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Imidazolidin-4-one Derivatives of Primaquine as Novel Transmission-Blocking Antimalarials

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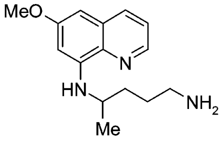
Imidazolidin-4-one derivatives of primaquine were synthesized as potential double prodrugs of the parent drug. The title compounds inhibit the development of the sporogonic cycle of *Plasmodium berghei*, affecting the appearance of oocysts in the midguts of the mosquitoes. The imidazolidin-4-ones are very stable, both in human plasma and in pH 7.4 buffer, indicating that they are active per se. Thus, imidazolidin-4-ones derived from 8-aminoquinolines represent a new entry in antimalarial structure−activity relationships.

Introduction

Malaria is the major life-threatening parasitic disease in tropical and subtropical regions. Worldwide, there are at least 300 million acute cases of malaria and more than 1−2 million deaths each year, mostly young children infected with *Plasmodium falciparum*.[1] Most of the drugs actually used in antimalarial chemotherapy are particularly active against the asexual blood forms of the parasite, which are responsible for the clinical symptoms of the disease. With the rapid spread of drug-resistant *P. falciparum* strains, the development of safe and effective antimalarials that prevent transmission, in addition to curing patients, has become an important strategy toward achieving an effective control of malaria.[2]

In contrast to the asexual blood forms of *Plasmodium*, the sexual form of the parasite is a much unexplored life-cycle target. Currently, primaquine, **1**, is the only available transmission-blocking antimalarial displaying a marked activity against gametocytes from all species of parasite causing human malaria, including chloroquine-resistant *P. falciparum*.[3] However, the use of primaquine is limited by its extensive conversion to carboxyprimaquine, **2**, and by its toxic effects, among them hemolytic anemia, particularly in patients who are deficient in glucose-6-phosphate dehydrogenase.[4−6]



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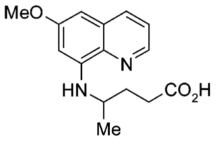

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Several peptide and amino acid derivatives of primaquine and other 8-aminoquinoline antimalarials have been synthesized to reduce the metabolic oxidative deamination pathway, as well as to reduce toxicity of the parent drug.[7−10] Such derivatives display improved activity/toxicity ratios when compared to primaquine, which can be ascribed either to a reduction in metabolic inactivation[8,9] or to a selective hydrolysis inside the parasite,[7] leading to a higher intracellular drug concentration. However, we, and others, have shown that amino acid and peptide derivatives of primaquine are rapidly hydrolyzed to primaquine by aminopeptidases and endopeptidases,[9,11] suggesting that they might undergo extensive hydrolysis to the parent drug in the GI tract when given orally. One approach to enhance the enzymatic stability of amino acid or peptide derivatives of primaquine toward proteolytic degradation at the mucosal absorption barrier or in the blood is the development of a double prodrug.[12] To this end, imidazolidin-4-one formation was introduced as a useful prodrug approach to protect the *N*-terminal amino acid residue of di- to pentapeptides against aminopeptidase-catalyzed hydrolysis.[13−16] Usually, peptide imidazolidin-4-one derivatives are quantitatively hydrolyzed to the parent peptide in pH 7.4 buffer at 37 °C with half-lives ranging from 1 to 30 h, depending on the *N*-terminal dipeptide sequence and on the imidazolidinone substituents.[13−16] Therefore, we reasoned that imidazolidin-4-one derivatives, **4**, of primaquine (see Scheme 1) would release the corresponding amino acid derivative via a nonenzymatic reaction, which, in turn, could be enzymatically hydrolyzed to primaquine. In this study, we report the reactivity in human plasma and gametocytocidal activity of imidazolidin-4-ones, **4**.

