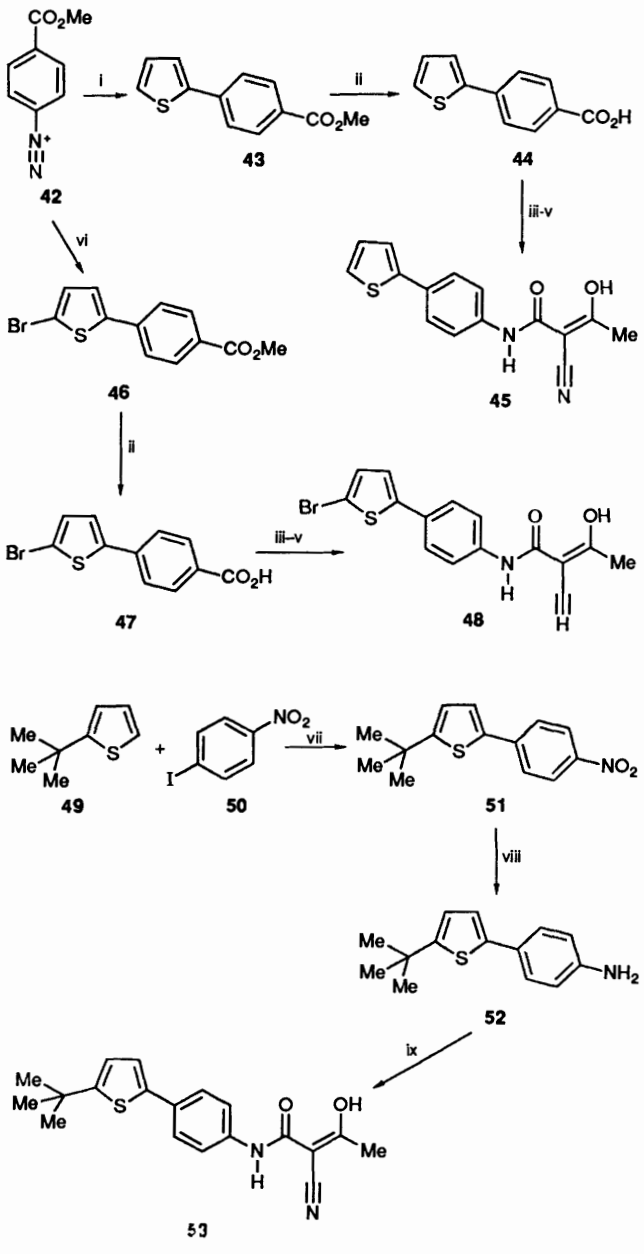
[a] Light petroleum (40–60 °C).

Table 2 ^{1}H NMR spectroscopic data (δ) of the *N*-alkyl- and thienyl-butenamides

Compound	Solvent	X	Me	OH	NH	H^3	H^4	$J_{3,4}$[a]	$H^{2'}$	$H^{3'}$	$J_{2',3'}$	R, δ (multiplicity, integration)
17	$CDCl_3$	S	2.38	15.14	8.34	6.78	7.07	4.0	7.51	7.39	8.0	Bu^t, 1.34 (9 H, s)
24[b]	$CDCl_3$		2.3	16.0	6.4							Me, 2.9 (3 H, s)
25[b]	$CDCl_3$		2.3	16.0	6.0							Cyclohexyl[c]
26[d]	$CDCl_3$		2.27	16.5	6.7							Hexyl[e]
27	$CDCl_3$		2.26	16.1	6.0							Dodecyl[f]
63	$(CD_3)_2SO$	S	2.27		11.7	6.88	7.25	3.8	7.58	7.38	m	H, 7.23 (1 H, m)
66	$(CD_3)_2SO$	S	2.25	11.73		7.25	*g*	m	7.54	7.40	8.0	Bu^t, 1.29 (9 H, s)
68	$CDCl_3$	S	2.30	16.6	*h*	6.90	7.12	3.8	7.52	7.40	8.0	Bu^t, 1.33 (9 H s)

[a] *J*-Values in Hz. [b] 60 MHz spectrum. [c] 1.0–2.0 (10 H, m), 4.0 (1 H, m). [d] 80 MHz spectrum. [e] 0.89 (3 H, s), 1.2–1.4 (8 H, m) and 3.30 (2 H, q). [f] 0.88 (3 H, t), 1.1–1.7 (20 H, m) and 3.32 (2 H, q). [g] H^5, 7.25 (1 H, m). [h] NMe, 3.42 (3 H, s).

available starting material for the synthesis of the butenamide **55**. The synthesis of the pyridine analogue was achieved from *N*-(4,4-dimethyl-3-oxopentyl)piperidine hydrochloride **56**,[28] which was converted *via* basification with aq. potassium hydroxide into the free amine and was then quaternised with iodomethane. The methiodide was treated directly with commercially available ethyl 4-ethylbenzoylacetate **57** and triethylamine to give ethyl 6,6-dimethyl-2-(4-nitrobenzoyl)-5-oxoheptanoate, which was hydrolysed and decarboxylated *in situ* to give 6,6-dimethyl-1-(4-nitrophenyl)heptane-1,5-dione **58**. Cyclisation with ammonium acetate in acetic acid[29] produced 2-(1,1-dimethylethyl)-6-(4-nitrophenyl)pyridine **59**, and this was then reduced in 63% yield using tin(II) chloride dihydrate in ethyl acetate[11] to give 4-[6-(1,1-dimethylethyl)pyridin-2-yl]-benzeneamine **60**. The aniline **60** was converted into the butenamide **61** *via* the amine route (Scheme 5). Compound **61**



Scheme 4 *Reagents and conditions:* i, KOAc, thiophene, MeCN; ii, Na_2CO_3, aq. 1,4-dioxane; iii, Et_3N, $(PhO)_2PON_3$, DMF; iv, toluene, 100 °C; v, **16**, THF; vi, KOAc, **11**, MeCN; vii, BuLi, $ZnBr_2$, $(Ph_3P)_2PdCl_2$, THF; viii, 10% Pd(C), H_2, EtOH; ix (a) **7**, pyridine; (b) NaOH, MeOH

