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Antimalarial Pyrido[1,2-*a*]benzimidazoles

Albert J. Ndakala,[†] Richard K. Gessner,[†] Patricia W. Gitari,[†] Natasha October,[†] Karen L. White,[§] Alan Hudson,[∥] Foluke Fakorede,[⊥] David M. Shackleford,[§] Marcel Kaiser,[#] Clive Yeates,[∞] Susan A. Charman,[§] and Kelly Chibale[*,†,‡]

[†]Department of Chemistry, and [‡]Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Rondebosch 7701, South Africa

[§]Centre for Drug Candidate Optimisation, Monash University (Parkville Campus), 381 Royal Parade, Parkville, Victoria 3052, Australia

[∥]Pharmacons, Kent, U.K.

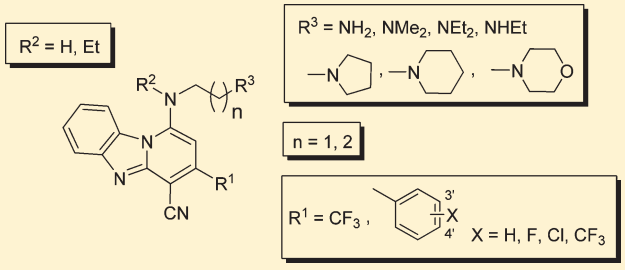
[⊥]Tropical Diseases Research, World Health Organization, 20 Avenue Appia, CH-1211, Geneva 27, Switzerland

[#]Swiss Tropical and Public Health Institute, Postfach, Socinstrasse 57, 4002 Basel, Switzerland

[∞]InPharma Consultancy, Herts, U.K.

Supporting Information

ABSTRACT: A novel class of antimalarial pyrido[1,2-*a*]benzimidazoles were synthesized and evaluated for antiplasmodial activity and cytotoxicity following hits identified from screening commercially available compound collections. The most active of these, TDR86919 (**4c**), showed improved in vitro activity vs the drug-resistant K1 strain of *Plasmodium falciparum* relative to chloroquine (IC_{50} = 0.047 μM v 0.17 μM); potency was retained against a range of drug-sensitive and drug-resistant strains, with negligible cytotoxicity against the mammalian (L-6) cell line (selectivity index of >600). **4c** and several close analogues (as HCl or mesylate salts) showed significant efficacy in *P. berghei* infected mice following both intraperitoneal (ip) and oral (po) administration, with >90% inhibition of parasitemia, accompanied by an increase in the mean survival time (MSD). The pyrido[1,2-*a*]benzimidazoles appeared to be relatively slow acting in vivo compared to chloroquine, and metabolic stability of the alkylamino side chain was identified as a key issue in influencing in vivo activity.



INTRODUCTION

Malaria continues to take an enormous toll on human health, particularly in tropical regions. The drugs used to treat this disease are far from ideal, and many of these were introduced decades ago. The utility of these medicaments in resource-poor settings is limited by a number of factors, such as high cost, poor compliance, drug resistance, low efficacy, and poor safety.[1]

Malaria results from infection with four different species of the genus *Plasmodium*, all transmitted by mosquitoes, namely, *falciparum*, *vivax*, *ovale*, and *malariae*. The most important of these in terms of virulence and mortality is *P. falciparum*, although *P. vivax* also has a huge impact on populations with regard to morbidity.[2] The enormous public health problem posed by malaria across the developing world is reflected by the grim estimates that each year the disease causes between 1.7 and 2.5 million deaths. Over the past few decades the mainstays of antimalarial chemotherapy, chloroquine and pyrimethamine/sulfadoxine, have been significantly compromised in many regions by the spread of drug-resistant parasites. To counter this, a range of newer drugs and combinations have gradually been introduced into use, e.g., mefloquine (1984), artemisinins (1994), artemether/lumefantrine (1999), atovaquone/proguanil (1999), chlorproguanil/dapsone (2003), but all come with some issue limiting use.[3] There is thus a clear need to develop new more affordable and effective antimalarials.[4]

As part of a TDR collaboration with the Belgian company Tibotec, in 2000 a small library of 1440 diverse nonpropriety compounds donated by SPECS were screened against a panel of protozoa in vitro. This led to the identification of a pyrido[1,2-*a*]-benzimidazole coded TDR15087 (**1**, Figure 1), with moderate in vitro activity toward *P. falciparum* GHA and W2 strains (IC_{50} = 0.17−0.37 μM), significant because there is no published prior art relating to the antimalarial activity of pyrido[1,2-*a*]benzimidazoles, although derivatives related to **1** have previously been investigated for antifungal,[5] antibacterial,[6] and antitumor[6−11] activity.

Other pyrido[1,2-*a*]benzimidazoles were then sought to further explore the in vitro antimalarial structure−activity relationships (SARs). Initially this involved the selection of 535

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commercially available analogues, primarily on the basis of providing diversity around the pyridobenzimidazole "core", which were evaluated in a medium throughput screen (MTS) using a multidrug resistant strain of *P. falciparum* (K1), with cytotoxicity assessed against the murine L-6 cell-line; actives from the MTS were re-evaluated for IC_{50} against both *P. falciparum* and L-6. From this exercise, 49 compounds were identified with *P. falciparum* IC_{50} of <0.1 μg/mL; notable were the *N*-benzylpiperazinyl derivatives TDR35885 (**2**) and TDR44047 (**3**) (Figure 1), which showed good selectivity and greater activity in vitro compared to **1** (Table 2).

However, **1**−**3** all proved to be inactive in the standard *P. berghei* mouse model at doses up to 4 × 100 mg/kg ip, most likely because of a combination of poor solubility and metabolic stability. To find compounds active in vivo in the *P. berghei* mouse model, we have investigated the antimalarial SAR of pyridobenzimidazoles further. Here we describe part of these investigations focused on 3-aryl derivatives, with alkylamino side chains (Figure 2). Metabolic stability emerged as a significant factor influencing activity in vivo, and in vitro microsome stability studies proved to be useful in guiding the selection of compounds for animal assessment.

