Eur J Med Chem (1992) 27, 503–509

Synthesis and anti-filarial activity of 6-(3-aminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole derivatives

JCW Comley, JP Court, WE Gutteridge, AT Hudson*, DC Jenkins, DD Miller, RH Nicol, AW Randall, JN Stables, R Thornton

Research Division, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS, UK

(Received 11 October 1991; accepted 30 January 1992)

Summary — Structure–activity studies against filariae are described for a series of derivatives of 6-(3-aminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole (**15**) derivatives. In an *in vivo* assay using gerbils implanted with both *Acanthocheilonema viteae* and *Brugia pahangi*, the former parasite was shown to be the more sensitive to a range of amides of **15**. None of these compounds, however, had any activity at 5×10^{-5} mol against the worms *in vitro* suggesting that they required *in vivo* activation. This was confirmed when the benzamido derivative (**6**) was shown to be rapidly metabolised in gerbils to the parent amine (**15**). The latter had potent effects on filariae both *in vivo* and *in vitro* but was also found to be toxic to mammals and unsuitable for further development. Unsuccessful attempts to improve upon the therapeutic index of **15** through the synthesis of various novel analogues are described.

antifilarial / 6-(3-aminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole / *A viteae* / *B pahangi* / filaricide

Introduction

Despite significant advances over the last two decades in helminth chemotherapy, hundreds of millions of people remain infected with filarial worms [1]. *Wucheria bancrofti* and *Brugia malayi* cause lymphatic filariasis, typified by acute adenolymphangitis and chronic lesions such as elephantiasis and hydrocoele, whilst *Onchocerca volvulus* produces onchocerciasis – 'river blindness'. The latter parasite is one of the world's leading causes of blindness affecting some eighteen million people. In filarial infections both adult worms (macrofilariae) and their larvae (microfilariae) are present in the body. The microfilariae occur in vast numbers and in onchocerciasis cause many of the disease symptoms. To date, chemotherapy has largely been confined to the use of diethylcarbamazine for lymphatic filariasis as this drug is very effective against the microfilariae which circulate in the blood [2]. It is not satisfactory, however, in the treatment of onchocerciasis as here the microfilariae are distributed throughout the body and diethylcarbamazine can induce an intense adverse effect, the so-called Mazzotti reaction [3]. A recent major advance has been the introduction of ivermectin for chemotherapy of onchocerciasis [4]. This compound is also a microfilaricide but its side effects are far less severe than those of diethylcarbamazine [5].

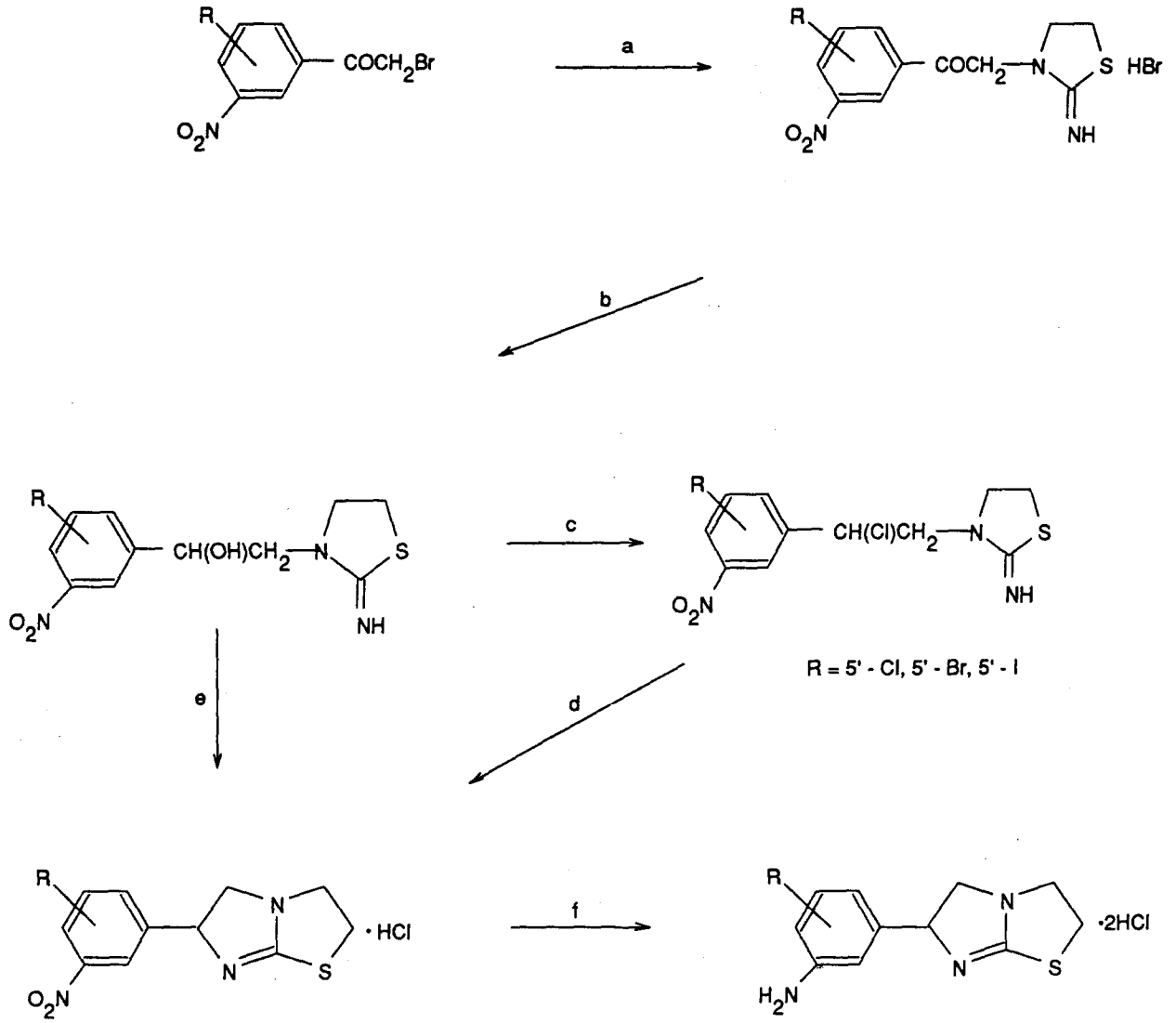
Although ivermectin represents a significant breakthrough in the treatment of onchocerciasis it has little effect on adult worms [6]. Consequently there is a continuing need for a safe macrofilaricide for both onchocerciasis and lymphatic filariasis. It was thus of interest when in 1982 a series of cycloalkenylcarboxamido derivatives of the anthelmintic drug tetramisole were claimed to be macrofilaricidal [7]. Specifically the spirocycloalkenyl analogue **1** was reported to be totally effective at 5 mg/kg for 5 days when administered sub-cutaneously to a cat infected with adult *Brugia pahangi*. Since no additional anti-filarial data were presented on any other member of the series we considered it worthwhile exploring structure-activity relationships for analogues of **1** with particular emphasis on varying the nature of the cycloalkenylcarboxamido moiety. In this manner we hoped to influence the bio-distribution of the compounds to direct them either to the lymphatic system for treatment of lymphatic filariasis, or to the fatty subcutaneous tissues harbouring adult *O volvulus*.

