Primaquine dipeptide derivatives bearing an imidazolidin-4-one moiety at the N-terminus as potential antimalarial prodrugs

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Primaquine dipeptide derivatives bearing an imidazolidin-4-one moiety at the N-terminus were synthesized and evaluated as potential transmission-blocking antimalarial prodrugs. All compounds were hydrolyzed to the parent dipeptide derivative of primaquine in neutral and basic solutions, with half-lives ranging from 0.7 to 31 h at 37 °C, depending on the nature of the substituents present in the imidazolidin-4-one moiety and in the C-terminal amino acid directly coupled to primaquine. The antimalarial activity was studied for selected compounds using a model consisting of Plasmodium berghei, BalbC mice and Anopheles stephensi mosquitoes. The imidazolidin-4-one–derivative from Ala-Ala-primaquine and acetone reduced the transmission of the infection to mosquitoes more efficiently than primaquine as shown by the significant decrease in the number of oocysts in the midguts of the mosquitoes at 10 and 50 μmol/kg when compared to the control.

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leaving group and formation of an iminium ion at N-imidazolidin-4-one derivatives of primaquine, evaluation of the chemical reactivity and antimalarial activity of novel dipeptide backbone, e.g. with half lives adequate for oral administration, we decided to (Scheme 2). We now report the synthesis, evaluation of the chemical reactivity and antimalarial activity of novel imidazolidin-4-one derivatives of primaquine, 5 (Table 1).

2. Chemistry

The synthesis of imidazolidin-4-one prodrugs 5 is illustrated in Scheme 2. Briefly, primaquine reacted with the appropriate N$_2$-Boc-protected amino acid (BocAAOH) using HOBr/DIPCI or HOBr/DCCI methods. The corresponding N$_2$-Boc-protected primaquine amino acid amides (6a–d) were isolated, Boc removed with neat trifluoroacetic acid (TFA) and the resulting trifluoroacetates were converted into the corresponding free bases, 7a–d, with sodium carbonate. Compounds 7 were reacted with the second N$_2$-Boc-protected amino acid (BocAAOH) in the presence of triethylamine (TEA) and HOBr/DIPCI or HOBr/DCCI, to yield the protected primaquine dipeptide amides 8a–4. These were converted to the deprotected products (9a–i) again with neat TFA followed by treatment with sodium carbonate. Finally, compounds 9 were deprotected into the corresponding imidazolidin-4-ones, 5a–s (Table 1), by reaction with the appropriate symmetric ketone.

The structure of imidazolidin-4-ones 5 follows from their spectroscopic data, which reveal the presence of two diastereomers resulting from using racemic primaquine as starting material. For example, the imidazolidin-4-one C-2 methyl proton signals of 5d and 5e clearly appear as two sets of two singlets with overall 1:1:1:1 integration. Similarly, the two methyl groups at C-2 are often visible as four signals in the $^{13}$C NMR spectra. In addition, the $^1$H NMR signal of the methylene protons on the glycine residue linked to primaquine in 5b–e, 5m–n and 5q–s reveals the diastereotopic nature of the two protons, which appear as an AB system at $\delta$ ca. 4 ppm, with $^3$J ca. 15 Hz. Similar observations were recently reported for the analogous peptidomimetic imidazolidin-4-ones 4 [27]. It should be outlined that identical spectroscopic behavior had already been found for the Boc-protected precursors 6, whose conversion into compounds 5 is carried out by standard N$_2$-Boc-amino acid acylolytic deprotection methods known to preserve the chiral purity of amino acids, peptides and other amino acid derivatives. In fact, it is well established that in what concerns chiral stability of their C$_2$, amino acids and their derivatives are fairly insensitive to acids [28,29].

Yields for the cyclization step range from reasonable to good and seem to be dependent on the nature of the R$_3$ substituent present in intermediates 9. For example, the cyclization yield is significantly lower when a bulky amino acid such as Leu or Phe is present as the C-terminal residue (i.e. R$^3$) (e.g. 5h versus 5d and 5i versus 5n). This effect might reflect the steric requirements for the cyclization of imine, 10 (Scheme 3), formed from the reaction of the amino group in 9 with the appropriate ketone [23,30,31]. It can be seen that conformation A (Scheme 3) of the imine 10 presents some repulsion between the R$_1$ group and the R$_2$ and R$_3$ substituents, thus favouring reactions in which R$^3$ = H. Bulkier R$^3$ substituents might also favour cyclization of the imine, as they force the amide nitrogen atom to get in closer proximity to the imine carbon atom. For example, 5d (R$^1$ = H, R$^4$ = Bu') was formed in 75% yield, while 5a (R$^3$ = R$_2$ = H) was formed only in 41% yield. Similar observations can be made for 5f versus 5g and for 5o versus 5p.