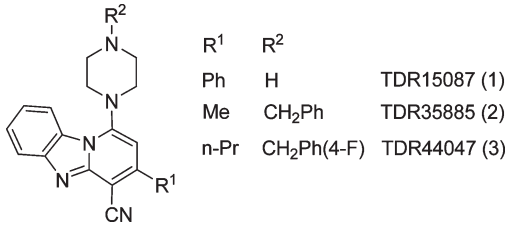


Figure 1. Pyrido[1,2-*a*]benzimidazole screening hits with potent activity toward *P. falciparum*.

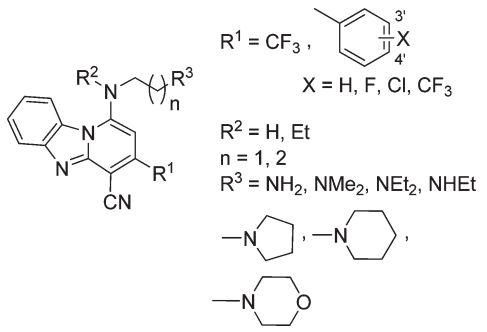


Figure 2. Target pyrido[1,2-*a*]benzimidazoles for SAR exploration.

RESULTS AND DISCUSSION

Chemistry. A relatively straightforward synthetic approach (Scheme 1) was followed for the synthesis of target pyrido-[1,2-*a*]benzimidazoles, adapting published procedures.[6−12]

The final stage involving nucleophilic substitution with the appropriate amine was initially carried out with external heating, requiring relatively long reaction times of up to 18 h; microwave irradiation was subsequently found to reduce the reaction time to approximately 20 min. As the pyridobenzimidazoles generally show low aqueous solubility, hydrochloride salts were made of compounds selected for in vivo evaluation to aid formulation; for a few compounds mesylate salts were also made for comparison to provide confidence that the salt form was not significantly affecting activity. Chloroquine, administered as the free base, shows significantly lower activity in the *P. berghei* mouse model compared to the diphosphate salt, the standard used in our studies.

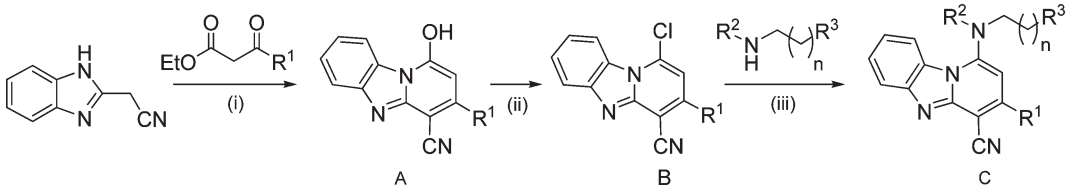
Hydrochloride salts were initially prepared by bubbling excess HCl(g) through a mixture of the free base in dichloromethane or more conveniently with HCl(aq) in methanol; the compounds in the free base form were generally insoluble in methanol, and solubilization occurred on addition of the aqueous acid. The latter method allowed for easy isolation of the salts by removal of the solvent followed by trituration with dichloromethane to remove any impurities. Mesylate salts were prepared by addition of methanesulfonic acid to a solution of the appropriate freebase in dichloromethane.

Biology. In Vitro Activity. Initial SAR studies (Figure 2, Table 1) involved exploration around the aminoalkyl side chain and variation in substitution at position R^1 (including CF_3 and positions 3′ and 4′ substituted aromatic groups). Compounds **4a**−**10f** were evaluated for antimalarial activity against the multidrug-resistant *P. falciparum* K1 strain and cytotoxicity against the mammalian L-6 cell line (Table 1).

Many of the compounds showed antimalarial activity comparable to that of chloroquine (IC_{50} = 0.17−0.20 μM), with compound **4c** being the most active (IC_{50} = 0.047 μM, ∼3.5 times more potent than chloroquine) and **6a** the least active (IC_{50} = 4.48 μM, ∼22 times less potent than chloroquine and ∼95 times less potent than **4c**). In general lipophilicity appears to be an important factor influencing in vitro activity and selectivity, although other structural features in the alkylamino side chain also seem to have a significant influence:

1. N-Alkylated derivatives (R^3 = $N(Me)_2$, $N(Et)_2$, or N(H)(Et)) showed greater potency (∼2−20 times) than their N-dealkylated (R^3 = NH_2) counterparts and also had more favorable selectivity to L-6.
2. Phenyl or monosubstituted aryl groups at R^1 in place of a CF_3 group showed superior potency of up to 10-fold in all cases, and activity was generally further enhanced when the R^1 aryl group was substituted (CF_3, Cl, or F) at the 4′ position.

Scheme 1. General Synthetic Approach to 4-Cyanopyrido[1,2-*a*]benzimidazoles[a]



[a] Reagents and conditions: (i) NH_4OAc, 140−150 °C, 30−60 min; (ii) $POCl_3$, reflux, 2 h; (iii) DMF or THF, Et_3N, 80−90 °C, 18 h or microwave (150 W), 20 min; R^1, R^2, R^3, *n* as defined in Table 1.