We report herein the synthesis, anti-filarial activity and metabolic fate of the above compounds.

*Correspondence and reprints

Chemistry

6-(3-Aminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]-thiazole (**15**), (and the dihydrochloride salt) [8], its (*S*)-isomer [8], and the analogues **1** [7], **4** [7], **6–8** [8], **23** [9], **27** [10] and **28** [8], were prepared according to the literature referenced. The 4'-methoxy and 5'-halogeno-6-(3-aminophenyl)-2,3,5,6-tetrahydroimidazo-[2,1-*b*]thiazoles (**30**, **20**, **21** and **22**) respectively, were obtained using similar methodology to that employed for **15** (see scheme 1). The amides **3** and **5** were synthesised by reaction of **15** with the appropriate ester in the presence of trimethylaluminium as for **1** and **4** *(method A)*. The other amides (**9–14**, **24**, **25**, **26**, **29** and **31**) were obtained by the more convenient procedure of condensing an aryl acid chloride with the appropriate aminophenyl-2,3,5,6-tetrahydroimidazo-[2,1-*b*]thiazole in aqueous methanol at pH 6 as outlined for **6–8** [8] *(method B)*. This route was also used to obtain the cyclohexyl analogue **2** but in this instance the method was unsatisfactory and the compound was only isolated in very poor yield. Compounds **16**, **17** and **19** were prepared by reaction of **15** with 4-chlorophenylmethylthioimidate, 2-hydroxybenzaldehyde and 4-chlorophenylsulphonylchloride, respectively, using standard methodology.



Scheme 1. Reagents (a) 2-aminothiazoline; (b) $NaBH_4$; (c) $SOCl_2$; (d) aq K_2CO_3; (e) conc H_2SO_4; (f) $SnCl_2 \cdot 2H_2O$, conc HCl. **15** R = H. **20** R = 5'-Cl. **21** R = 5'-Br. **22** R = 5'-I. **30** R = 4'-OMe.

Results and discussion

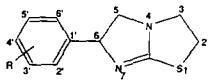
Initially, analogues of **1**, in which the somewhat esoteric spirocycloalkenyl moiety was replaced with more synthetically accessible cycloalkyl and aryl groups, were synthesised. These compounds (**2–6**), were evaluated against *B pahangi* in gerbils but were devoid of any activity when 5 daily doses of 50 mg/kg were given subcutaneously (sc). This prompted resynthesis of **1**, but this to proved to be inactive in this model. However, when **1** was tested according to the original literature report [7], *ie B pahangi* in cats, significant though variable activity was observed. At 5 x 5 mg/kg sc parasite reductions of 84 and 24% were obtained in individual animals whilst at 5 x 20 mg/kg sc the corresponding values were 72 and 24%. Although these results were not as promising as previously reported (number of test animals not specified) they did provide sufficient inducement to investigate the series further. Consequently, two further analogues **5** and **6**, were tested in cats at 5 x 20 mg/kg sc. In 3 animals **6** was found to give consistently over 90% reduction in the worm burden whilst in one cat **5** totally eliminated the parasites, thus substantiating the anti-filarial promise of this series.