Results and Discussion

Chemistry. The synthesis and characterization of imidazolidin-4-ones **4** has been described elsewhere.[17] In short, compounds **4** can be synthesized in good yields from the corresponding amino acid derivatives AA-PQ,

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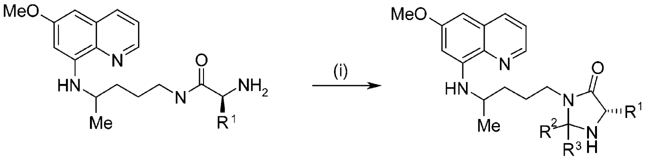
Scheme 1[a]



3 **4**

[a] (i) Me_2CO or C_5, C_6, or C_7 cyclic ketones in refluxing MeOH, TEA, molecular sieves.

Table 1. Percentage of Hydrolysis of Imidazolidin-4-ones **4** to the Corresponding Amino Acid Products **3**, in 80% Human Plasma and in pH 7.4 Phosphate Buffer at 37 °C after 3 Days of Incubation, with Half-Lives for the Hydrolysis in pH 7.4 Buffer in Parentheses

				% hydrolysis to **3** after 3 days	
compd	R^1	R^2	R^3	80% human plasma	pH 7.4 buffer ($t_{1/2}$/d)
4a	H	$(CH_2)_5$		stable[a]	11 (18)[d]
4b	H	Me	Me	16[b]	19 (9.8)[c]
4c	Me	$(CH_2)_5$		9[b]	16 (12)[c]
4d	CH_2Ph	$(CH_2)_6$		stable[a]	10 (20)[d]
4e	CH_2Ph	$(CH_2)_5$		stable[a]	17 (11)[d]
4f	CH_2Ph	$(CH_2)_4$		3[b]	28 (6.4)[c]
4g	CH_2Ph	Me	Me	stable[a]	6 (31)[c]
4h	$CHMe_2$	$(CH_2)_6$		nd[e]	nd[e]
4i	$CHMe_2$	$(CH_2)_5$		stable[a]	8 (26)[c]
4j	$CHMe_2$	$(CH_2)_4$		5[b]	21 (8.8)[c]
4k	$CHMe_2$	Me	Me	stable[a]	16 (12)[c]
4l	CH_2CHMe_2	Me	Me	stable[a]	10 (20)[d]

[a] No degradation after 3 days of incubation. [b] Percentage of hydrolysis after 3 days of incubation. [c] From ref 17. [d] This study. [e] Not determined due to solubility problems.

3, by refluxing with an excess of the appropriate ketone in methanol in the presence of triethylamine (TEA) and 4 Å molecular sieves.

In Vitro Stability Studies. The hydrolyses of imidazolidin-4-ones **4** in 80% human plasma were monitored by HPLC for the simultaneous disappearance of substrate and formation of the amino acid derivative **3** and primaquine. With the exception of compound **4b**, imidazolidin-4-ones **4** display unusually high stability when incubated in 80% human plasma (Table 1), with no significant disappearance of the starting material over 3 days of incubation. The stability of **4** in human plasma is not significantly affected either by the nature of the amino acid R^1 substituent or by the R^2 and R^3 substituents in the imidazolidin-4-one moiety. This contrasts with the behavior of the corresponding amino acid intermediates, **3**, which are hydrolyzed quantitatively to primaquine with rates depending on the nature of the amino acid side chain (Figure 1: **4c** versus Ala-PQ). Thus, while simple amino acid derivatives behave as prodrugs of primaquine, the corresponding imidazolidin-4-ones **4** are too stable to be considered prodrugs.