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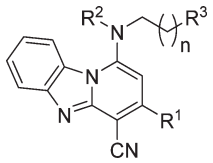
Table 1. In Vitro Antimalarial Activity Evaluation of Pyrido[1,2*a*]benzimidazole Derivatives



						IC$_{50}$ (μM)[a]		
compd	R^1	R^2	R^3	*n*	log $D_{pH7.4}$[b]	*P. fal* (K1)	cytotox (L6)	SI
chloroquine						0.17−0.20		
artemisinin						0.006−0.011		
podophyllotoxin							0.009−0.014	
4a	4-CF_3Ph	H	NH_2	1	1.80	1.49	5.11	3.4
5a	3-CF_3Ph	H	NH_2	1	1.80	1.00	11.7	11.7
6a	Ph	H	NH_2	1	0.83	4.48	18.9	4.2
7a	CF_3	H	NH_2	1	1.37	3.09	40.1	13
4b	4-CF_3Ph	H	$N(Me)_2$	1	2.45	0.13	>210	>1600
5b	3-CF_3Ph	H	$N(Me)_2$	1	2.45	0.29	7.14	24.6
6b	Ph	H	$N(Me)_2$	1	1.48	1.49	>80	>50
7b	CF_3	H	$N(Me)_2$	1	2.01	3.43	44.7	13
8b	4-FPh	H	$N(Me)_2$	1	1.41	0.28	19.6	70
9b	3-FPh	H	$N(Me)_2$	1	1.50	0.84	5.88	7
10b	4-ClPh	H	$N(Me)_2$	1	2.05	0.31	19.5	62.9
4c	4-CF_3Ph	H	$N(Et)_2$	1	2.94	0.047	28.8	612
5c	3-CF_3Ph	H	$N(Et)_2$	1	2.94	0.42	102	243
6c	Ph	H	$N(Et)_2$	1	1.89	1.11	120	108
7c	CF_3	H	$N(Et)_2$	1	2.51	1.90	121	63.7
8c	4-FPh	H	$N(Et)_2$	1	1.90	0.25	56.5	226
9c	3-FPh	H	$N(Et)_2$	1	1.99	0.55	124	225
10c	4-ClPh	H	$N(Et)_2$	1	2.54	0.06	41	683
4d	4-CF_3Ph	H	NH_2	2	1.46	0.92	6.26	6.8
5d	3-CF_3Ph	H	NH_2	2	1.46	0.63	4.33	6.9
6d	Ph	H	NH_2	2	0.49	2.27	11.9	5.2
4e	4-CF_3Ph	H	$N(Me)_2$	2	2.49	0.44	>200	455
5e	3-CF_3Ph	H	$N(Me)_2$	2	2.49	0.38	180	474
6e	Ph	H	$N(Me)_2$	2	1.52	1.52	108	71
7e	CF_3	H	$N(Me)_2$	2	2.06	3.16	166	52.5
8e	4-FPh	H	$N(Me)_2$	2	1.45	0.27	11.6	43
9e	3-FPh	H	$N(Me)_2$	2	1.54	0.53	15.4	29
10e	4-ClPh	H	$N(Me)_2$	2	2.09	0.21	>210	>1000
4f	4-CF_3Ph	H	$N(Et)_2$	2	2.97	0.30	>190	>633
5f	3-CF_3Ph	H	$N(Et)_2$	2	2.97	0.19	24.3	128
6f	Ph	H	$N(Et)_2$	2	2.00	1.53	>230	>150
7f	CF_3	H	$N(Et)_2$	2	2.54	2.08	87.8	42.2
8f	4-FPh	H	$N(Et)_2$	2	1.93	0.71	86.6	122
9f	3-FPh	H	$N(Et)_2$	2	2.02	0.46	55.7	121
10f	4-ClPh	H	$N(Et)_2$	2	2.57	0.17	48.8	287
4g	4-CF_3Ph	Et	N(H)(Et)	1	3.52	0.94	21.5	22.9
4h	4-CF_3Ph	H	N(H)(Et)	1	2.01	0.12	2.46	20.5
4i	4-CF_3Ph	H	piperidine	1	3.78	0.054	193	3581
4j	4-CF_3Ph	H	pyrrolidine	1	2.58	0.053	146	2751
4k	4-CF_3Ph	H	morpholine	1	3.72	0.49	33.3	68
4l	4-CF_3Ph	H	morpholine	2	3.79	1.22	98.3	80.6

[a] Mean from $n \geq 2$ independent experiments. Individual values varied by less than a factor of 2. [b] The log $D_{pH7.4}$ values were calculated using the ACD LogD Suite of software (version 9.0, Advanced Chemistry Development Inc., Toronto, Canada).

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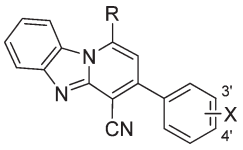
Table 2. In Vitro Activity against Drug-Sensitive and Drug-Resistant Strains of *P. falciparum*

	P. falciparum IC_{50} (μM)						
	W2 mef	HB3	NF54	FC27	FCR3	MAD20	K1
chloroquine	0.118	0.041	0.006	0.004	0.085	0.010	0.151−0.173
artemesinin	0.008	0.003	0.005	0.008	0.005	0.008	0.005
mefloquine	0.011	0.012	0.013	<0.040	0.005	0.013	0.008
pyrimethamine	29.5	1.146	0.019	<0.060	0.012	<0.060	9.9
1	ND	ND	ND	ND	ND	ND	0.17−0.36
2	ND	ND	0.052	ND	ND	ND	0.05−0.078
3	ND	ND	0.053	ND	ND	ND	0.023−0.095
4c	0.084	0.060	0.11	0.175	0.137	0.104	0.047−0.058
4h	0.123	0.090	0.11	0.11	0.154	0.106	0.116

Table 3. In Vivo Antimalarial Activity of Pyrido[1,2*a*]benzimidazole Aminoalkyl Derivatives and Corresponding Salts