In the cat assay *B pahangi* larvae are injected and allowed to mature into adult worms over a 6–8-week period before being used to evaluate compounds. The adult worms by then are widely distributed in the lymphatic system of the animal. This contrasts with the gerbil screen where adult worms are transplanted into the peritoneal cavity where they remain for the duration of the test period. It seems reasonable that the discrepancy in activity of **1** and **6** against *B pahangi* in cats and gerbils could result from the different parasite localisations. Since the target human parasite, adult *O volvulus*, resides primarily in subcutaneous nodules, the systemic parasite distribution in the cat assay is probably more relevant than the peritoneal location in gerbils. However, it is impractical to use the cat assay as a primary screen because large numbers of infected animals are needed to allow collection of statistically valid data. It is also difficult to establish *B pahangi* subcutaneously in gerbils and only poor worm recoveries are obtained. In view of this it was decided to investigate the use of the so-called 'dual-implant' assay whereby gerbils are infected with both *B pahangi* and another filarial species *Acanthocheilonema viteae*. In this screen *B pahangi* adults as before are transplanted into the peritoneal cavity where they remain. The same animals also receive *A viteae* implanted into subcutaneous pockets in the neck from where they migrate subcutaneously. When **6** was tested in this assay at 5 x 50 mg/kg *po* or sc complete elimination of *A viteae* resulted. Oral administration also gave rise to significant effects against *B pahangi*, an 88% worm reduction being achieved compared to only 50% when the drug was given by the subcutaneous route (see table I). In view of this, all further analogues were tested orally unless otherwise stated. In order to develop structure–activity relationships, the amides **7–14** were synthesised. At 5 x 50 mg/kg all showed good activity against *A viteae* (table I) with **7**, **8** and **12** also being effective against *B pahangi*. Further evaluation of several of the compounds was then carried out by giving a single oral dose of 25 mg/kg and autopsying the animals after 2 weeks instead of the normal 6-week period. In this assay **8** again showed good activity against *A viteae* but was less effective against *B pahangi*. In an attempt to determine whether these discrepancies in activity resulted from differences in tissue distribution or inherent parasite susceptibility, **8** was tested *in vitro* against adult *B pahangi* and *A viteae*. No effects on motility were observed at a concentration of 5×10^{-5} M maintained over 120 h. A similar observation was made with other members of the series, strongly suggesting that all these compounds were acting as pro-drugs, possibly of 6-(3-aminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole (**15**) . This was confirmed when oral administration of tritium-labelled **6** to a male gerbil revealed rapid metabolism to **15**. Fifteen min post dosing, 75% of the radioactivity in plasma was associated with **15**, whilst after 30 min complete conversion had occurred. Evaluation of **15** against adult worms *in vitro* provided further evidence that this was indeed the active species of the 3'-amido derivatives. Immediate flaccid paralysis of *B pahangi* and *A viteae* occurred at 10^{-5} M but both species regained motility by 18 h. In contrast *Onchocerca gibsoni* and *O volvulus* never recovered motility after the initial paralysis.

The above results contrast with those of Weikert *et al* who recently reported [11] that the anthelmintic activity of a related series of 3'-benzoylurea derivatives of **15** was not due to metabolism to the latter. These data were, however, obtained against non-filarial worms in other hosts (mice, sheep), which may account for the differences observed.

When **15** was tested in the dual implant model host toxicity was evident at 5 x 25 mg/kg *po* or sc, whilst at 1 x 25 mg/kg *po* activity similar to that of **6** at the same dose was seen. The finding that the active species of the 3'-amido analogues was a compound with significant mammalian toxicity was a cause of some concern and prompted a 14-day rat toxicity study of **6** and **15**. This showed the latter compound to be overtly toxic, all animals treated with 33 mg/kg *po* dying within 5 min. In contrast, **6** was well tolerated at this dose and after 14 days no ill effects were observed. However, at 100 mg/kg *po* 4 out of 5 animals had died by day 6. In an effort to improve further

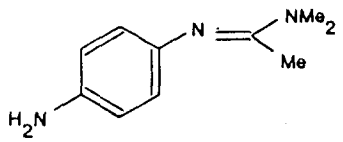
Table I. Structure and activity of 6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazoles.