In contrast to their behavior in plasma, the imidazolidin-4-ones **4** hydrolyze to the corresponding amino acid derivatives in pH 7.4 buffer with half-lives ranging from 9 to 30 days (Table 1). Moreover, compounds **4** are hydrolyzed 50−100 times slower than the imidazolidin-4-one counterparts derived from dipeptides or pentapeptides.[13−16] We were surprised by such large differences in reactivity, though one possible explanation might lie in the mechanism of hydrolysis. The pH-independent hydrolysis of imidazolidin-4-ones (i.e. at pH > 4) occurs by an S_N1-type mechanism which involves the departure of an amide leaving group (Scheme 2).[13] The amides resulting from the rate-limiting ring opening of **4** are much poorer leaving groups than those from dipeptide imidazolidin-4-ones (the difference in pK_a is about 3.3 units[18]). Assuming that the pH-independent hydrolysis of imidazolidin-4-ones has the same susceptibility to the leaving group effect as the analogous acyclic *N*-Mannich bases,[19] i.e. with a Brönsted β_{lg} value of ca. −1, then it would be expected that compounds **4** would hydrolyze ca. 10^3 times slower[20] than their counterparts derived from dipeptides. The smaller differences reported herein might be attributed to the fact that the amino acid chain affects both the amide leaving group ability and the ability of the imidazolidin-4-one N^1 amino nitrogen atom to expel the amide. A remark should be made on the higher stability of **4** in human plasma when compared to pH 7.4 phosphate buffer. This might be ascribed to the binding to plasma proteins. A decrease in reactivity in human plasma when compared to pH 7.4 buffers has also been reported for imidazolidin-4-ones derived from dipeptides and pentapetides.[14,15]

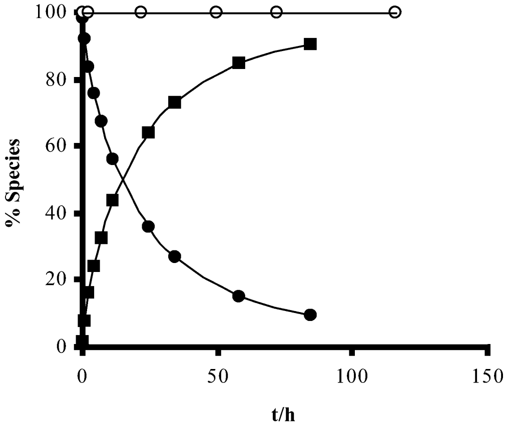


Figure 1. Time courses for compounds **4c** (○), Ala-PQ (●), and primaquine (■) when **4c** (open symbols) and Ala-PQ (closed symbols) were incubated in 80% human plasma at 37 °C.

In Vivo Gametocytocidal Studies. The potential of compounds **4** to prevent the transmission of malaria was studied using a model consisting of BalbC mice infected with *Plasmodium berghei* and *Anopheles stephensi* mosquitoes. The two criteria used to assess the antimalarial activity of each compound were (i) the percentage of mosquitoes with oocysts and (ii) the mean number of oocysts per infected mosquito. These in vivo screening assays are of major support in analyzing the effect of drugs in the sporogonic cycle, since no equivalent in vitro assays exist.[21] Although this model cannot specifically attribute the drug effect to either gametocytocidal activity (i.e. affects parasite development by killing gametocytes) or sporontocidal activity (i.e. affects directly the development of oocysts on the stomach wall of the mosquitoes), it can clearly show if a compound is effective at interrupting the transmission of the infection to mosquitoes by interference with the cycle in these insects.[22,23]

The antimalarial activity data are presented in Table 2, and from these the following observations can be made. First, primaquine (PQ) and the imidazolidin-4-one derivatives **4a−g** (i.e. those derived from Gly-PQ, Ala-PQ, and Phe-PQ) completely inhibited the produc-

Scheme 2

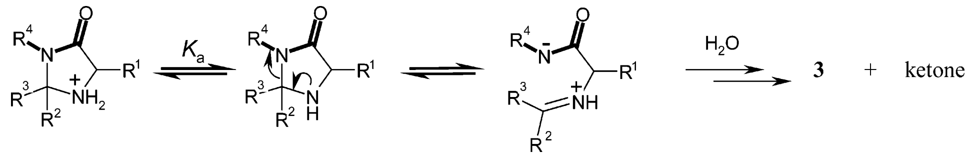


Table 2. Effect of Compounds **4** and Primaquine on the Sporogonic Development of *Plasmodium berghei* ANKA in *Anopheles stephensi* Mosquitoes