			IC_{50} (μM)		*P. berghei* in vivo % reduction parasitemia (MSD)[a]	
compd	X	R	*P. fal* (K1)	cytotox (L6)	ip	po
chloroquine[b] control					99.6% (20) (5−7)	99.9% (17)
4b	4′-CF_3	$-NH(CH_2)_2N(Me)_2$	0.13	>210		
4ba	4′-CF_3	2HCl salt of **4b**	0.1	147.2	0%[c]	
4bb	4′-CF_3	mesylate salt of **4b**	0.14	>175	94.1% (11)	96.8% (13.7)
4c	4′-CF_3	$-NH(CH_2)_2N(Et)_2$	0.05	28.8		
4ca	4′-CF_3	2HCl salt of **4c**	0.08	89.6	91.9% (10)	96.2% (16)
4cb	4′-CF_3	mesylate salt of **4c**	0.09	123.6	95.2% (11)	97.4% (11)
4e	4′-CF_3	$-NH(CH_2)_3N(Me)_2$	0.44	>200		
4ea	4′-CF_3	2HCl salt of **4e**	0.26	21.1	30.7%[c]	
5e	3′-CF_3	$-NH(CH_2)_3N(Me)_2$	0.38	180		
5ea	3′-CF_3	2HCl salt of **5e**	0.49	19.2	0%[c]	
4f	4′-CF_3	$-NH(CH_2)_3N(Et)_2$	0.30	>190		
4fa	4′-CF_3	2HCl salt of **4f**	0.16	2.23	72.6% (9)	93.4% (11)
5f	3′-CF_3	$-NH(CH_2)_3N(Et)_2$	0.19	24.3		
5fa	3′-CF_3	2HCl salt of **5f**	0.25	11.8	0%[c]	

[a] MSD = mean survival time (in days). 50 (mg/kg)/day × 4 ip or 100 (mg/kg)/day × 4 po (formulated in 10% aq DMSO). [b] Chloroquine diphosphate at 10 (mg/kg)/day × 4 ip or po. [c] Mice euthanized on day 4, 24 h after last treatment, because of inactivity.

3. Variation in the alkyl chain length had practically no significant effect on the in vitro antiplasmodial activity, although this was only varied by one methylene unit.
4. The morpholino derivative **4k** was ∼10× less active than other cycloalkylamino derivatives **4i** and **4j.**
5. In the one example in which there was additional alkyl substitution on the N attached to the ring (**4g**), activity was reduced compared to the des-ethyl derivative (**4h**).
6. **4h**, the *N*-des-ethyl metabolite of compound **4c**, retained its activity to an extent, albeit with reduced selectivity vs L-6.The activity of compounds **4c** and **4h** was found to be more or less invariant across a broad range of drug sensitive and drug resistant strains of *P. falciparum* (Table 2).

In Vivo Studies. Six compounds with the most favorable activity and selectivity from the in vitro screen (**4b**, **4c**, **4e**, **5e**, **4f**, and **5f**) were chosen for preliminary in vivo evaluation in *P. berghei* infected mice. The corresponding hydrochloride salts (**4ba**−**5fa**), as well as two mesylate salts (**4bb** and **4cb**), were used for the in vivo studies. At a repeat dose of 50 (mg/kg)/day ip for 4 days four of these (**4bb**, **4ca**, **4cb**, and **4fa**) showed significant efficacy. Subsequently they were also found to be active following oral (po) administration with 100 (mg/kg)/day × 4, giving >90%

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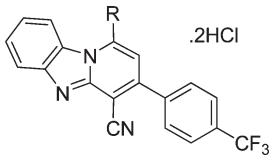
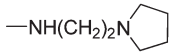
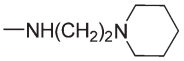
Table 4. Oral Efficacy of Selected Compounds (Bis-hydrochloride Salts) in the *P. berghei* Mouse Model[a]



Compound	R	Dose (mg/kg)	% reduction parasitemia Day 2	Day 3	Day 4	Day 5	MSD (days)
4ca	—$NH(CH_2)_2N(Et)_2$	1x50	5.15	29.76	59.97	78.22	8
4ca	"	1x25	13.07	24.38	51.13	70.33	8
4ha	—$NH(CH_2)_2N(H)(Et)$	1x50	30.78	50.24	71.22	79.79	12.7
4ha	"	1x25	23.51	55.45	62.25	68.92	13.3
4ja	—$NH(CH_2)_2N$ 	1x50	14.04	47.92	73.01	88.53	8.7
4ja	"	1x25	14.75	43.20	63.73	63.72	7.7
4ia	—$NH(CH_2)_2N$ 	1x50	23.38	9.30	0.00	0.00	5
4ia	"	1x25	28.44	28.07	38.72	46.41	12.7
chloroquine		1x10	99.9				9
control		-	-	-	-	-	5

[a] Test compounds were formulated in hydroxypropyl methylcellulose (HPMC) and administered po (3 mice per group).

inhibition of *P. berghei* parasitemia and a significant increase in the mean survival time (MSD) of the mice from 10 to 16 days, relative to controls (5−7 days, Table 3). The lack of in vivo activity with the hydrochloride salt of **4b** (**4ba**) compared to the mesylate salt (**4bb**) when given ip may be due to a poorer solubility or dissolution rate adversely affecting distribution.

In single dose studies in the *P. berghei* mouse model (Table 4), the hydrochloride salts of four of the most active compounds **4ca**, **4ha**, **4ja**, **4ia** showed significant activity at 25 mg/kg, which was not significantly improved on going to a higher dose of 50 mg/kg, presumably because of saturation of systemic exposure; **4ia** was ineffectual at 50 mg/kg, possibly because of a problem with formulation arising from poor solubility (cloudiness of the solution was observed during administration of **4ia** at both doses). In any case the rate of reduction in parasitemia after treatment with the pyridobenzimidazoles was significantly slower than with chloroquine, with maximum reduction generally observed on day 5 compared to day 2 with chloroquine.

In Vitro Metabolic Stability. Studies were conducted using human, mouse, and rat liver microsomes (Table 5). Compounds **4ca** and **4ja**−**4ka** exhibited moderate to high rates of metabolic degradation, while degradation of **4ha** was in the low to moderate range. Generally for the alkylated derivatives **4ca** and **4ha**, N-dealkylated metabolites were observed, and for the cycloalkylated derivatives (**4ja**−**4la**), there was evidence for deamination, N-dealkylation, or ring cleavage.