No	*R*[a]	*mp, °C*	*Formula*[b]	*Dose Days x mg/kg*[c]	*% Reduction A viteae*	*B pahangi*
1[d]	6-(*S*)-3'-NHCO	110–112	$C_{23}H_{25}N_3OS{\cdot}0.4C_6H_5CH_3$	5 x 50 sc	NT[e]	0
				5 x 5 sc (2 cats)	NT	84*, 24
				5 x 20 sc (2 cats)	NT	72*, 24
2[f]	3'-NHCO-c-C_6H_{11}	124–125	$C_{18}H_{23}N_3OS$	5 x 50 sc	NT	0
3	3'-NHCOCH_2-c-C_6H_{11}	140–142	$C_{19}H_{25}N_3OS$	5 x 50 sc	NT	0
4[g]	3'-NHCO-1"-c-C_6H_9	96–99	$C_{18}H_{21}N_3OS$	5 x 50 sc	NT	0
5	3'-NHCO-1"-c(4,4-Et_2)C_6H_9	178–180	$C_{22}H_{31}N_3OS$	5 x 50 sc	NT	7
				5 x 20 sc (1 cat)	NT	100**
6[h]	3'-NHCOC_6H_5	145–147	$C_{18}H_{17}N_3OS$	5 x 50 sc	100**	50*
				5 x 50	100**	88**
				5 x 12.5	96**	37
				1 x 25	46	8
				5 x 20 sc (3 cats)	NT	91**, 92**, 92**
7[i]	3'-NHCO-(4-Cl)C_6H_4	174–176	$C_{18}H_{16}ClN_3OS$	5 x 50	100**	98**
				1 x 25	42	39
8[j,k]	3'-NHCO-(3,4-Cl_2)C_6H_3	145–147	$C_{18}H_{15}Cl_2N_3OS$	5 x 50	100**	97**
				5 x 6.25	100**	25
				1 x 25	88**	39
9	3'-NHCO-(3,4-Br_2)C_6H_3	188–191	$C_{18}H_{15}Br_2N_3OS$	5 x 50[l]	89**	33
10	3'-NHCO-(3,4,5-Cl_3)C_6H_2	248–249	$C_{18}H_{14}Cl_3N_3OS$	5 x 50	100**	9
11	3'-NHCO- -co- -Cl	179–183	$C_{18}H_{16}ClN_3OS$	5 x 50	100**	8
12	3'-NHCO-(4-O'Pr)C_6H_4	152–154	$C_{21}H_{23}N_3O_2S$	5 x 50	100**	85**
13	3'-NHCO-(3,4-Me_2)C_6H_4	124–126	$C_{20}H_{21}N_3OS$	5 x 50	100**	50*
				1 x 25	79*	29
14	3'-NHCO-	184–185	$C_{16}H_{14}ClN_3OS_2$	5 x 50	100**	45*
				1 x 25	46	3
15[m,n]	3'-NH_2·2HCl	dec 200	$C_{11}H_{13}N_3S{\cdot}2HCl$	5 x 25[o]	87*	38
				5 x 25 sc[p]	toxic	toxic
				5 x 12.5	53*	16
				1 x 25	52*	8
16	3'-NH(C=NH)-(4-Cl)C_6H_4HI	210–213	$C_{18}H_{17}ClN_4S{\cdot}HI$	5 x 50	84*	85*
17	3'-N=CH-(2-OH)C_6H_4	97–105	$C_{18}H_{17}N_3OS$	5 x 50	83*	93**
18[q]	3'-NHC$H_2C_6H_5$	98–101	$C_{18}H_{19}N_3S$	5 x 50	100**	74**
19	3'-NHSO_2(4-Cl)C_6H_4	236–240	$C_{17}H_{16}ClN_3O_2S_2$	5 x 50 sc	75*	7
20	3'-NH_2-5'-Cl·2HCl	247–251	$C_{11}H_{12}ClN_3S{\cdot}2HCl$	5 x 50	67*	20
21	3'-NH_2-5'-Br·2HCl	249–253	$C_{11}H_{12}BrN_3S{\cdot}2HCl$	5 x 50[e]	100**	37
22	3'-NH_2-5'-I·2HCl	253–255	$C_{11}H_{12}IN_3S{\cdot}2HCl$	5 x 50	23	12
23[o]	4'-NH_2·2HCl	237–249	$C_{11}H_{13}N_3S{\cdot}2HCl$	1 x 25	29	21
24	3'-NHCO(4-Cl)C_6H_4-5'-Cl	173–176	$C_{18}H_{15}Cl_2N_3OS{\cdot}0.2MeCN$	1 x 25	0	50
25	3'-NHCO(4-Cl)C_6H_4-5'Br	212–215	$C_{18}H_{15}BrClN_3OS$	5 x 50	63*	22
26	3'-NHCO(4-Cl)C_6H_4-5'-I	217–219	$C_{18}H_{15}ClIN_3O_3S$	5 x 50	67*	3
27[r,k]	3'-NH_2-6'-I	161–162	$C_{11}H_{12}IN_3S$		NT	NT
28[s,k]	3'-NH_2-4-Br·2HCl	dec 100	$C_{11}H_{12}BrN_3S{\cdot}2HCl$		NT	NT
29	3'-NHCOC_6H_5-6'-I	231–232	$C_{18}H_{16}IN_3OS$		NT	NT
30	3'-NH_2-4'-OMe·2HCl	[t]	$C_{12}H_{15}N_3OS$		NT	NT
31	3'-NHCO(4-Cl)C_6H_4-4'-OMe	185–187	$C_{19}H_{18}ClN_3O_2S$	5 x 50	33	2

[a]All compounds are racemic mixtures except **1** which is the *S*-isomer. For the sake of clarity, IUPAC nomenclature rules have not been strictly followed. [b]C, H, N analyses were obtained with values within ± 0.4% of theoretical on all compounds except for (30) which was analysed by high-resolution mass spectrometry and gave an accurate mass measurement of 249.0938; calculated value for $C_{12}H_{15}N_3OS$ is 249.0936. Infra-red and NMR-spectra were consistent with the structures shown and with published data for closely related compounds. [c]Activity recorded against both *A viteae* and *B pahangi* indicates that compound was evaluated in a dual-implant assay. All compounds were administered orally to infected gerbils unless otherwise stated. The statistical significance of the reduction in parasite burden was calculated using the Student's *t*-test: $*P < 0.05$; $**P < 0.01$. [d]Literature [7] mp 92–95°C. [e]NT = not tested. [f]Literature [8] mp 130–132°C. [g]Literature [7] mp 91–93°C. [h]Literature [8] mp 101–105°C. [i]Literature [8] mp 142–144°C. [j]Literature [8] mp 150–153°C. [k]5 x 10^{-5} mol failed to immobilise adult *A viteae* and *B pahangi in vitro*. [l]2 out of 4 test animals died. [m]Literature [8] dec 198–201°C. [n]10^{-5} mol induced immediate flaccid paralysis of adult *A viteae, B pahangi, O gibsoni, O volvulus in vitro*; after 18 h *A viteae* and *B pahangi* recovered motility. [o]1 out of 4 test animals died. [p]All test animals died. [q]Literature [8] mp 114–116°C. [r]Synthesis detailed in [10] but no mp given. [s]Literature [8] free base mp 129–130°C. [t]Mp not determined as the solid was deliquescent.