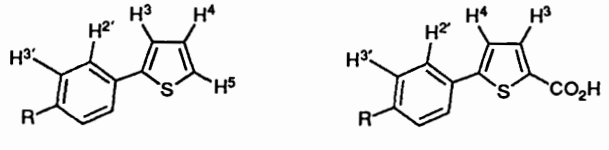
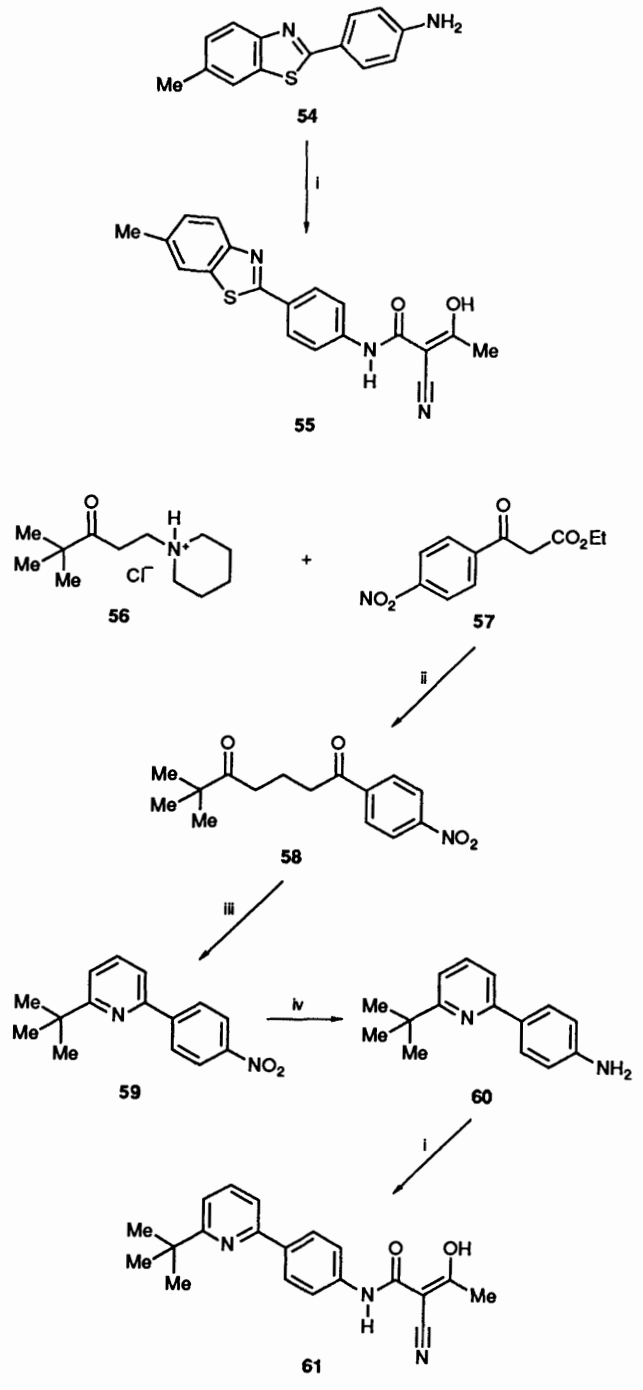


Fig. 2 2-Phenylthiophene products and their atom-labelling schemes for use in conjunction with Tables 4 and 5.

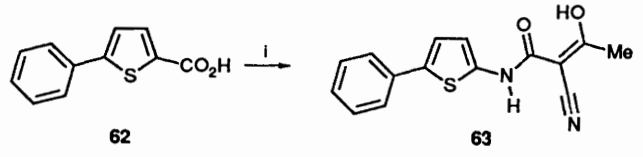
was an active immunosuppressant at 0.1 μmol (see Table 6).

Interchanging the thiophene and phenyl rings of the butenamide **45** produced 2-cyano-3-hydroxy-*N*-(5-phenyl-2-thienyl)-but-2-enamide **63**, which proved to be active *in vitro* (see Table 6) and was synthesized from the commercially available 5-phenylthiophene-2-carboxylic acid **62** (Scheme 6). This prompted the synthesis of the *tert*-butyl derivative **17**.

Microanalytical or accurate mass measurements (Table 4) together with examination (Fig. 2) of the ^1H NMR spectrum in



Scheme 5 *Reagents:* i (a) **7**, Pyridine, CH_2Cl_2; (b) NaOH, MeOH; ii (a) KOH, Et_2O; (b) MeI, MeOH; (c) Et_3N, **57**; (d) HCl; iii, H_4NOAc, AcOH; iv, $SnCl_2{\cdot}2H_2O$, EtOAc



Scheme 6 *Reagents and conditions:* i (a) $(PhO)_2PON_3$, Et_3N, DMF; (b) toluene, 110 °C; (c) **16**, THF

$CDCl_3$ (Table 5) established the structures of the arylthiophenes and 5-arylthiophene-2-carboxylic acids. A characteristic coupling of 3.8 Hz was noted in the ^1H NMR spectrum in $CDCl_3$ for the coupling between protons 3 and 4 of the 2,5-disubstituted acids.

Table 3 ^{1}H NMR spectroscopic data (δ) for 4-substituted phenylbutenamides

Compound	Solvent	Me	OH	NH	H^2	H^3	$J_{2,3}$ (Hz)	R, δ (multiplicity, integration)
9	$CDCl_3$	2.37	15.5	9.89	7.48	7.57	8.0	CMe_2CN, 1.73 (6 H, s)
28	$CDCl_3$	2.24	15.7	7.8	7.15	7.3	8.2	Bu, 0.8 (3 H, s), 1.1–1.7 (6 H, m)
29	$CDCl_3$	2.28	15.8	7.7	7.10	7.40	8.0	Pr^i, 1.21 (6 H, d), 2.89 (1 H, sept.)
30	$(CD_3)_2SO$	2.29		10.15	7.35	7.44	8.6	Bu^t, 1.27 (9 H, s)
31	$CDCl_3$	2.35	15.8	7.6	7.32	7.40	8.3	CMe_2Et, 0.67 (3 H, t), 1.27 (6 H, s), 1.64 (2 H, q)
32	$CDCl_3$	2.36	15.8	7.6	7.35	7.40	8.0	CMe_2Pr, 0.81 (3 H, t), 1.05 (2 H, m), 1.28 (6 H, s), 1.6 (2 H, t)
38	$CDCl_3$	2.36	15.7	7.5	7.4		m	CMe_2CH_2OMe, 1.32 (6 H, s), 3.31 (3 H, s), 3.38 (2 H, s)
41	$CDCl_3$	2.36	15.7	7.5	7.35		m	$CMe_2CH{=}CH_2$, 1.39 (6 H, s), 5.05 (2 H, m), 6.0 (1 H, dd)
45	$(CD_3)_2SO$	2.34		10.0	7.57	7.63	7.9	2-Thienyl, 7.08 (1 H, d), 7.35 (2 H, m)
48	$(CD_3)_2SO$	2.28		10.5	7.56	7.61	8.2	5-Bromo-2-thienyl, 7.20 (1 H, d), 7.27 (1 H, d)
53	$(CD_3)_2SO$	2.33		10.3	7.6		m	5-Bu^t-2-thienyl, 1.40 (9 H, s), 6.89 (1 H, d), 7.27 (1 H, d)
55	$(CD_3)_2SO$	2.24		10.98	7.73	8.08	8.0	6-Me-benzothiazol-2-yl, 2.45 (3 H, s), 7.32 (1 H, dd), 7.89 (1 H, m), 7.89 (1 H, m)
61	$CDCl_3$	2.36	15.78	7.75	7.60	8.12	8.0	6-Bu^t-pyridin-2-yl, 1.42 (9 H, s), 7.25 (1 H, m), 7.53 (1 H, m), 7.65 (1 H, m)

Compound **17** was of particular interest as no significant cyclooxygenase enzyme inhibition could be demonstrated at 30 μmol (Table 6), whilst immunosuppressive activity at 0.1 μmol dm^{-3} was noted.