In Vivo Pharmacokinetic Studies. The in vivo pharmacokinetic properties of both **4c** and **4h** were assessed following administration of the bis-hydrochloride salts (i.e., **4ca** and **4ha**) at dose levels of 5 mg/kg intravenously and 20 mg/kg orally to male Sprague−Dawley rats (Table 6).

For compound **4c**, the apparent half-life ranged between 6 and 8 h; volume of distribution was high, and plasma clearance was moderate. The rate of absorption was relatively slow after oral administration, and the apparent oral bioavailability was ∼22%. The des-ethyl derivative **4h** was identified as a metabolite of **4c** in plasma samples following both routes of administration, in agreement with the in vitro microsome studies. The in vivo conversion of **4c** to **4h** was estimated to be approximately 70%, suggesting that N-dealkylation is likely to be a major in vivo clearance pathway for **4c**. Direct urinary excretion of **4c** was negligible following both intravenous and oral administration. When **4h** was administered intravenously as the bis-hydrochloride salt (**4ha**) at a dose of 5 mg/kg, it exhibited a long apparent half-life, high volume of distribution, and low plasma clearance; direct urinary excretion of **4h** was negligible. Compound **4h** exhibited high protein binding in human and mouse plasma with fraction bound values being in the ranges 98.3−99.9% and 95.3−96.9%, respectively. In comparison **4c** had 96.7−99.2% (human) and 97.9% (mouse) protein binding. On the basis of the high conversion of **4c** to **4h** observed in vivo, the long half-life, and low blood clearance of **4h** and its intrinsic activity, it is likely that the N-dealkylation product **4h** contributes significantly to the efficacy of **4c**.

Single-dose and multidose oral efficacy studies of **4ha** against *P. berghei* ANKA GFP in mice indicated that the activity plateaus (reaches a limit) with dose concentrations higher than 25 mg/kg

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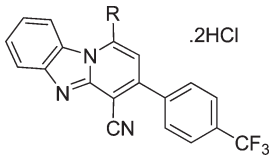
Table 5. In Vitro Microsomal Stability



compound	R	Species	Degradation half-life (min)	*In vitro* CL_{int} (μL/min/mg protein)	Microsome-Predicted E_H[a]	Metabolites detected[b]
4ca	—$NH(CH_2)_2N(Et)_2$	Human	13.7	126.7	0.88	P-28; P-56
		Rat	24.8	69.8	0.69	"
		Mouse	24.1	71.8	0.76	"
4ha	—$NH(CH_2)_2N(H)(Et)$	Human	136.8	12.7	0.41	P-28
		Rat	284.4	6.1	0.17	"
		Mouse	321.2	5.4	0.19	"
4ja	—$NH(CH_2)_2N$ (pyrrolidine)	Human	19.6	88.4	0.83	P+14; P-53; P-97
		Rat	67.2	25.8	0.53	"
		Mouse	67.6	25.6	0.46	"
4ia	—$NH(CH_2)_2N$ (piperidine)	Human	37.9	45.8	0.72	P+16; P-68
		Rat	64.2	27	0.54	P-68
		Mouse	59.5	29.1	0.49	P+16; P-68
4ka	—$NH(CH_2)_2N$ (morpholine)	Human	15.9	108.9	0.86	P-26; P-70; P-113
		Rat	16	108.4	0.83	"
		Mouse	26.6	65.2	0.68	"
4la	—$NH(CH_2)_3N$ (morpholine)	Human	10.4	166.2	0.90	P-70; P-127
		Rat	7.4	234.4	0.91	"
		Mouse	19.9	87.0	0.74	"

[a] E_H (hepatic extraction ratio) = fraction of dose entering liver, which is metabolized during one pass through the liver. [b] P-28: monodeethylation. P-56: bis-deethylation. P + 14: carbonyl addition. P-53: deamination of the pyrrolidine ring. P-97, P-68, P-127, P-113: N-dealkylation of the respective R side chain. P + 16: oxygenation. P-70: morpholine dealkylation. P-26: morpholine ring cleavage.

Table 6. Pharmacokinetic Parameters for 4c and 4h after iv and Oral Administration of Their Bis-hydrochloride Salts (4ca and 4ha) to Male Rats

	4c		**4h** (after administration of **4ca**)		**4h**	
parameter	iv[a]	oral[a]	iv[a]	oral[a]	iv[a]	oral[a]
nominal dose (mg/kg)[b]	4.3	19.0			2.6	19.1
apparent $t_{1/2}$ (h)	7.3	6.4	cnc[d]	cnc[d]	~16.3	cnc[d]
plasma CL_{total}	30				5.4	
V_Z (L/kg)	18.9				7.5	
% dose in urine[c]	0.39	0.05	0.08	0.05	1.13	0.21
C_{max} (μM)		0.38	0.54	0.92		0.95
T_{max} (min)		248	640	1200		960
$AUC_{0-tlast}$ (μM·min)	304	290	666	1032	1000	2076
bioavailability (%)		>22				>28[e]

[a] Compounds were formulated as suspensions in 0.5% hydroxypropyl methylcellulose. Values are the mean from two animals. [b] As the bishydrochloride salt. [c] % of dose present in pooled urine (collected over 0−24 or 0−48 h). [d] Given the flat nature of the profile, the terminal elimination half-life could not be determined. [e] The terminal elimination half-life and oral bioavailability could not be accurately determined. On the basis of the dose-normalized $AUC_{0-tlast}$, the exposure after oral administration was approximately 25−30% of that after iv administration; however, this will likely be an underestimation of the actual oral bioavailability.

in vivo (Tables 7 and 8). A similar plateau in efficacy was also observed following subcutaneous administration.