on the therapeutic index of **6** several other potential pro-drugs of **15** were synthesised (**16–19**) but none of these was markedly better than **6** in the dual implant assay. A variety of analogues of **15** were then prepared (**20–31**) but the activities of these against *B pahangi* or *A viteae*, *in vitro* or *in vivo*, were not sufficiently encouraging to warrant proceeding further. Other workers [12] have experienced similar difficulties in attempting to optimise the anthelmintic properties of derivatives of **15**. In view of this, work on 6-(aminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazoles was terminated and effort redirected to different structural types having the potential to act at the same site as **15**. For example, the amidantel analogue **32** [13] like **15** [14] has been shown to be a potent cholinergic agonist on the nematode *Caenorhabditis elegans*. Work in this area will be reported in a later paper.



Experimental protocols

Chemistry

Melting points were determined using an electrothermal melting point apparatus and are uncorrected. 'Flash' chromatography was carried out according to the procedure of Still [15] using silica gel 60, particle size 0.04–0.06 mm. Thin-layer chromatography (TLC) was on silica gel 60 F254 (Merck). 6-(3-Benzamido-6-tritiophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole was prepared at Amersham International by reduction of **29** with tritium gas using the procedure detailed for the synthesis of tritium-labelled **15** [10]. Non-commercially available intermediate acids required for the synthesis of novel derivatives of **15** were obtained as referenced: -3,4-dibromobenzoic acid [16], 4-isopropoxybenzoic acid [17], 3,4,5-trichlorobenzoic acid [18], 4-(4-chlorobenzoyl)benzoic acid [19], 4,4-diethylcyclohexylcarboxylic acid [20]. 3-Amino-5-nitrobenzoic acid was prepared from 3,5-dinitrobenzoic acid by the action of hydrazine hydrate and Raney Nickel in aqueous solution [21]. This was then converted into the 5-nitro-3-halogenobenzoic acids by the method of Gunstone and Tucker [22] to give products with similar characteristics to those previously reported [23]. The acid chlorides used in *method B* were obtained by heating the acids at reflux in thionyl chloride for 2 h. The thionyl chloride was removed *in vacuo* to give the crude products which were used without further purification. Ethyl 4,4-diethylcyclohexanecarboxylate for use in the synthesis of **5** *(method A)* was prepared by heating the acid at reflux for 4 h in ethanol saturated with hydrogen chloride. Removal of the solvent afforded an oil which was purified by distillation, bp 95–100°C @ 4 mmHg.

*Method A. Preparation of **1**, **3**, **4**, **5***

Trimethylaluminium (8 ml of a 25% w/v solution in hexane, Fluka) was added under nitrogen to a stirred solution of **15** (or the (*S*)-isomer for the synthesis of **1**) (12 mmol) in dry dichloromethane (20 ml). The mixture was stirred for 1.5 h and then the appropriate ethyl ester (6 mmol) in dry benzene (25 ml) added and the mixture heated at 50°C for approximately 22 h. The reaction mixture was cooled, poured onto ice (50 g), 2 M HCl (20 ml) added, and then basified with 2 M NaOH. The mixture was extracted with chloroform (4 x 50 ml) which was then dried ($MgSO_4$) and evaporated. The residue was flash chromatographed on silica eluting the desired product with ethyl acetate/methanol (*ca* 9/1). Crystallisation from ethyl acetate/petrol (boiling range 60–80°C) gave the following yields of pure amides: **3**, 24%; **4**, 15%; **5**, 56%. **1** was purified by crystallisation from toluene and was obtained from various preparations in yields of 28 to 95% depending on the age of the trimethylaluminium solution. The product always contained approximately 0.4 mol toluene of crystallisation.

*Method B. Preparation of **2**, **6–14**, **24–26**, **29** and **31***

The amine dihydrochloride (14 mmol) was dissolved in methanol (40 ml)/water (40 ml) and the pH adjusted to 6 by the addition of 2 M NaOH. The appropriate acid chloride was then added portionwise with stirring over 10 min and the reaction left at room temperature overnight. Water (40 ml) and dichloromethane (100 ml) were then added along with 2 M NaOH to adjust the pH to 10. The dichloromethane phase was removed and the aqueous layer extracted further with solvent. The dichloromethane extracts were combined, dried ($MgSO_4$) and evaporated. The residues were then crystallised from acetonitrile unless otherwise indicated to give the following yields: **2**, 4%; **6**, 63%; **7**, 30%; **8**, 36%; **9**, 29% (triturated with boiling dichloromethane); **10**, 7%; **11**, 22% (dichloromethane); **12**, 10%; **13**, 16%; **14**, 39%; **24**, 55%; **25**, 50%; **26**, 37%; **29**, 74% (purified by flash chromatography on silica eluting with chloroform/methanol, 19/1); **31**, 54%.