3. Results and discussion

3.1. Kinetics and products of hydrolysis

As revealed by HPLC analysis, compounds 5 hydrolyze quantitatively at 37 °C to the corresponding dipeptide derivative 9 over the pH range 0.5–12. Products 9 were stable in these conditions and in the time-scale of the decomposition of 5. The rates of hydrolyses of imidazolidin-4-ones 5 at a fixed pH value were found to be independent of buffer concentration over a 10-fold buffer concentration range, indicating the absence of general acid or base catalysis (e.g. Table 2). The influence of pH on the rate of hydrolysis is shown in Fig. 1 for compounds 5a and 5s, where the logarithm of the observed pseudo-first-order rate constant, $k_{obs}$, is plotted against pH. The pH-rate profiles for compounds 5 have a sigmoid shape, with two pH-independent regions. Such sigmoid pH–rate profile have been reported for other imidazolidin-4-ones [19,32] as well as for their acyclic counterparts, N-Mannich bases [33], and can be accounted for by assuming that both the protonated, SH-, and the unionized, S, forms of the substrate undergo spontaneous hydrolysis (Scheme 4). The best computer fit (solid line) to the experimental data for 5a and 5s in Fig. 1 was achieved using Eq. (1):
\[ k_1 = k_{\text{neut}} \left( \frac{K_a}{K_a + [H^+]} \right) + k_{\text{prot}} \left( \frac{[H^+]}{K_a + [H^+]} \right) \]

where \( k_{\text{neut}} \) and \( k_{\text{prot}} \) are the apparent first-order rate constants for the decomposition of neutral and protonated forms of 5, \( K_a \) is the acid dissociation constant of the protonated 5, and \( [H^+] / ([K_a + [H^+]] \) and \( K_a([K_a + [H^+]] \) represent, respectively, the fraction of the protonated and neutral forms of 5 present in solution. The \( k_{\text{neut}} \) values derived either from the pH-rate profiles or determined at pH 8 are presented in Table 1. The kinetically determined \( pK_a \) values for 5a and 5s are 3.78 and 3.06, respectively, i.e. ca. 4 units lower than those of Gly-Gly and Phe-Gly (\( pK_a \) 8.2 and 7.6, respectively [34]). A similar observation has been reported for imidazolidin-4-ones derived from peptides [20].

3.2. Structural effects on chemical reactivity

The imidazolidin-4-one moiety. Inspection of the kinetic data presented in Table 1 reveals that the pH-independent pathway rate constant for the neutral produrg, \( k_{\text{neut}} \) (Scheme 4), has a small dependence on the size of the amino acid substituent \( R^4 \) at the imidazolidin-4-one C-5. For example, the order of half lives for the AA–Gly–PQ series derived from acetone is Gly-Gly–PQ, 5a > Ala-Gly–PQ, 5b > Phe-Gly–PQ, 5c with 5a being only ca. 10 times more reactive than 5c. The exception is compound 5d, derived from Leu-Gly–PQ and acetone, which is more reactive than expected based on the size of the Leu side chain, which might reflect the importance of polar effects on reactivity. The decrease of \( k_{\text{neut}} \) with the size of the amino acid substituent \( R^4 \) at the imidazolidin-4-one C-5 is also observable for the cyclohexanone series: Gly-Gly–PQ, 5i > Ala-Gly–PQ, 5m > Phe-Gly–PQ, 5n. These results are consistent with those reported for imidazolidin-4-ones derived on amino acid derivatives of primaquine and for imidazolidin-4-ones derived from dipeptides [20,26] and can be ascribed to unfavourable steric interactions between the amino acid substituent and the \( R^2 \) and \( R^3 \) substituents in the iminium ion (i.e. the protonated form of 10; Scheme 3).