As a preliminary indication of systemic exposure, in vivo studies in mice following oral and subcutaneous administration of **4ha** at

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Table 7. Single-Dose Oral Efficacy of 4ha against *P. berghei* ANKA GFP in Mice

		% reduction parasitemia				
compd	dose (mg/kg)[a]	day 2	day 3	day 4	day 5	MSD (day)
4ha	1 × 25	23.51	55.45	62.25	68.92	13.3
4ha	1 × 50	30.78	50.24	71.22	79.79	12.7
chloroquine	1 × 10	99.9				9
control						5

[a] Compounds were formulated in HPMC.

Table 8. Multidose Oral Efficacy of 4ha against *P. berghei* ANKA GFP in Mice

		% reduction	
4ha (mg/kg)[a]	cured/infected	parasitemia at day 4	MSD (day)
4 × 3	0/3	0	4[b]
4 × 6	0/3	38.35	7
4 × 12.5	0/3	80.73	14
4 × 25	0/3	95.72	14.3
4 × 50	0/3	95.56	14
control			6.5

[a] Compounds were formulated in HPMC. [b] Mice euthanized on day 4, 24 h after last treatment, because of inactivity.

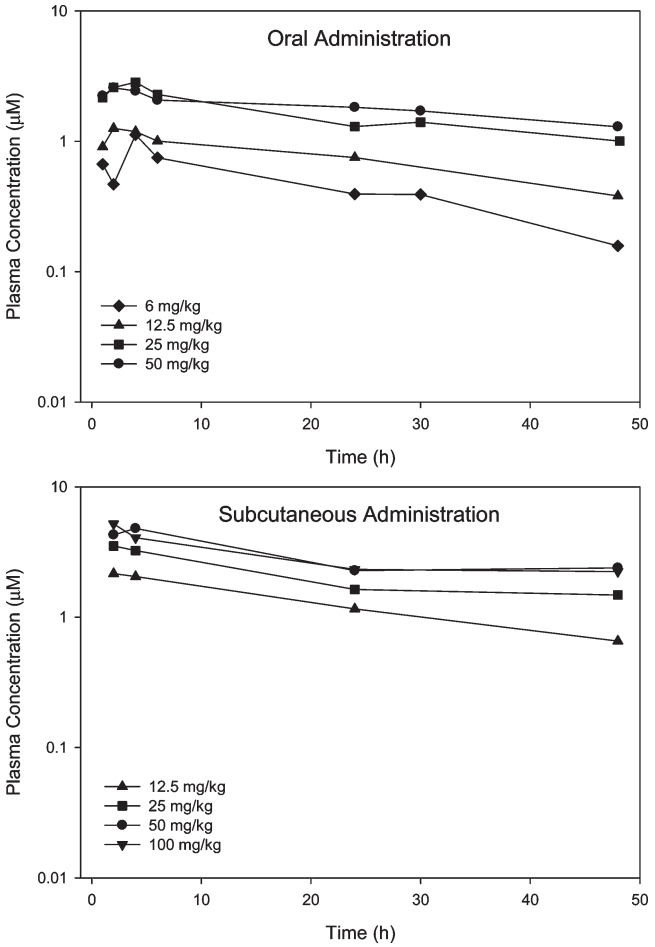

Figure 3. Systemic exposure of **4h** following single dose oral and subcutaneous administration of the bis-hydrochloride salt (**4ha**) to mice.

escalating dose levels (6−100 mg/kg) were conducted. Exposure was found to become saturated at dose levels above 25 mg/kg (orally) or 50 mg/kg (subcutaneously), consistent with the observed plateau in efficacy (Figure 3). It is likely that solubility or dissolution-limited absorption is a contributory factor in limiting systemic exposure and efficacy with increasing dose.

CONCLUSIONS

A novel series of pyrido[1,2-*a*]benzimidazole derivatives have been identified that combine good in vitro activity against *P. falciparum* with oral efficacy in a *P. berghei* mouse model. The pyridobenzimidazoles appear to be slower acting in vivo relative to chloroquine, pointing to a different mode of action, which has not been established. The most significant feature of the series is that the pharmacokinetic profile of the lead compounds needs considerable improvement if a pyridobenzimidazole is to be identified as worthy of further progression. Although **4h** shows good stability in rat and mouse microsomes and has a long half-life in rats, pharmacokinetic studies indicate oral absorption becomes saturated at relatively low doses, most likely because of poor dissolution or solubility. Further work is needed to identify compounds that have the potential for improved pharmacokinetics, most likely achievable through a combination of improved solubility and metabolic stability.

EXPERIMENTAL SECTION

Chemistry. All commercially available chemicals were purchased from either Sigma-Aldrich or Merck. All solvents were dried by appropriate techniques. Unless otherwise stated, all solvents used were anhydrous. Reactions were monitored by TLC using Merck silica gel plates (60 F-254), and were visualized by ultraviolet light. Silica gel chromatography was performed using Merck Kieselgel 60: 70−230 mesh for gravity columns. Melting points were determined on a Reichert-Jung Thermovar hotstage microscope and are uncorrected. Infrared spectra were recorded on a Thermo Nicolete FTIR instrument in the 4000−500 cm^{-1} range using KBr disks. Microanalyses were determined using a Fisons EA 1108 CHNO-S instrument. Mass spectra were recorded at the School of Chemistry, University of the Witwatersrand, South Africa. NMR spectra were recorded on either a Varian Mercury 300 (^{1}H, 300.13 MHz; ^{13}C, 75.5 MHz) or a Varian Unity 400 (^{1}H, 400.13 MHz; ^{13}C, 100.6 MHz) spectrometer. Chemical shifts (δ) are given in ppm downfield from TMS as the internal standard. Coupling constants, *J*, are recorded in hertz (Hz). LC purity traces were obtained using the Kinetex C18 (2.1 mm × 150 mm, 2.6 mm fused-core particles) column, 1 mL injection volume, flow of 0.4 mL/min, gradient 0−100% B in 9 min (hold 3 min) (mobile phase A of 10 mM ammonium formate, pH 3, in 10% MeCN and mobile phase B of 10 mM ammonium formate, pH 3, in 90% MeCN) with a diode array detector operating at a wavelength range from 190 to 400 nm.