*6-(3-Amino-4-methoxyphenyl)-2,3,5,6-tetrahydroimidazo[2,1-b]thiazole dihydrochloride (**30**)*

A solution of bromine (32 g, 0.2 mol) in chloroform (35 ml) was added dropwise over 1 h to a stirred solution of 4-methoxy-3-nitroacetophenone (39 g, 0.2 mol). The reaction was initiated by heating to 50°C then cooled in ice as the remainder of the bromine was added. The reaction mixture was stirred for approximately 0.75 h in ice, then for a further 0.5 h at room temperature before being washed with 5% (w/v) sodium bicarbonate solution (350 ml), water (350 ml) and dried ($MgSO_4$). Evaporation gave a brown solid which was crystallised from ethanol to give crude 4-methoxy-3-nitrophenacyl bromide as a pale brown powder (44 g). 27.5 g (0.1 mol) of this was dissolved in acetone (150 ml) and added to a stirred solution of 2-amino-2-thiazoline (10.2 g, 0.1 mol) in acetone (400 ml). The reaction mixture was stirred at room temperature for 1 h, refluxed for 0.5 h, filtered to give 2-imino-3-(4-methoxy-3-nitrobenzoylmethyl)thiazole hydrobromide as a pale yellow powder (31 g, 83%). The hydrobromide (11.3 g, 30 mmol) was suspended in methanol (300 ml) and stirred and cooled in ice as sodium borohydride (1.1 g, 30 mmol) was added over 1 h. After stirring at room temperature for a further 2 h, the reaction mixture was added to 5% hydrochloric acid (800 ml) and left to stand for 1 h, basified with 0.88 sg ammo-

nia to pH 10 and extracted with dichloromethane (2 x 500 ml). The combined organic extracts were washed with water (500 ml), dried ($MgSO_4$) and evaporated to give 7.8 g of a sticky yellow solid. This crude material (7.7 g) was added portionwise over 1.5 h to concentrated sulphuric acid (30 ml) with vigorous stirring and cooling in ice. The resulting red solution was stirred overnight under a dry nitrogen atmosphere before being poured onto crushed ice (220 g). The solution was basified to pH 10 with 0.88 sg ammonia and extracted with dichloromethane (3 x 150 ml). The combined organic extracts were washed with water (250 ml), dried and evaporated to give a dark coloured oil which was dissolved in boiling methanol (10 ml) and 2-propanolic hydrogen chloride (10 ml). After cooling at 0°C overnight 6-(4-methoxy-3-nitrophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole hydrochloride was obtained as a fawn coloured powder (2.8 g, 34%). Reduction of this compound was achieved as follows: stannous chloride dihydrate (60 mmol) was added slowly to stirred concentrated hydrochloric acid (60 ml) and when fully dissolved the nitro compound (17 mmol) was added over 1 h with vigorous stirring. The temperature was raised to 40°C for 1 h and then to 70°C for a further 1.5 h. The reaction mixture was poured onto crushed ice (240 g) and basified with 10 N sodium hydroxide to pH 10. The product was extracted into chloroform (3 x 200 ml) and the combined organic fractions washed with water (200 ml), dried ($MgSO_4$) and evaporated. The residue was dissolved in boiling methanol (15 ml) and propanolic HCl (15 ml) added to give, on cooling, the title compound in 49% yield; the overall yield from the acyl bromide was 12%.

*6-(3-Amino-5-chlorophenyl)-2,3,5,6-tetrahydroimidazo[2,1-b]-thiazole dihydrochloride (**20**)*

3-Chloro-5-nitrobenzoic acid (60 mmol) was dissolved in thionyl chloride (50 ml) and a catalytic amount of *N,N*-dimethylformamide added. The resulting solution was stirred and refluxed for 3 h before excess thionyl chloride was removed by evaporation at reduced pressure. The oily residue was redissolved in benzene and evaporated to give a brown oil which was dissolved in chloroform (15 ml) and added dropwise to a cooled (– 50°C) solution of diazomethane (150 mmol) in ether (500 ml). The reaction mixture was allowed to warm to ambient temperature overnight before being evaporated at room temperature under reduced pressure. The residue was dissolved in dichloromethane (200 ml) and cooled to – 30°C and stirred at this temperature as 48% HBr (30 ml) was added. After stirring at room temperature for 1 h the solution was washed with 5% sodium bicarbonate solution (4 x 100 ml), and water (3 x 100 ml) before being dried ($MgSO_4$) and evaporated to give 3-chloro-5-nitrophenacyl bromide as a brown oil which was used without further purification. The phenacyl bromide in acetone (75 ml) was added to a stirred solution of 2-amino-2-thiazoline (57 mmol) in acetone (150 ml). The reaction mixture was stirred at room temperature for 1 h, refluxed for 0.5 h and then filtered to give 2-imino-3-(3-chloro-5-nitrobenzoylmethyl)thiazoline hydrobromide as a white powder (78%). The hydrobromide (30 mmol) was suspended in methanol (300 ml) and stirred and cooled in ice as sodium borohydride (30 mmol) was added portionwise over 0.5 h. After stirring at room temperature for 1 h 5% HCl (800 ml) was added and the mixture left to stand for 0.5 h with occasional stirring. The mixture was basified with 0.88 sg ammonia to pH 9 and extracted with chloroform (3 x 500 ml). The combined organic extracts were washed with water (3 x 500 ml), dried ($MgSO_4$) and evaporated to give 2-imino-1-(3-chloro-5-nitrophenyl)-3-thiazolidineethanol as a cream coloured solid (98%). Thionyl chloride (22 ml) was added to a suspension of 2-imino-1-(3-chloro-5-nitrophenyl)-3-thiazolidineethanol (36 mmol) in chloroform (350 ml) and the resulting mixture refluxed for 1 h before being evaporated to give a solid residue. This was resuspended in chloroform (350 ml) and to this a solution of potassium carbonate (140 mmol) in water (72 ml) was added dropwise. The mixture was stirred and refluxed for 1 h, cooled, separated and the organic layer washed with water, dried ($MgSO_4$) and evaporated to give a brown oil. This oil was dissolved in methanol (10 ml) and an equal volume of propanolic hydrogen chloride added. After cooling at 4°C 6-(3-chloro-5-nitrophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole was obtained as yellow needles (73%). Reduction with stannous chloride as in the preparation of **30** gave 6-(3-amino-5-chlorophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole dihydrochloride (**20**) as a yellow powder, purified by crystallisation from methanol; the overall yield from the acyl bromide was 32%.