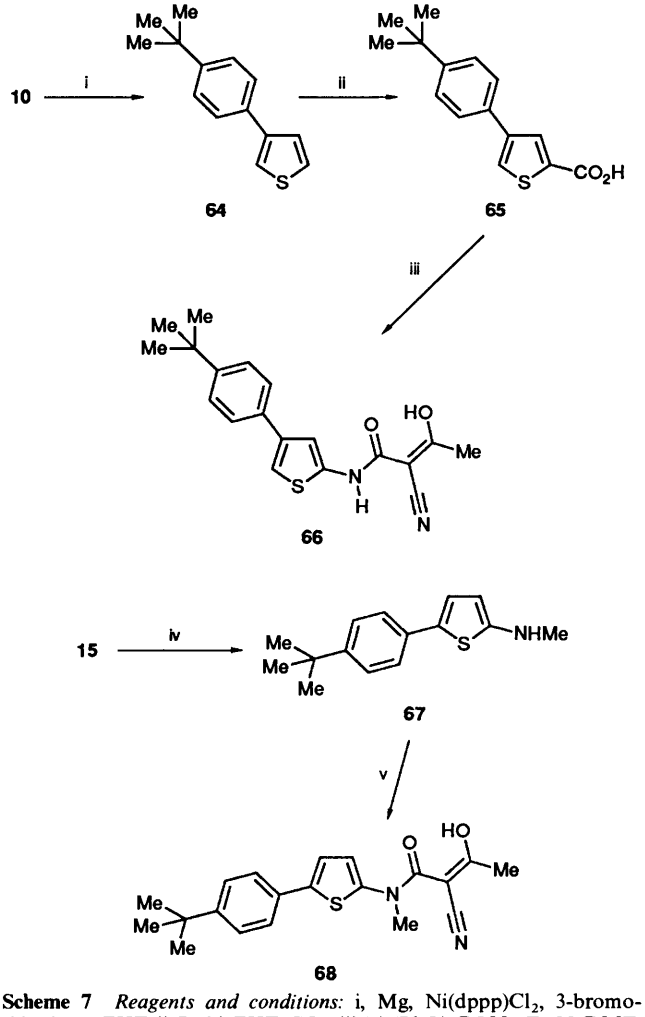
The 2,4-disubstituted analogue of **17** was also synthesized, using a nickel-catalysed coupling reaction between the Grignard reagent derived from bromide **10** and 3-bromothiophene to produce, after chromatography and recrystallization, 3-[4-(1,1-dimethylethyl)phenyl]thiophene **64**, which was converted *via* acid **65** into the butenamide **66** (Scheme 7) in an analogous fashion to its positional isomer **17**.

N-Demethylation of amides can occur *in vivo*;[30,31] consequently the *N*-methylated analogue of compound **17** was synthesized as a potential pro-drug. The synthesis of the potential pro-drug was facilitated by the avilability of the isocyanate **15** used in the synthesis of compound **17**. Isocyanate **15** was reduced with lithium aluminium hydride in diethyl ether to give 5-[4-(1,1-dimethylethyl)phenyl]-*N*-methylthiophen-2-amine **67**, which was converted into butenamide *68 via* the amine route.

Biology.—The effects of the butenamides **3**, **17**, **30**, **61** and Leflunomide **1** on Con A-induced proliferation of lymphocytes are shown in Table 6. Significant inhibition of proliferation was observed with all four butenamides **3**, **17**, **30** and **61** and Leflunomide **1** at a concentration of 1 μmol, whilst with compound **17** at 30 μmol and compound **61** at 10 μmol no significant inhibition of the cyclooxygenase enzyme was noted.

In summary we have shown that these new heterocyclic butenamides have immunosuppressive activity on the *in vitro* activation of T-lymphocytes.

The data from the immunosuppressive tests, together with the lack of activity of some of the butenamides (notably compounds **17** and **61**) as cyclooxygenase enzyme inhibitors, demonstrate that these molecules are immunosuppressive agents that could be applied to the treatment of both rheumatoid arthritis and other immunological diseases, and as an adjunct to therapy used during organ transplanation.



Scheme 7 *Reagents and conditions*: i, Mg, $Ni(dppp)Cl_2$, 3-bromothiophene, THF; ii, BuLi, THF, CO_2; iii (a) $(PhO)_2PON_3$, Et_3N, DMF; (b) toluene, 110 °C; (c) **16**, THF; iv, $LiAlH_4$, Et_2O; v (a) **7**, pyridine, CH_2Cl_2; (b) NaOH, MeOH

Experimental

Chemistry.—M.p.s were determined on a Reichert melting point apparatus and are uncorrected. IR spectra were recorded using a Bruker IFS 48 spectrometer. ^{1}H NMR spectra were determined using a Bruker AM 300 spectrometer. Dilute solutions in deuteriochloroform were used throughout (unless otherwise noted) with tetramethylsilane as internal standard. All *J*-values are in Hz. Molecular weights and mass spectra were measured using a VG 7070E spectrometer. Elemental analysis were within ±0.3% of the theoretical values except where noted.

Table 4 Physical and analytical data for the thiophenes and thiophene carboxylic acids

Compound	Yield (%) (Method)	M.p. T/°C (lit. °C)	Solvent	Formula	Found (%) (Required) C	H
12	52(B)	*a*		$C_{14}H_{16}S$		*b*
13	86(C)	240–241	Amorphous	$C_{15}H_{16}O_2S$	69.4 (69.20)	6.4 (6.19)
64	40(B)	77–78	MeOH	$C_{14}H_{16}S$	77.9 (77.73)	7.4 (7.45)
65	28(C)	214–215	aq. MeOH	$C_{15}H_{16}O_2S$	69.4 (69.20)	6.3 (6.19)

[a] B.p. 110–112 °C/0.35 mmHg. [b] Found: M^+, 216.097 209. $C_{14}H_{16}S$ requires M, 216.097 272.

THF, dimethylformamide (DMF), pyridine and dichloromethane were dried using 4 Å molecular sieves.