compd	dose/ μmol· kg^{-1}	% infected mosquitoes[a]	mean no. of oocysts/ mosquito (±SE)	compd	dose/ μmol·kg^{-1}	% infected mosquitoes[a]	mean no. of oocysts/ mosquito (±SE)
4a	0	65	105 (22)	**4h**	0	100	120 (8.0)
	10	25	7.4 (9.3)		10	65	17.7 (6.5)
	50	0	0		50		
4b	0	65	105 (22)	**4i**	0	100	51.0 (9.1)
	10	21	6.1 (3.5)		10	85	61.1 (6.7)* [b]
	50	0	0		50	25	0.4
4c	0	93	83.2 (7.1)	**4j**	0	100	51.0 (9.1)
	10	20	0.8 (1.1)		10	35	4.2 (3.3)
	50	0	0		50	15	0.5 (0.2)
4d	0	100	120 (8.0)	**4k**	0	65	105 (22)
	10	90	78.9 (6.6)*		10	25	5.0 (2.5)
	50	0	0		50	5	0.9 (0.5)
4e	0	93	83.2 (7.1)	**4l**	0	100	120 (8.0)
	10	83	71.0 (6.5)*		10	30	13.7 (10.9)
	50	0	0		50	20	1.0 (0.9)
4f	0	80	66.1 (9.1)	primaquine	0	93	83.2 (7.1)
	10	65	51.6 (8.8)*		10	28	2.6 (2.0)
	50	0	0		50	0	0
4g	0	80	66.1 (9.1)				
	10	76	61.2 (7.5)*				
	50	0	0				

[a] Counting of oocysts was carried out at day 10 post-feed. [b] (*) $P > 0.05$ versus control, by Student's *t* test.

tion of oocysts at a dose of 50 μmol/kg. Second, imidazolidin-4-ones derived from Val-PQ and Leu-PQ, **4i**–**l**, although significantly ($P < 0.05$) affecting the sporogonic development of *P. berghei*, did not completely inhibit the production of oocysts at 50 μmol/kg. Third, the mean number of oocysts was also significantly affected by compounds **4a**–**c** and **4j**–**l** at a dose of 10 μmol/kg. Primaquine was similarly effective at this dose level. In contrast, at a dose of 10 μmol/kg, derivatives **4d**–**g** (derived from Phe-PQ) and **4h**,**i** (derived from Val-PQ) did not significantly ($P > 0.05$) reduce the oocyst production when compared with the control.

From these results it can be concluded that the imidazolidin-4-ones derived from Gly-PQ, **4a**,**b**, and Ala-PQ, **4c**, are the most effective gametocytocidal agents, displaying an antimalarial activity comparable to that of primaquine. The activity of **4a** and **4b** compares to the activity reported previously for Gly-PQ, thus suggesting that incorporation of the imidazolidin-4-one scaffold does not alter substantially the antimalarial activity. In contrast, imidazolidin-4-ones **4** derived from the more lipophilic amino acids phenylalanine, valine, and leucine were less active when compared to primaquine. A similar observation has been reported for dipeptide derivatives of primaquine, AA-Gly-PQ,[9] for which it was found that the most active compound was AA = Gly, while those containing the bulky and lipophilic amino acids (*R*)-Phe and (*S*)-Phe were less active. More recently, Vangapandu et al. reported that the attachment of a hydrophobic amino acid to the terminal amino group of primaquine analogues results in decreased blood-schizontocidal antimalarial activity.[10] Taken together, these suggest that hydrophobic amino acid side chains have a detrimental effect on the activity of 8-aminoquinoline derivatives against both blood schizonts and gametocytes.

The effect of the substituents at the C-2 position of imidazolidin-4-ones **4** on the gametocytocidal activity is less clear. For example, there is no difference in activity between the imidazolidin-4-ones prepared from Phe-PQ and cycloheptanone, **4d**, cyclohexanone, **4e**, cyclopentanone, **4f**, and acetone, **4g**, at 10 or 50 μmol/kg. Similarly, no difference in activity is observed between the Gly-PQ derivatives **4a** and **4b**. In contrast, the cycloheptanone, cyclopentanone, and acetone derivatives of Val-PQ (**4h**, **4j**, and **4k**, respectively) are active at 10 μmol/kg, while their cyclohexanone counterpart **4i** is inactive at this dose level. This warrants further studies to disclose the effect of the imidazolidin-4-one C-2 substituents on antimalarial activity.