Purity was determined by combustion analysis and/or HPLC, and all compounds were confirmed to have >95% purity.

General Procedure for the Synthesis of 1-Oxo-3-alkyl/aryl-5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitriles (A). A mixture of 2-benzimidazole acetonitrile (1.0 g, 6.36 mmol), NH_4OAc (0.98 g, 12.72 mmol), and ethyl (4-alkanoyl/aroyl)acetate (7.63 mmol) was heated to reflux at 150 °C for 1 h and allowed to cool to 100 °C. MeCN (10 mL) was added. The mixture was stirred for 15 min, allowed to cool to room temperature, and then cooled on ice. The cold mixture was filtered and the residue washed with cold MeCN (4 × 10 mL), dried in vacuo, and used without further purification.

1-Oxo-3-[4-(trifluoromethyl)phenyl]-5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile. Silvery tan powder, mp 341−342 °C

(ethanol); IR (KBr) 3250−2500 bm, 2203 m (CN), 1664 s (CO), 1548 s, 1509 s, 1458 m, 1324 s, 1169 m, 1107 s, 1075 m, 1064 s cm^{-1}; ^1H NMR (400 MHz, DMF-d_7) δ 8.64 (1H, d, J = 7.3 Hz, ArH), 7.93 (4H, s, ArH), 7.64 (1H, d, J = 7.3 Hz, ArH), 7.57 (1H, dd, J = 7.3, 7.3 Hz, ArH), 7.42 (1H, dd, J = 8.8, 7.3 Hz, ArH), 6.11 (1H, s, =C*H*−); ^{13}C NMR (100 MHz, DMF-d_7) δ 157.9, 150.9, 147.2, 140.9, 131.6, 130.0, 129.7, 128.4, 127.6, 126.4, 125.1, 122.5, 122.3, 115.9, 115.7, 111.0, 104.6, 67.9; LRMS (EI) m/z 354.2 (M + H).

General Procedure for the Synthesis of 1-Chloro-3-alkyl/arylpyrido[1,2-*a*]benzimidazole-4-carbonitriles (B). A mixture of 1-oxo-3-alkyl/aryl-5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (2.83 mmol) and $POCl_3$ (8.69 g, 5.28 mL, 56.68 mmol) was heated to reflux at 130 °C for 2 h. Excess $POCl_3$ was removed under reduced pressure and ice-cold water (20 mL) added to the residue, stirring to yield a precipitate. The mixture was neutralized with saturated $NaHCO_3$ and filtered. The resultant solid was washed with ice-cold water (4 × 15 mL), dried in vacuo, and used without further purification.

1-Chloro-3-[4-(trifluoromethyl)phenyl]pyrido[1,2-*a*]benzimidazole-4-carbonitrile. Yellow solid, mp 247−248 °C (ethanol); IR (KBr) 3094 w, 3065 w, 2217 m (CN), 1617 w, 1591 m, 1487 s, 1444 s, 1339 s, 1172 s, 1129 s, 1067 s cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.59 (1H, d, J = 8.8 Hz, ArH), 8.10 (1H, d, J = 7.8 Hz, ArH), 7.85 (4H, s, ArH), 7.67 (1H, dd, J = 8.8, 6.8 Hz, ArH), 7.49 (1H, dd, J = 8.8, 5.9 Hz, ArH), 7.05 (1H, s, =C*H*−); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.2, 145.3, 138.6, 134.5, 132.7, 132.3, 132.0, 129.7, 129.1, 127.3, 126.2, 125.0, 123.0, 122.3, 120.8, 115.4, 114.2, 111.8, 98.4; LRMS (EI) m/z 372.1 (M + H), 374.0 (M + 2 + H);

General Procedure for the Synthesis of 1-[(Alkylamino/piperido/morpholino/pyrrolidino)ethyl/propylamino]-3-alkyl/arylpyrido[1,2-*a*]benzimidazolecarbonitriles (C). *Method 1.* The appropriate amine (2.69 mmol) was added to a stirred mixture of the 1-chloro-3-alkyl/arylpyrido[1,2-*a*]benzimidazole-4-carbonitrile (1.345 mmol) and triethylamine (0.27 g, 0.37 mL, 2.69 mmol) in THF or DMF (10 mL). The mixture was heated at 80−90 °C for 18 h, filtered hot, and allowed to cool. The solvent was removed in vacuo, and the residue was washed with minimum amounts of ice-cold ethanol. The resulting solid was recrystallized from acetone or ethanol.

Method 2. Microwave irradiation (150 W) was substituted for external heating, reducing the reaction time to approximately 20 min. Workup followed the same protocol as method 1.

1-(2-Diethylaminoethylamino)-3-[4-(trifluoromethyl)phenyl]-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (4c). Yellow powder, mp 219−220 °C (ethanol); purity 98% by LC (t_R = 5.82 min); IR (KBr) 3333 bm, 2971 m, 2841 w, 2210 s (CN), 1624 s, 1595 s, 1552 s, 1458 m, 1371 m, 1371 m, 1324 s, 1165 m, 1129 s, 1067 s, 1014 m cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.13 (1H, d, J = 7.8 Hz, ArH), 8.05 (1H, d, J = 7.8 Hz, ArH), 7.83 (2H, d, J = 7.8 Hz, ArH), 7.77 (2H, d, J = 8.8 Hz, ArH), 7.59 (1H, t, J = 7.8 Hz, ArH), 7.44 (1H, br s, NH), 7.36 (1H, dd, J = 7.8, 6.8 Hz, ArH), 5.90 (1H, s, =C*H*−), 3.49 (2H, t, J = 5.9 Hz, $CH_2CH_2N(Et)_2$), 2.97 (2H, t, J = 5.9 Hz, $CH_2CH_2N(Et)_2$), 2.73 (4H, q, J = 7.8 Hz, $N(CH_2CH_3)_2$), 1.16 (6H, t, J = 7.8 Hz, $N(CH_2CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.5, 149.0, 147.9, 145.7, 141.0, 129.0, 128.0, 126.0, 125.7, 121.0, 120.1, 116.7, 112.8, 89.3, 50.1, 46.0, 39.9, 11.7; LRMS (EI) m/z 452.2 (M + H).