2-Methyl-2-(4-nitrophenyl)propionitrile **5**.—4-Nitrophenylacetonitrile **4** (9.0 g, 55.5 mmol) and iodomethane (26.0 g, 183 mmol) were dissolved in dichloromethane (75 cm^3) and the solution was stirred vigorously with a solution of sodium hydroxide (6.0 g, 150 mmol) in water (75 cm^3). Tetrabutylammonium bromide (1.0 g, 3.1 mmol) was added and the reaction mixture was stirred overnight. The organic phase was separated, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The resulting solid was dissolved in diethyl ether, and the solution was filtered through Celite and evaporated under reduced pressure to give the crude product (10.8 g). This material was recrystallised from diethyl ether–hexane to give the *nitrophenylpropionitrile* **5** (10.23 g, 97%) as a solid, m.p. 77–78 °C; ν_{max}(KBr)/cm^{-1} 2240 (CN), 1520 (NO_2) and 1350 (NO_2); δ_H 1.79 (6 H, s, CMe_2), 7.68 (2 H, m) and 8.27 (2 H, m) (Found: C, 63.2; H, 5.1; N, 14.6. $C_{10}H_{10}N_2O_2$ requires C, 63.15; H, 5.30; N, 14.73%).

2-(4-Aminophenyl)-2-methylpropionitrile **6**.—Tin(II) chloride dihydrate (27.31 g, 121 mmol) was added to a solution of the nitro compound **5** (4.6 g, 24.21 mmol) in ethyl acetate (150 cm^3) and the mixture was heated under reflux under nitrogen for 3 h. The clear solution was poured into ice–water (150 cm^3), basified with 50% aq. sodium hydroxide. The organic phase was separated and the aqueous phase was extracted with ethyl acetate (2 × 150 cm^3), the organic phases were combined and dried ($MgSO_4$), and the solvent was evaporated off under reduced pressure to give the *aminophenylpropionitrile* **6** (3.22 g, 83%) as an oil, m.p. of hydrochloride 186–190 °C; ν_{max}(KBr)/cm^{-1} 3466 (NH_2), 3373 (NH_2) and 2245 (CN); δ_H 1.67 (6 H, s, CMe), 3.7 (2 H, exchangeable, NH_2), 6.68 (2 H, m) and 7.25 (2 H, m) (Found: M^+, 160.100 011. $C_{10}H_{12}N_2$ requires M, 160.100 248).

Method A.—5-Methylisoxazole-4-carbonyl chloride **7**[12] (2.1 g, 14.4 mmol) was added at room temperature to a stirred solution of the amine **6** (5.0 mmol) and pyridine (1.5 g, 18.9 mmol) in dry dichloromethane (40 cm^3) and the solution was stirred at room temperature overnight. The reaction mixture was poured into dil. hydrochloric acid (2 mol dm^{-3}), and the organic phase was separated, dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give the *N*-substituted 5-methylisoxazole-4-carboxamide **8** as a solid, which was used without further purification in the next step. The isoxazole (12.96 mmol), sodium hydroxide (0.57 g, 14.25 mmol) and methanol (30 cm^3) were heated under reflux for 1 h and then poured into hydrochloric acid (2 mol dm^{-3}; 150 cm^3). The mixture was extracted with ethyl acetate (3 × 75 cm^3) and the combined organic phases were dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give the *butenamide* **9** as a solid (see Table 1 for analytical data).

Method B.—The bromobenzene **10** (469.7 mmol) as a solution in dry, distilled THF (150 cm^3) was added dropwise under nitrogen to a magnetically stirred suspension of magnesium metal (11.67 g, 480 mmol) in dry distilled THF (100 cm^3). After initiation of the Grignard reaction by heating, the bromobenzene **10** was added at such a rate that the solvent boiled. The reaction mixture was then cooled to room temperature and poured cautiously into a cooled (4 °C) solution of 2-bromothiophene **11** (469.7 mmol) in dry, distilled THF (250 cm^3) containing 1,3-bis(diphenylphosphino)propanenickel(II) chloride [Ni(dppp)$_2$Cl$_2$] (0.15 g, 77 μmol) under nitrogen. After the solution of the arylmagnesium bromide had been added, the cooling bath was removed and then more 1,3-bis(diphenylphosphino)propanenickel(II) chloride (2.75, 5.08 mmol) was added in 0.1–0.2 g portions during 0.75 h, with ice-bath cooling where necessary, to bring the reaction mixture back to room temperature. The stirred mixture was then heated under reflux under nitrogen for 2 h and then kept for 16 h. The precipitated magnesium bromide was separated by decantation, washed with THF, and separated by decantation. The magnesium bromide was then dissolved in water (500 cm^3) and the aqueous solution was extracted with ethyl acetate (2 × 200 cm^3). The extracts were then combined with the decanted THF washings and diluted with ethyl acetate (1100 cm^3). This was washed with aq. sodium hydrogen carbonate, dried ($MgSO_4$), decolourised with charcoal, filtered (3 ×) and the solvent was evaporated off under reduced pressure to give the *arylthiophene* **12**, which was distilled using a Claisen stillhead, then redistilled through a Vigreux column (2 × 20 cm) jacketed with cotton wool and fitted with an air condensor (analytical MS in Table 4).

Method C.—Butyllithium (1.55 mol dm^{-3} in hexane; 458 cm^3, 709.9 mmol) was added dropwise with mechanical stirring at 0–19 °C during 0.75 h to a solution of the arylthiophene **12** (516.66 mmol) in dry diethyl ether (1145 cm^3). The mixture was cooled to −19 °C and was then allowed to warm to 0 °C during 2 h. The reaction mixture was transferred during 0.25 h under nitrogen *via* a glass tube into a mechanically stirred slurry of carbon dioxide pellets (~1730 g) in diethyl ether (575 cm^3). The mixture was stirred for 1.25 h, then water (2.88 dm^3) was added cautiously followed by aq. sodium hydroxide (2 mol dm^{-3}; 576 cm^3). After being stirred for 0.5 h the mixture was stored overnight and the lower alkaline layer was separated and adjusted to pH 1 [pH determined using Whatman narrow range pH papers (pH 1–5)] with conc. hydrochloric acid. The resulting precipitate was filtered, washed with water (2 dm^3), and dried *in vacuo* to give the *arylthiophenecarboxylic acid* **13** as a solid (analytical data in Table 4).