The imidazolidin-4-one N^1 nitrogen atom of **4** is substantially less basic (pK_a ca. 3)[13,24] than that of primaquine (pK_a 10) or its amino acid or peptide derivatives (pK_a 8–8.5). Consequently, a major finding that emerges from this study is that the presence of a terminal, strongly basic amino group, as found in primaquine or its amino acid and peptide derivatives, is not a necessary requirement for gametocytocidal activity.

Conclusion

The reported imidazolidin-4-ones prepared from amino acid derivatives of primaquine exhibit potent gametocytocidal activity against *P. berghei*. In general, those derivatives **4** containing small amino acid chains (Gly and (*S*)-Ala) are superior to those containing bulky/hydrophobic side chains ((*S*)-Phe, (*S*)-Val, and (*S*)-Leu).

These imidazolidin-4-ones **4** are very stable both in chemical and in enzymatic conditions, suggesting that they are active per se. Thus, the imidazolidin-4-ones **4** can be considered as a novel type of 8-aminoquinoline antimalarial. Recent reports indicate that adequate substitution at the C-2, C-4, and C-5 positions of the quinoline moiety can lead to potent 8-aminoquinoline blood-schizontocidal antimalarials devoid of significant blood toxicity. Therefore, combination of the imidazolidin-4-one scaffold with the appropriately substituted quinoline moiety deserves further attention.

Experimental Section

HPLC Analysis. High-performance liquid chromatography (HPLC) measurements were carried out using a Waters assembly equipped with a model 600 controlled pump and a model 991 photodiode-array detector. A Rheodyne 7725 injection valve equipped with 20-μL sample loop was used. Acquisition and treatment of data were made by means of NEC for MS-DOS, version 3.30 software. The separation was performed on a Purospher, 250 × 4.0-mm i.d. 5 μm (Merck, Germany) analytical column. A LiChrospher 100 RP-8 5 μm (Merck, Germany) was employed as precolumn. The solvent system used was a gradient of sodium acetate buffer (pH 4.75; 0.05 M) (A) and acetonitrile (B); 10^{-3} M triethylamine was added to the aqueous mobile phase in order to improve peak shape. The gradient was as follows: 0 min, 50% B; 4.5 min, 50% B; 5.0 min, 10% B; 20 min, 10% B. For the imidazolidin-4-one derivatives of valine a second gradient was developed: 0 min, 40% B; 5.5 min, 40% B; 6.0 min, 10% B; 20 min, 10% B. Elution was performed at a solvent flow rate of 1 mL/min, and a 15 mL/min nitrogen sparging was applied to remove dissolved gases. Chromatographic separation was monitored by UV detection at 265 nm. All analyses were performed at room temperature.

Hydrolysis in Human Plasma. The compounds **4** were incubated at 37 °C in human plasma (from heparinized blood of healthy donors) diluted to 80% (v/v) with pH 7.4 isotonic phosphate buffer. At appropriate intervals, aliquots were added to acetonitrile to quench the reaction and precipitate plasma proteins. These samples were centrifuged and the supernatant was analyzed by the HPLC method described above for the presence of substrate and products.

Hydrolysis in Aqueous Solution. The rates of hydrolyses of compounds **4** were determined in pH 7.4 phosphate saline buffer, at 37 °C. Usually, a 10 μL aliquot of a 10^{-2} M stock solution of substrate in acetonitrile was added to 10 mL of the appropriate thermostated buffer solution. At regular intervals, samples of the reaction mixture were analyzed by HPLC. All reactions followed first-order kinetics over four half-lives.