*6-(3-Amino-5-bromophenyl)-2,3,5,6-tetrahydroimidazo[2,1-b]thiazole dihydrochloride (**21**)*
This was obtained as for **20** in overall yield of 20%.

*6-(3-Amino-5-iodophenyl)-2,3,5,6-tetrahydroimidazo[2,1-b]-thiazole dihydrochloride (**22**)*
This was obtained as for **20** in overall yield of 24%.

*6-[3-(4-Chlorobenzamidino)phenyl]-2,3,5,6-tetrahydroimidazo [2,1-b]thiazole hydroiodide (**16**)*
4-Chlorophenylmethylthioimidate hydroiodide (2.8 g, 8.93 mmol) and 6-(3-aminophenyl)2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole (**15**) (1.9 g, 8.67 mmol) were heated at reflux in ethanol (30 ml) for 6 h. The reaction mixture was cooled, filtered and the resulting white solid washed with ethanol and dried *in vacuo* to afford 2.5 g (59%) of **16** as the hydroiodide, mp 210–213°C.

*6-[3-(2-Hydroxybenzylideneamino)phenyl]-2,3,5,6-tetrahydroimidazo[2,1-b]thiazole (**17**)*
2-Hydroxybenzaldehyde (0.42 g, 3.4 mmol) and 6-(3-aminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole (**15**) (0.5 g, 2.28 mmol) in toluene (25 ml) were heated under reflux for 4 h collecting the water formed in a Dean-Stark head. The toluene was removed *in vacuo* and the resulting oily residue dissolved in hot methanol, treated with activated charcoal, filtered and the filtrate evaporated to give the product as a yellow solid, 0.4 g (54%), mp 97–105°C.

*6-[3-(4-Chlorobenzenesulphonamido)phenyl]-2,3,5,6,-tetrahydroimidazo[2,1-b]thiazole (**19**)*
6-(3-Aminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole **15** dihydrochloride (5.8 g, 20 mmol) was dissolved in water (45 ml)/methanol (45 ml) and the solution adjusted to pH 6 by the dropwise addition of 2 N NaOH. 4-Chlorobenzenesulphonyl chloride (12.6 g, 60 mmol) was added portionwise with stirring over 20 min. After a further 4 h stirring the mixture was left at room temperature overnight. Water (120 ml) and dichloromethane (240 ml) were added and the pH brought to 10 by the addition of 2 N NaOH solution. The white precipitate was filtered off and dried to give **19**, 3.6 g (45.7%), mp 236–240°C.

Biological evaluations

In vitro assays were carried out as previously described using adult *B pahangi*, *A viteae*, *O gibsoni* [24] and *O volvulus* [25].

In vivo evaluations against *B pahangi* in cats [26] and gerbils [27] and *B pahangi* plus *A viteae* in gerbils [28] were exactly as published except that in the latter dual implant assay when a single compound dose of 25 mg/kg was given the animals were killed after 14 days rather than the normal 35 days.

*Metabolism of 6-(3-benzamido-6-tritiophenyl)-2,3,5,6-tetrahydroimidazo[2,1-b]thiazole (**6**)*

The title compound, specific activity 50 μCi/mg, was ball-milled overnight with 1% Tween 80 in water. Gerbils were dosed by gavage with 2.5 mCi/kg drug and killed at 15-min intervals using groups of 3 animals for each time point. They were bled out completely by cardiac puncture and the plasma separated. This was then diluted with 20% potassium acetate solution (1 ml to each ml of plasma) and extracted with dichloromethane (2 x 5 ml). The organic extract was concentrated and cold samples of **6** and **15** added as carrier. The mixtures were then run in bands on either Merck Silica gel F254 plates using a solvent of chloroform/methanol/0.880 ammonia 90/10/1 or on Whatman KC18F254 plates with a solvent of methanol/water/acetic acid 80/20/5. Audioradiography was carried out using Ultrafilm (LKB) and the plates scraped and the radioactive incorporations determined. This revealed that after 15 min at least 75% of the radioactivity cochromatographed with the amino derivative **15** while by 30 min post-drug administration this was the only compound observable.