Method D.—The arylthiophenecarboxylic acid **13** (443.3

Table 5 ^{1}H NMR spectroscopic data (δ) of the thiophenes and thiophene carboxylic acids

Compound	Solvent	H^3	H^4	H^5	$H^{2'}$	$H^{3'}$	$J_{2',3'}$ [a]	R, δ (multiplicity, integration)
12	$CDCl_3$	7.24	7.00	7.17	7.52	7.36	8.4	Bu^t, 1.34 (9 H, s)
13 [b]	$CDCl_3$	7.7	7.29		7.58	7.44	8.5	Bu^t, 1.34 (9 H s)
64	$CDCl_3$	*c*	7.3	7.5	7.43	7.31	8.4	Bu^t, 1.33 (9 H, s)
65	$CDCl_3$	8.15		7.71	7.75	7.46	8.1	Bu^t, 1.33 (9 H, s)

[a] *J*-Values in Hz. [b] J_{34} 3.8. [c] H_2, 7.4 (1 H, s).

mmol) was added with mechanical stirring to a solution of triethylamine (61.7 cm^3, 44.8 g, 443.3 mmol) in dry DMF (346 cm^3). A solution of diphenyl phosphorazidate (121.82 g, 485.3 mmol) in dry DMF (58 cm^3) was added dropwise during 0.25 h to the mixture at 2.5–15 °C then the mixture was stirred at 37 ± 2 °C for 1.75 h before being poured into crushed ice (2 kg)–water (1 dm^3). The precipitated solid was filtered off and then mechanically stirred in aq. sodium hydroxide (0.1 mol dm^{-3}; 800 cm^3) for 0.16 h, filtered off, washed with water (2 dm^3), and dried *in vacuo* over silica gel to give the carbonyl azide **14** and this was used without further purification.

A solution of the carbonyl azide **14** (432.7 mmol) in dry toluene (1 dm^3) was heated under reflux for 1.25 h. Evaporation of the solvent under reduced pressure gave the crude isocyanatothiophene **15**, which was used without further purification.

A solution of the isocyanatothiophene **15** (389.1 mmol) in dry THF (400 cm^3) was added dropwise with mechanical stirring at 3–5 °C during 0.7 h to a suspension of sodium 1-cyanoprop-1-ene 2-oxide **16**[9] (40.98 g, 390.3 mmol) in dry THF (200 cm^3). After 0.35 h at 3–5 °C the mixture was stirred for 52 min at ambient temperature then at 55 ± 2 °C for 1.5 h. The solvent was evaporated off under reduced pressure and the resulting solid was stirred in aq. sodium hydroxide (0.7 mol dm^{-3}; 975 cm^3) for 0.5 h, filtered, and the alkaline filtrate was adjusted to pH 1 with conc. hydrochloric acid and the precipitated solid was filtered off, washed with water (3 dm^3) and dried *in vacuo* at 60 °C. This solid was recrystallised from ethanol and then from ethyl acetate followed by suspension in diethyl ether (750 cm^3) and aq. sodium hydroxide (1 mol dm^{-3}; 750 cm^3). The alkaline layer was separated, filtered, and then adjusted to pH 1 with conc. hydrochloric acid. The precipitated solid was filtered off, washed with water (2 dm^3), and dried *in vacuo* at 60 °C, to give the *butenamide* **17** as a solid (see Table 1 for analytical data).

Method E.—A solution of the isocyanate **15** (571 mmol) in dry THF (185 cm^3) was added dropwise during 40 min to a mechanically stirred suspension of sodium 1-cyanoprop-1-ene 2-oxide **16**[9] (60 g, 571 mmol) in dry THF (830 cm^3) at 6–8 °C and the mixture was stirred for 15 min at room temperature then for 1 h at 52 °C. The mixture was then allowed to cool to room temperature and stirred for 16 h, the solvent was evaporated off under reduced pressure, water (1 dm^3) was added followed by aq. sodium hydroxide (2 mol dm^{-3}; 25 cm^3), and the aqueous solution was washed with diethyl ether (2 × 250 cm^3), then acidified with hydrochloric acid (10 mol dm^{-3}; 60 cm^3). The resultant precipitate was filtered off, washed with water, and dried *in vacuo* at 46 °C to give the butenamide **17** as a solid.

Ethyl 3-Hydroxy-2-(4-trifluoromethylphenylcarbamoyl)but-2-enoate **22**.—Ethyl acetoacetate (5.99 g, 46 mmol) was added to a solution of ethoxide sodium [sodium 1.06 g, 46 mmol) in ethanol (100 cm^3)]. The solvent was then evaporated off under reduced pressure to give a solid, which was dissolved in dry, distilled THF (70 cm^3) and the solution was cooled to 2 °C. A solution of 4-trifluoromethylphenyl isocyanate* (8.61 g, 46 mmol) in dry THF (35 cm^3) was added dropwise during 20 min to the mixture at 2–8 °C. After the addition of the isocyanate the mixture was warmed to 50 °C for 20 min, then was cooled, and the solvent was removed under reduced pressure to give a solid. Water (100 cm^3) and methanol (30 cm^3) were added and the suspension was adjusted to pH 2 with conc. hydrochloric acid. The solid thus produced was filtered off, and dried *in vacuo* to give the *butenamide* **22** (5.74 g, 39.3%) as a solid, m.p. 76–77 °C (from aq. acetone); ν_{max}(KBr)/cm^{-1} 1680 (CO); δ_H 1.15 (3 H, t, *J* 7, Me), 2.45 (3 H, s, Me), 4.28 (2 H, q, *J* 7, CH_2), 7.58 (4 H, m, ArH) and 11.35 (1 H, OH) (Found: C, 53.0; H, 4.3; N, 4.4. $C_{14}H_{14}F_3NO_4$ requires C, 53.00; H, 4.45; N, 4.41%).

2-(4-Bromophenyl)-2-methylpropyl Methyl Ether **36**.—Sodium hydride (50% dispersion in oil; 1.7 g, 35 mmol) was added in portions to a stirred solution of 2-(4-bromophenyl)-2-methylpropan-1-ol **35**[23] (7.55 g, 32.75 mmol) in dry THF (200 cm^3) at 0 °C under nitrogen. After 30 min iodomethane (2.244 cm^3, 5.11 g, 36.02 mmol) was added, and the mixture was warmed to room temperature and stirred for 16 h before water (5 cm^3) was added. The mixture was partitioned between water and ethyl acetate, separated, and the organic layer was dried ($MgSO_4$), then filtered, and the solvent was removed under reduced pressure to give a yellow oil which, after chromatography on flash silica with hexane as eluent, gave the *methyl ether* **36** (6.152 g, 77%) as an oil, b.p. 130–132 °C/0.25 mmHg; δ_H 1.30 (6 H, s, 2 × Me), 3.30 (3 H, s, OMe), 3.36 (2 H, s, CH_2O), 7.25 (2 H, d, *J* 8, 2 × *m*-H) and 7.41 (2 H, d, *J* 8, 2 × *o*-H) (Found: M^+, 242.010 956. $C_{11}H_{15}{}^{79}BrO$ requires *M*, 242.030 626).