In Vivo Gametocytocidal Activity. BalbC mice were infected by intraperitoneal inoculations of 10^7 erythrocytes parasitized with *P. berghei* ANKA. After 4 days, when the presence of gametocytes and exflagellation was observed by microscopic observation of Giemsa stained blood films, mice were randomly separated into five different groups of six animals. Each group was treated by intraperitoneal administration with one single dose of each compound **4** and primaquine (10 and 50 μmol/kg in inoculation volumes of 0.1−0.2 mL; controls consisted of mice given a PBS solution). Two hours after administration, mice were anesthetized and placed on top of individual cages containing ca. 50 glucose-starved *Anopheles stephensi* female mosquitoes, which were allowed to feed for 2 h. After the blood meal, unfed females mosquitoes were removed from each cage. Ten days after the blood meal, 10 mosquitoes of each cage were randomly collected and dissected for microscopic detection of oocysts in midguts. For further details see ref 9.

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Supporting Information Available: HPLC chromatograms of incubation mixtures in 80% human plasma and in pH 7.4 buffer for compounds **4f** and **4j**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) *WHO Expert Committee on Malaria: Twentieth Report*; WHO Technical Report Series, Vol. 892, World Health Organization: Geneva, 2000.

(2) Beales P. F. The use of drugs for malaria control. In *Malaria, Principles and Practice of Malariology*; Wernsdofer, W. H., McGregor, I., Eds.; Churchill Livingstone: New York, 1988; pp 1263−1285.

(3) Rieckmann, K. H.; McNamara, J. V.; Frischer, H.; Stockert, T. A.; Carson, P. E.; Powel, R. D. Gametocytocidal and sporontocidal effects of primaquine and of sulfadiazine with pyrimethamine in a chloroquine-resistant strain of *Plasmodium falciparum*. *Bull. W.H.O.* **1968**, *38*, 625−632.

(4) Brueckner, R. P.; Ohrt, C.; Baird, J. K.; Milhous, W. K. 8-Aminoquinolines. In *Antimalarial Chemotherapy*; Rosenthal, P. J., Ed.; Humana Press: Totowa, 2001; pp 123−151.

(5) Mihaly, G. W.; Ward, S. A.; Edwards, G.; Orme, M. L'E.; Breckenridge, A. M. Pharmacokinetics of primaquine: identification of the carboxylic acid derivative as a major plasma metabolite. *Br. J. Clin. Pharmacol.* **1984**, *17*, 441−446.

(6) Constantino, L.; Paixão, P.; Moreira, R.; Portela, M. J.; Rosário, V. E.; Iley, J. Metabolism of primaquine by liver homogenate fractions. Evidence for monoamine oxidase and cytochrome P450 involvement in the oxidative deamination of primaquine to carboxyprimaquine. *Exp. Toxicol. Pathol*. **1999**, *51*, 299−303.

(7) Trouet, A.; Pirson, P.; Steiger, R.; Masquelier, M.; Baurain, R.; Gillet, J. Development of new derivatives of primaquine by association with lysosomotropic carriers. *Bull. W.H.O.* **1981**, *59*, 449−458.

(8) Philip, A.; Kepler, J. A.; Johnson, B. H.; Carroll, F. Y. Peptide derivatives of primaquine as potential antimalarial agents. *J. Med. Chem*. **1988**, *31*, 870−874.

(9) Portela, M. J.; Moreira, R.; Valente, E.; Constantino, L.; Iley, J.; Pinto, J.; Rosa, R.; Cravo, P.; do Rosário, V. E. Dipeptide derivatives of primaquine as transmission-blocking antimalarials. Effect of aliphatic side-chain acylation on the gametocytocidal activity and on the formation of carboxyprimaquine in rat liver homogenates. *Pharm. Res.* **1999**, *16*, 949−955.

(10) Vangapandu, S.; Sachdeva, S.; Jain, R.; Jain, M.; Singh, S.; Singh, P. P.; Kaul, C. L.; Jain, R. 8-Quinolinamines conjugated with amino acids are exhibiting potent blood-schizontocidal antimalarial activities. *Bioorg. Med. Chem.* **2004**, *12*, 239−247.

(11) Borissova, R.; Stjarnkvist, P.; Karlsson, M. O.; Sjoholm, I. Biodegradable microspheres. 17. Lysosomal degradation of primaquine-peptide spacer arms. *J. Pharm. Sci*. **1995**, *84*, 256−262.

(12) Bundgaard, H. Prodrugs as a means to improve the delivery of peptide drugs. 1. *Adv. Drug. Del. Rev.* **1992**, *8*, 1−38.