*Toxicity of 6-(3-aminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-b]-thiazole (**15**) dihydrochloride and 6-(3-benzamidophenyl)-2,3,5,6-tetrahydroimidazo[2,1-b]thiazole (**6**)*

The title compounds were ball-milled in 0.25% aqueous methyl cellulose and administered by gavage at dose levels of 300, 100 and 30 mg/kg to groups of 5 male and 5 female Wistar(COBS) rats. Compound **6** at 300 mg/kg and **15** at all dose levels killed all animals after a single dose. Rats dosed daily with 100 mg/kg of **6** survived only until day 3. However, at 30 mg/kg, 14 daily doses of **6** failed to induce any treatment related effects in any of the animals.

Acknowledgements

We thank Miss R Balsara, Mr J Barlas, Miss P Bartlett, Mr M Beston, Miss DJ Bluff, Mr D Dunstone, Mr D Gower, Mrs L Holmes, Mrs R Lancaster, Mrs G Lees, Mr M Martin-Short, Mr D Moseley, Mr R Murgatroyd, Mr A O'Dowd, Mr R Rankin, Mr M Reddy, Mr M Rees, Mr G Riley, Miss J Rowley and Miss C Turner for skilled technical assistance. The general contributions of Drs Hayes, Townson and Mendis to this work are acknowledged. We also thank Mr CJ Port and Dr P Greaves for carrying out the toxicological evaluation of **6** and **15**, and staff of the Department of Physical Sciences for the provision of analytical and spectral data. Additional facilities and help were provided by Dr DB Copeman (James Cooke University of North Queensland, Townsville, Australia) and Dr DW Buttner (Bernard-Hocht Institut, Hamburg, Germany). This study was supported by the World Health Organisation Onchocerciasis Chemotherapy Project of the Onchocerciasis Control Program in the Volta River Basin, OCT contract no 82005.

References

1 World Health Organisation. Review of the Steering Committee of the Scientific Working Group on Filariasis, 1983-1988, TDR/FIL/SC-SWG (83-88)/88.3
2 Ottesen EA (1985) *Rev Infect Dis* 7, 341–356
3 Bell D (1985) *Br Med J* 290,1450–1451
4 Lindley D (1987) *Nature* 329, 752
5 Greene BM, Taylor HR, Cupp EW, Murphy RP, White AT, Aziz MA, Shulz-Key H, D'Anna SA, Newland HS, Goldschmidt LP, Auer C, Hanson AP, Freeman SV, Reber EW, Williams PN (1985) *N Engl J Med* 313, 133–138
6 Awadzi K, Dadzie KY, Shulz-Key H, Haddock DRW, Gilles HM, Aziz MA (1985) *Ann Trop Med Parasitol* 79, 63–78
7 Dorgan RJ (1982) Europ Pat 0,046,377
8 Spicer LD, Hand JJ (1976) US Pat 3, 989, 835
9 Raeymaekers AHM, Allewijn FTN, Vandenberk J, Demoen PJA, Offenwert TTTV, Janssen PAJ (1966) *J Med Chem* 9, 545–551
10 Lewis JA, Paterson I (1984) *J Labelled Compds Radiopharm* 21, 945–959
11 Weikert RJ, Bingham S, Emanuel MA, Fraser-Smith EB, Loughhead DG, Nelson PH, Poulton AL (1991) *J Med Chem* 34, 1630–1633
12 Brewer MD, Dorgan RJJ, Manger BR, Mamalis P, Webster RAB (1987) *J Med Chem* 30, 1848–1853
13 Tomlinson G, Albuquerque CA, Woods RA (1985) *Eur J Pharmacol* 113, 255–262
14 Lewis JA, Fleming JT, McLafferty S, Murphy H, Wu C (1987) *Mol Pharmacol* 31, 185–193
15 Still WC, Kahn M, Mitra A (1978) *J Org Chem* 43, 2923–2925
16 Miller AK (1892) *J Chem Soc* 1023–1035
17 Stanley G, Shorter J (1958) *J Chem Soc* 256–259
18 Chiavarelli S (1955) *Gazz Chim Ital* 85 1405–1410
19 Robinson GE, Vernon JM (1970) *J Chem Soc* (C) 2586–2591
20 Hudson AT, Randall AW (1984) *Eur Pat App* EP123, 238
21 Pitre D, Lorenzotti E (1965) *Chimia (Aarau)* 19, 462 (1965) *Chem Abs* 63, 13128h
22 Gunstone FD, Tucker SH (1963) *Organic Syntheses Coll Vol 4* 160–161
23 Buckingham J, ed (1982) *Dictionary of Organic Compounds, 5th ed* Chapman and Hall, London
24 Comley JCW, Rees MJ, O'Dowd AB (1988) *Trop Med Parasitol* 39, 221–226
25 Comley JCW, Szopa TM, Strote G, Buttner M, Darge K, Bunner DW (1989) *Parasitology* 99, 417–425
26 Denham DA (1979) *J Helminthol* 53, 175–187
27 Suswillo RR, Denham DA (1977) *J Parasitol* 63, 591–592
28 Court JP, Stables JN, Lees GM, Martin-Short MR, Rankin R (1988) *J Helminthol* 62, 1–9