4-(2-Methoxy-1,1-dimethylethyl)benzoic Acid **37**.—Butyllithium (1.55 mol dm^{-3} in hexane; 33 cm^3, 51.15 mmol) was added dropwise under nitrogen to a stirred solution of compound **36** (6.15 g, 25.3 mmol) in dry THF (250 cm^3) at −78 °C, and then the mixture was allowed to warm to room temperature, was recooled to −78 °C, then poured over a mixture of solid carbon dioxide (100 g) in THF (200 cm^3). The mixture was allowed to warm to room temperature and the solvent was then removed under reduced pressure, the residue was dissolved in aq. sodium hydroxide (2 mol dm^{-3}; 100 cm^3), and the mixture was washed with diethyl ether (100 cm^3), acidified with hydrochloric acid (5 mol dm^{-3}; 30 cm^3), and extracted with ethyl acetate (3 × 100 cm^3). The combined extracts were washed with water, dried ($MgSO_4$), and filtered, and the solvent was removed under reduced pressure to give the *benzoic acid* **37** (4.52 g, 86%) as a solid, m.p. 94–95 °C (from aq. MeOH); ν_{max}(KBr)/cm^{-1} 1684 (CO); δ_H 1.35 (6 H, s, 2 × Me), 3.31 (3 H, s, OMe), 3.44 (2 H, s, CH_2O), 7.48 (2 H, d, *J* 8, 2 × *m*-H), 8.04 (2 H, d, *J* 8, 2 × *o*-H) and 12.0 (1 H, s, CO_2H) (Found: C, 69.2; H, 7.8. $C_{12}H_{16}O_3$ requires C, 69.21; H, 7.74%).

4-(1,1-Dimethylprop-2-enyl)benzoic Acid **40**.—Butyllithium

* Aldrich Chemical Co., Gillingham, Dorset, UK.

Table 6 Cyclooxygenase enzyme inhibition and inhibition of T-cell proliferation

Compound	Dose (μmol dm^{-3})	Cycloxygenase % Reduction of metabolites[a] $PGF_{2\alpha}$	PGE_2	TxB_2	T-Cell Proliferation % Reduction[b] 0.1 μmol dm^{-3}	1.0 μmol dm^{-3}	10.0 μmol dm^{-3}
1	30				42.9	36.6	51.7
2	0.30	52.2	76.6	90			
3	30	66.7	74.6	90.1	46.9	62.7	70.4
17	30				69.5	79.3	77.3
21	30	41.2		68.8		56.4	69.7
28	10					81.8	94.2
29	10					88.7	89.6
30	10				39.6	51.5	68.5
53	10			51.2	47.5	60.7	64.4
61	10				84.6	88.1	88.9
63	10				14.1	61.0	72.6

[a] $p < 0.02$. [b] $p < 0.002$.

(1.55 mol dm^{-3} in hexane; 30 cm^3, 46.5 mmol) was added dropwise under nitrogen to a stirred solution of 3-(4-bromophenyl)-3-methylbut-1-ene **39**[24] (5.025 g, 22.33 mmol) in dry THF (200 cm^3) at −70 °C, then the mixture was allowed to warm to room temperature, was recooled to −70 °C, then poured over a slurry of solid carbon dioxide (100 g) in THF (200 cm^3). The mixture was allowed to warm to room temperature and the solvent was evaporated under reduced pressure, the residue was dissolved in aq. sodium hydroxide (2 mol dm^{-3}; 100 cm^3), and the solution was washed with diethyl ether (100 cm^3), acidified with hydrochloric acid (5 mol dm^{-3}; 30 cm^3), and extracted with dichloromethane (3 × 100 cm^3). The combined extracts were washed with water, dried ($MgSO_4$), then filtered, and the solvent was evaporated off under reduced pressure to give the *benzoic acid* **40** (3.40 g, 80%) as a solid, m.p. 105–107 °C (from aq. MeOH); ν_{max}(KBr)/cm^{-1} 1685; δ_H 1.43 (6 H, s, 2 × Me), 5.08 (1 H, d, *J* 16, CH=C*H*$_t$H$_c$), 5.09 (1 H, d, *J* 10, CH=CH$_t$*H*$_c$), 6.02 (1 H, d, *J* 16 and 10, C*H*=CH$_t$H$_c$), 7.45 (2 H, d, *J* 8, 2 × *m*-H), 8.04 (2 H, d, *J* 8, 2 × *o*-H) and 12.0 (1 H, s, CO_2H) (Found: C, 75.8; H, 7.6. $C_{12}H_{14}O_2$ requires C, 75.76; H, 7.42%).

4-(*Methoxycarbonyl*)*benzenediazonium Tetrafluoroborate* **42**.—Sodium tetrafluoroborate (8.75 g, 79.7 mmol) was added to a mechanically stirred solution of methyl 4-aminobenzoate (9.45 g, 62.5 mmol) in hydrochloric acid (4 mol dm^{-3}; 37.5 cm^3) at 5 °C. Aq. sodium nitrite (7.163 mol dm^{-3}; 8.75 cm^3) was added dropwise during 11 min to the mixture at 5–11 °C. The resultant thick paste was stirred vigorously for 20 min at 4 °C, then was filtered, washed successively with water (2 × 10 cm^3), ice-cold methanol (2 × 10 cm^3), and diethyl ether (3 × 10 cm^3) and then dried *in vacuo* to give the tetrafluoroborate **42** (13.36 g, 85.49%) as a solid, m.p. 123 °C (decomp.), which was used without further purification.

Methyl 4-(*5-Bromo-2-thienyl*)*benzoate* **46**.—Potassium acetate (7.85 g, 80 mmol), dried by heating until molten then allowed to cool in a desiccator, was added to a mechanically stirred mixture of 2-bromothiophene **11** (65.2 g, 400 mmol) and 4-(methoxycarbonyl)benzenediazonium tetrafluoroborate **42** (10 g, 40 mmol) in dry acetonitrile (16.4 g, 400 mmol). The mixture was stirred for 2 h by which time the temperature of the reaction mixture was 65 °C. The mixture was then filtered and the contents of the filter were washed with diethyl ether (2 × 100 cm^3). The filtrate and washings were combined and diluted with light petroleum (40–60 °C; 100 cm^3), then were cooled at 4 °C, and the precipitated solid was removed by filtration to give the *ester* **46** (1.325 g, 11.15%) as a solid, m.p. 155 °C; ν_{max}(KBr)/cm^{-1} 1720; δ_H 3.93 (3 H, s, CO_2Me), 7.07 (1 H, d, *J* 3.9, 3-H), 7.16 (1 H, d, *J* 3.9, 4-H), 7.57 (2 H, d, *J* 8, 2 × *o*-H) and 8.03 (2 H, d, *J* 8, 2 × *m*-H) (Found: $[M + NH_4]^+$, 313.863 953. $C_{12}H_9{}^{79}BrO_2S$ requires $[M + NH_4]$, 313.985 037).