1-(2-Ethylaminoethylamino)-3-[4-(trifluoromethyl)phenyl]-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (4h). Yellow fluffy powder, mp 222−224 °C (dec). Purity >99% by LC (t_R = 5.94 min). ^1H NMR (400 MHz, $CDCl_3$) δ: 8.57 (1H, d, J = 8.4 Hz, ArH), 7.94 (4H, m, ArH), 7.79 (1H, d, J = 8.0 Hz, ArH), 7.52 (1H, m, ArH), 7.33 (1H, m, ArH), 6.18 (1H, s, =C*H*-), 3.11 (2H, t, J = 6.2 Hz, -$NHCH_2C$−), 2.83 (2H, q, J = 7.2 Hz, -$NHCH_2CH_3$), 2.48 (2H, m, -$NHCH_2C$−), 1.14 (3H, t, J = 7.2 Hz, −CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 150.5, 149.1, 147.9, 145.8, 141.1, 129.0, 128.1, 126.1, 125.8, 121.0, 120.3, 116.7, 112.9, 89.3, 50.1, 46.1, 40.0, 11.8. m/z (EI, positive ion) 423.5 (M^+), 417, 352, 333, 274, 237, 210, 162, 88.

General Procedure for the Synthesis of 1-(Alkylamino/piperido/morpholino/pyrrolidino)ethyl/propylamino)-3-alkyl/arylpyrido[1,2-*a*]benzimidazolecarbonitrile Bis-hydrochloride Salts. HCl in methanol (1.25 M, 0.57 mL, 1.32 mmol) was added to a stirred mixture of the pyrido[1,2-*a*]benzimidazole-carbonitrile derivative (0.354 mmol) in methanol (20 mL). After the mixture was stirred at room temperature for 2.5 h, the solvent was removed in vacuo and the residue washed with minimum amounts of ice-cold methanol followed by DCM (4 × 3 mL), dried in vacuo, and used without further purification.

1-(2-Diethylaminoethylamino)-3-[4-(trifluoromethyl)phenyl]-pyrido[1,2-*a*]benzimidazole-4-carbonitrile Bis-hydrochloride Salt (4ca). Pale yellow, mp 161−162 °C; ^1H NMR (300 MHz, DMSO) δ 11.07 (1H, broad s, NH), 8.88 (1H, d, J = 8.4 Hz, ArH), 8.00 (4H, q, J = 8.4 Hz, ArH), 7.87 (1H, d, J = 7.5 Hz, ArH), 7.68 (1H, d, J = 7.7 Hz, ArH), 7.48 (1H, ddd, J = 1.2, 7.6, 8.4, ArH), 6.74 (1H, s, =C*H*−), 4.14 (2H, t, J = 6.6 Hz), 3.47 (2H, td, J = 5.1, 6.0 Hz), 3.23 (2H, quintet, J = 4.8, 7.2 Hz), 1.25 (6H, t, J = 7.2 Hz). ^{13}C NMR (100 MHz, DMSO): δ 151.2, 148.4, 147.6, 130.0, 127.1, 125.5, 121.7, 116.2, 93.4, 48.9, 46.4, 8.1 (CH_3). LRMS (APCI): m/z 452 (M^+ + 1 − HCl).

1-(2-Ethylaminoethylamino)-3-[4-(trifluoromethyl)phenyl]-pyrido[1,2-*a*]benzimidazole-4-carbonitrile Bis-hydrochloride Salt (4ha). Pale yellow solid, mp 181−183 °C; purity 97.8% by LC (t_R = 5.94 min); ^1H NMR (300 MHz, DMSO) δ 9.36 (2H, broad s), 8.85 (1H, d, J = 8.4 Hz, ArH), 8.00 (5H, m, ArH), 7.88 (1H, d, J = 8.1 Hz, ArH), 7.68 (1H, t, J = 7.5 Hz, ArH), 7.48 (1H, t, J = 7.5 Hz, ArH), 6.70 (1H, broad s, =C*H*−), 4.08 (2H, m, −$NHCH_2C$−), 3.31 (2H, m, −$NHCH_2CH_3$), 3.02 (2H, m, −$NHCH_2C$−), 1.25 (3H, m, −CH_3); ^{13}C NMR (100 MHz, DMSO) δ 151.0, 148.5, 147.6, 140.3, 129.7, 127.1, 126.8, 125.4, 121.4, 116.5, 116.0, 92.8, 44.3, 42.0, 10.7.

■ ASSOCIATED CONTENT

Supporting Information. Additional details of the characterization of selected compounds and the procedures used for the in vitro and in vivo antimalarial studies and cytotoxicity assays. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +27 21 650 2553. Fax: +27 21 650 5195. E-mail: Kelly.Chibale@uct.ac.za.

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■ ABBREVIATIONS USED

ip, intraperitoneal; po, oral administration; HPLC, high pressure liquid chromatography; HPMC, hydroxypropyl methylcellulose;

MSD, mean survival time; MTS, medium throughput screen; SAR, structure−activity relationship; TDR, tropical diseases research; TLC, thin layer chromatography; TMS, tetramethylsilane; WHO, World Health Organization

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