(13) Klixbull, U.; Bundgaard, H. Prodrugs as drug delivery systems. 30. 4-Imidazolidinones as potential bioreversible derivatives for the alpha-aminoamide moiety in peptides. *Int. J. Pharm.* **1984**, *20*, 273−284.

(14) Rasmussen, G. J.; Bundgaard, H. Prodrugs of peptides. 10. Protection of dipeptides and tripeptides against aminopeptidase by formation of bioreversible 4-imidazolidinone derivatives. *Int. J. Pharm.* **1991**, *71*, 45−53.

(15) Rasmussen, G. J.; Bundgaard, H. Prodrugs of peptides. 15. 4-Imidazolidinone prodrug derivatives of enkephalins to prevent aminopeptidase-catalyzed metabolism in plasma and absorptive mucosae. *Int. J. Pharm.* **1991**, *76*, 113−122.

(16) Bak, A.; Fich, M.; Larsen, B. D.; Frokjaer, S.; Friis, G. J. N-Terminal 4-imidazolidinone prodrugs of Leu-enkephalin: synthesis, chemical and enzymatic stability studies. *Eur. J. Pharm. Sci*. **1999**, *7*, 317−323.

(17) Gomes, P.; Araújo, M. J.; Rodrigues, M.; Vale, N.; Azevedo, Z.; Iley, J.; Chambel, P.; Morais, J.; Moreira, R. Synthesis of imidazolidin-4-one and 1*H*-imidazo[2,1-*a*]isoindole-2,5(3*H*,9b*H*)-dione derivatives of primaquine: scope and limitations. *Tetrahedron* **2004**, *60*, 5551−5562.

(18) The pK_a values for the dissociation of amides R^1CONHR^2 can be calculated using the equation $pK_a = 22 - 3.1\Sigma\sigma^*$ (Perrin, D. D.; Dempsey, B.; Serjeant, E. P. *pK_a Prediction for Organic Acids and Bases*; Chapman and Hall: London, 1981). The difference between the amide derived from primaquine and those from peptides is in the R^2 group. For simplicity, we considered the R^2 group for primaquine to be butyl (σ^* −0.23) and for the peptides to be $MeNHCOCH_2$ (for which σ^* can be estimated to be 0.84); thus, the difference in pK_a is $3.1(0.84 - (-0.23)) \approx 3.3$.

(19) Bundgaard, H.; Johansen, M. Hydrolysis of N-Mannich bases and its consequences for the biological testing of such agents. *Int. J. Pharm.* **1981**, *9*, 7−16.

(20) Assuming that the equation log $k = -\mathrm{p}K_\mathrm{a} + C$ holds for compounds **4**, then $\log(k_1/k_2) = \mathrm{p}K_\mathrm{a}^2 - \mathrm{p}K_\mathrm{a}^1$.

(21) Coleman, R. E.; Vaughan, J. A.; Hayes, D. O.; Hollingdale, M. R.; Do Rosário, V. E. Effect of mefloquine and artemisinin on the sporogonic cycle *of Plasmodium berghei* ANKA in *Anopheles stephensi* mosquitoes. *Acta Leiden.* **1988**, *57*, 61−74.

(22) Coleman, R. E.; Clavin, A. M.; Milhous, W. K. Gametocytocidal and sporontocidal activity of antimalarials against *Plasmodium berghei* ANKA in ICR mice and *Anopheles stephensi* mosquitoes. *Am. J. Trop. Med. Hyg.* **1992**, *46*, 169−182.

(23) Coleman, R. E.; Nath, A. K.; Schneider, I.; Song, G.-H.; Klein T. A.; Milhous, W. K. Prevention of sporogony of *Plasmodium falciparum* and *P. berghei* in *Anopheles stephensi* mosquitoes by transmission-blocking antimalarials. *Am. J. Trop. Med. Hyg.* **1994**, *50*, 646−653.

(24) For example, the pK_a value for the derivative **4f**, kinetically determined from a pH−rate profile, is 3.5 (data not shown).

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