4-(*5-Bromo-2-thienyl*)*benzoic Acid* **47**.—Sodium carbonate (6.68 g, 63 mmol) and methyl 4-(5-bromo-2-thienyl)benzoate **46** (6.2 g, 21 mmol) in water (88 cm^3)–1,4-dioxane (72 cm^3) were stirred and heated together under reflux for 10 h, cooled, and acidified with hydrochloric acid (10 mol dm^{-3}; 25 cm^3) and the resultant solid was removed by filtration, washed with water, and dried *in vacuo* at 46 °C to give the *acid* **47** (5.3 g, 89%) as a solid, m.p. >260 °C; ν_{max}(KBr)/cm^{-1} 1680; δ_H 7.32 (1 H, d, *J* 3.9, 3-H), 7.52 (1 H, d, *J* 3.9, 4-H), 7.74 (2 H, d, *J* 8, 2 × *o*-H) and 7.96 (2 H, d, *J* 8, 2 × *m*-H) (Found: $[M + NH_4]^+$, 299.892 365. $C_{11}H_7{}^{79}BrO_2S$ requires $[M + NH_4]$, 299.969 387).

2-(1,1-*Dimethylethyl*)-5-(4-*nitrophenyl*)*thiophene* **51**.—Butyllithium (1.6 mol dm^{-3} in hexane; 31.25 cm^3, 50 mmol) was added dropwise during 0.5 h to a stirred solution of 2-(1,1-dimethylethyl)thiophene **49** * (7.013 g, 50 mmol) in dry THF (140 cm^3) at −70 °C under nitrogen, then the mixture was allowed to warm to room temperature, was stirred for 1 h, then was added to a mixture of anhydrous zinc bromide (11.26 g, 50 mmol) in dry THF (34 cm^3) during 0.5 h at room temperature. After 1 h at room temperature the thienylzinc bromide solution was added dropwise at room temperature to a stirred solution of 4-iodonitrobenzene **50** (10.83 g, 43.5 mmol) and bis(triphenylphosphine)palladium(II) chloride (0.24 g, 0.341 mmol) in dry THF (100 cm^3). After 67 h at room temperature the reaction mixture was poured into hydrochloric acid (2 mol dm^{-3}; 550 cm^3)–diethyl ether (550 cm^3) and separated, the organic layer was washed successively with saturated aq. sodium hydrogen carbonate (2 × 150 cm^3) and water (4 × 250 cm^3), dried ($MgSO_4$), and filtered, and the solvent was evaporated off under reduced pressure to give an oil, which on recrystallisation from ethanol (2 ×) gave the *nitrophenylthiophene* **51** (2.79 g, 21.35%) as a solid, m.p. 151 °C; ν_{max}(KBr)/cm^{-1} 1510 and 1340; δ_H 1.39 (9 H, s, Bu^t), 6.87 (1 H, d, *J* 3.9, 3-H), 7.29 (1 H, d, *J* 3.9, 4-H), 7.67 (2 H, d, *J* 8, 2 × *o*-H) and 8.19 (2 H, d, *J* 8, 2 × *m*-H) (Found: C, 64.15; H, 5.8; N, 5.4. $C_{14}H_{15}NO_2S$ requires C, 64.34; H, 5.79; N, 5.36%).

4-[5-(1,1-*Dimethylethyl*)-2-*thienyl*]*aniline* **52**.—2-(1,1-Dimethylethyl)-5-(4-nitrophenyl)thiophene **51** (8.29 g, 31.7 mmol) in ethanol (300 cm^3) was hydrogenated over 10% palladium on

* See footnote on page 2205.

charcoal (0.5 g) at 60 psi at room temperature until the theoretical amount of hydrogen uptake had occurred. The catalyst was removed by filtration under nitrogen through Celite. The filtrate was evaporated under reduced pressure to give the *amine* **52** (7 g, 95.44%) as a solid, m.p. 51 °C; ν_{max}(KBr)/cm^{-1} 3400 and 3310; δ_H 1.39 (9 H, s, Bu^t), 3.6 (2 H, br s, NH_2), 6.66 (2 H, d, *J* 8, 2 × *m*-H), 6.73 (1 H, d, *J* 3.9, 3-H), 6.93 (1 H, d, *J* 3.9, 4-H) and 7.36 (2 H, d, *J* 8, 2 × *o*-H) (Found: M$^+$, 232.115 997. $C_{14}H_{17}NS$ requires *M*, 231.965 22).

6,6-*Dimethyl*-1-(4-*nitrophenyl*)*heptane*-1,5-*dione* **58**.—Aq. potassium hydroxide (85%; 2.404 g, 42.836 mmol in 50 cm^3) was added to *N*-(4,4-dimethyl-3-oxopentyl)piperidine hydrochloride **56**[28] (10 g, 42.836 mmol) followed by diethyl ether (100 cm^3), the two-phase mixture was stirred vigorously for 10 min, the ether layer was separated, and the aqueous layer was washed with diethyl ether (100 cm^3). The combined ether layers were washed with water (50 cm^3), dried ($MgSO_4$), and filtered, and the solvent was removed under reduced pressure to give a red oil (5.935 g), which was dissolved in methanol (40 cm^3) containing iodomethane (4.29 g, 1.88 cm^3, 30.22 mmol). The mixture was stirred for 16 h and then ethyl 4-nitrobenzoylacetate **57** (7.162 g, 30.22 mmol) and triethylamine (5.46 cm^3, 3.967 g, 39.29 mmol) were added. The mixture was then stirred for 24 h at room temperature, the solvent was removed under reduced pressure, and the residue was partitioned between ethyl acetate (100 cm^3) and hydrochloric acid (2 mol dm^{-3}; 100 cm^3). The ethyl acetate layer was washed with aq. sodium hydrogen carbonate, dried ($MgSO_4$), and filtered, and the solvent was removed under reduced pressure to give a red oil (7.853 g). This oil was immediately treated with hydrochloric acid (10 mol dm^{-3}; 70 cm^3) and the mixture was heated under reflux for 24 h, then was cooled, and the acid solution was extracted with diethyl ether (3 × 100 cm^3). The diethyl ether layer was washed with aq. sodium hydrogen carbonate, dried ($MgSO_4$), and filtered, and the solvent was evaporated off under reduced pressure to give a red oil which, after column chromatography on flash silica and elution with light petroleum (40–60 °C)–ethyl acetate (5:1), gave the *diketone* **58** (2.464 g, 20%) as a solid, m.p. 51–53 °C (from diethyl ether–pentane); ν_{max}(KBr)/cm^{-1} 1699 and 1687; δ_H 1.14 (9 H, s, Bu^t), 2.03 (2 H, m, $CH_2CH_2CH_2$), 2.66 (2 H, *J* 7, Me_3CCOCH_2), 3.06 (2 H, *J*, 7, CH_2COAr), 8.13 (2 H, *J* 9, 2 × *m*-H) and 8.32 (2 H, *J* 9, 2 × *o*-H) (Found: C, 64.95; H, 7.1; N, 5.15. $C_{15}H_{19}NO_4$ requires C, 64.97; H, 6.91; N, 5.05%).

2-(1,1-*Dimethylethyl*)-6-(4-*nitrophenyl*)*pyridine* **59**.—Ammonium acetate (2.243 g, 29.132 mmol) and acetic acid (7 cm^3) were added to 6,6-dimethyl-1-(4-nitrophenyl)heptane-1,5-dione **58** (2 g, 7.22 mmol) and the mixture was heated under reflux under nitrogen for 2 h, then was cooled, the solvent was evaporated off under reduced pressure, and the residue was partitioned between ethyl acetate (100 cm^3) and aq. sodium hydroxide (0.5 mol dm^{-3}; 20 cm^3), then was separated, and the ethyl acetate layer was washed successively with more aq. sodium hydroxide (0.5 mol dm^{-3}; 20 cm^3) and water (50 cm^3), dried ($MgSO_4$), and evaporated under reduced pressure to give a solid, which was chromatographed on flash silica and eluted with tetrachloromethane, to give the *nitrophenylpyridine* **59** (0.771 g, 42%) as a solid, m.p. 107–109 °C [from light petroleum (40–60 °C)]; δ_H 1.43 (9 H, s, Bu^t), 7.37 (1 H, dd, *J* 8 and 1, 3-H), 7.63 (1 H, m, *J* 8 and 1, 5-H), 7.74 (1 H, m, 4-H), 8.26 (2 H, d, *J* 9, 2 × *m*-H) and 8.32 (2 H, d, *J* 9, 2 × *o*-H) (Found: C, 70.1; H, 6.4; N, 11.2. $C_{15}H_{16}N_2O_2$ requires C, 70.29; H, 6.29; N, 10.93%).

4-[6-(1,1-*Dimethylethyl*)*pyridin*-2-*yl*]*aniline* **60**.—Tin(II) chloride dihydrate (3.38 g, 14.97 mmol) was added to a mixture of 2-(1,1-dimethylethyl)-6-(4-nitrophenyl)pyridine **59** (0.661 g, 2.582 mmol) in ethyl acetate (20 cm^3) and the mixture was heated under reflux under nitrogen for 2 h, cooled, poured over ice (30 g), basified with aq. sodium hydroxide (2 mol dm^{-3}), and extracted with ethyl acetate (2 × 50 cm^3); the extract was washed with water (100 cm^3), dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give an orange oil, which was distilled to give the *aminophenylpyrimidine* **60** (0.365 g, 63%) as an oil, b.p. 170–175 °C/0.2 mmHg; ν_{max}(KBr)/cm^{-1} 3420; δ_H 1.39 (9 H, s, Bu^t), 6.75 (2 H, d, *J* 8, 2 × *o*-H), 7.14 (1 H, d, 5-H), 7.42 (1 H, m, 3-H), 7.57 (1 H, d, 4-H) and 7.93 (2 H, d, *J* 8, 2 × *m*-H) (Found: M$^+$, 226.145 981. $C_{15}H_{18}N_2$ requires *M*, 226.146 998 7).

5-[4-(1,1-*Dimethylethyl*)*phenyl*]-N-*methylthiophen*-2-*amine* **67**.—A suspension of 2-[4-(1,1-dimethylethyl)phenyl]-5-isocyanatothiophene **15** (2.5 g, 9.7 mmol) in dry diethyl ether (50 cm^3) was added during 20 min to a stirred suspension of lithium aluminium hydride (0.95 g, 25 mmol) in dry diethyl ether (37 cm^3) under N_2 at 3.5–10 °C. The mixture was allowed to warm to room temperature, was stirred for 1.5 h, and was then heated under reflux for 1 h. After the mixture had cooled, water (0.95 cm^3) was added during 10 min, then aq. sodium hydroxide (15%; 0.95 cm^3) during 10 min, then water (2.85 cm^3) during 6 min. The suspension was then filtered and the resultant solid was washed with diethyl ether (3 × 50 cm^3). The combined filtrate and washings were dried ($MgSO_4$), and then evaporated under reduced pressure to give an oil, which was distilled in a Büchi Kugelrohr at 260 °C/0.25 mmHg to give a cream coloured distillate which solidified on storage to give the *amine* **67** (1.416 g, 59.6%) as a solid, m.p. 62–64 °C; ν_{max}(KBr)/cm^{-1} 3400; δ_H 1.23 (9 H, s, Bu^t), 2.77 (3 H, s, NMe), 3.8 (1 H, br s, NH), 5.85 (1 H, d, *J* 3.8, 3-H), 6.85 (1 H, d, *J* 3.9, 4-H), 7.25 (2 H, d, *J* 8, 2 × *m*-H) and 7.3 (2 H, d, *J* 8, 2 × *o*-H) (Found: C, 73.2; H, 7.8; N, 5.9. $C_{15}H_{19}NS$ requires C, 73.42; H, 7.81; N, 5.71%).

Biology. Concanavalin A-induced T-cell Proliferation.—The mitogenic response of T-lymphocytes to Con A was measured by incubating unfractionated spleen cells at 2 × 10^6 cm^{-3} in complete Medium [RPMI 1640 containing 5% foetal calf serum, 2-mercaptoethanol (20 μmol), glutamine (20 mmol), penicillin (100 units mm^{-3}) and streptomycin (100 μg cm^{-3})]. Con A was added to the cells to give a final concentration of 3 μg cm^{-3}. All compounds were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 40 μmol dm^{-3}. Solutions of compounds were diluted with complete medium for use in cell culture assays. Owing to the possibility that DMSO might affect the assays, experiments included a DMSO control in which DMSO was added at levels equal to those in compound-treated cultures. Compounds under test were prepared in complete medium to give final concentrations of 0.1, 1.0 and 10 μmol dm^{-3}. After 48 h culture at 37 °C under an atmosphere of 5% CO_2, 95% air, the cells were pulse labelled with tritiated thymidine (^{3}H-Tdr) (0.1 μCi) and incubated for a further 4 h before being harvested. The incorporation of ^{3}H-Tdr into DNA was determined by liquid scintillation spectrometry. The results presented in Table 6 are shown as percentage reduction in cell numbers versus controls.

Assay of Cyclooxygenase Metabolites of Arachidonic Acid.—Peritoneal polymorphonuclear leukocytes incubated with [^{14}C]arachidonic acid were stimulated with the ionophore A23187. Compounds were used at 10 or 30 μmol dm^{-3} in sodium hydroxide–Krebs Ringer buffer (0.1 mol dm^{-3}). Indomethacin was used as standard. The radiolabelled products as cyclooxygenase products were identified using HPLC and GC-MS. TLC was used to separate these metabolites which were then assayed by automatic quantitative scanning. Results

were expressed as a percentage of change of the radioactivity in each metabolite compared with the stimulated controls. The results are presented in Table 6 in terms of $PGF_{2\alpha}$, PGE_2 and TxB_2 as cyclooxygenase metabolites.

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