The rate data presented in Table 1 show that the nature of the substituents at C-2 of the imidazolidin-4-one moiety, and thus the ketone starting material, also affects the reactivity of the neutral form of the imidazolidin-4-ones 5. The order of reactivity (\( k_{\text{neut}} \) for
the Phe-Gly–PQ imidazolidin-4-ones, according to the ketone starting material, is cycloheptanone > cyclopentanone > cyclohexanone > acetone, the cycloheptanone compound 5s being ca. 20 times more reactive than its acetone counterpart, 5e. A similar trend has been reported for imidazolidin-4-ones derived from Gly–PQ, 3 [26], and from enkephalins, 11.[21] A good correlation ($r^2 = 0.99$, Fig. 2) was observed between the log $k_{\text{neut}}$ values for compounds 5e, 5n, 5q, 5r and those of their counterparts 11. This strongly suggests that decomposition of 5 and 11 follow similar pathways and present the same structural requirements. The higher reactivity of the seven- and five-membered ring derivatives, 5s and 5r, when compared with the six-membered ring, 5n, can be ascribed to a decrease in internal strain resulting from a change in coordination number of the imidazolidin-4-one C-2 carbon atom from four to three in the five-member ring-opening process. A similar observation was reported for compounds 3. [26].

The C-terminal amino acid. The effect of z-carbon atom substituents ($R^1$) in the C-terminal amino acid on the reactivity depends on the nature of the $R^3$ substituent at imidazolidin-4-one C-5 position. For example, the order of $k_{\text{neut}}$ for the Gly–AA1–PQ series (i.e. $R^1 = H$) derived from cyclohexanone is Gly-Gly–PQ, 5s > Gly-Phe–PQ, 5f > Gly-Leu–PQ, 5k = Gly-Ala–PQ, 5j, with 5i being only ca. 4 times more reactive than 5j. This suggests that electron-donating $R^1$ substituents, although having only a minor influence on the stability, retard the decomposition of imidazolidin-4-ones 5 in neutral and basic conditions ($\sigma^+ \text{H} = 0.49$; $\sigma^+ \text{CH}_2\text{Ph} = 0.21$; $\sigma^\prime \text{Me} = 0$; $\sigma^\prime \text{Bu} = -0.12$). In contrast, compound 5o, derived from Ala-Ala–PQ and cyclohexanone, is ca. 7 times more reactive than its Ala-Gly–PQ counterpart, 5m. Similarly, compound 5f, derived from Ala-Ala–PQ and acetone, is ca. 15 times more reactive than its Ala-Gly–PQ counterpart, 5b. This is consistent with the finding that rate of decomposition of imidazolidin-4-ones derived from dipeptides is accelerated when sterically-hindered amino acids are present as C-terminal residues [20]. For example, the imidazolidin-4-one derived from Phe-Ala and acetone is ca. 37 times more reactive than its Phe-Gly counterpart [20].

3.3. Antimalarial activity

The potential of compounds 5a, 5b and 5f (i.e. half lives of 3, 16 and 1 h, respectively) to prevent the transmission of malaria was studied using a model consisting of BalbC mice infected with P. berghei and Anopheles stephensi mosquitoes and compared to that of primaquine. The antimalarial activity was assessed based on the percentage of mosquitoes with oocysts and the mean number of oocysts per infected mosquito (Table 3). Although this model cannot specifically attribute the drug effect to either gametocytocidal activity or sporontocidal activity, it can clearly show if compounds are effective at interrupting the transmission of the infection to mosquitoes by interference with the cycle in these insects [35,36]. Compound 5f was the most potent compound at both dose levels, reducing significantly ($P < 0.05$) the sporogonic development of P. berghei when compared with the control. Notably, 5f was more potent than primaquine, inhibiting almost completely the production of oocysts at 50 $\mu$mol/kg. Compound 5b was the least active in this screen. A dose of 10 $\mu$mol/kg of 5b did not significantly ($P > 0.05$) affect oocyst production when compared with the control. This lack of activity is unlikely to reflect the slow hydrolysis of 5b to the parent active dipeptide derivative, Ala-Gly–PQ (more than 99% of 5b is converted to the active at the time of oocyst counting), but might reflect the poor gametocytocidal activity of the parent dipeptide at lower doses [18]. The decomposition of compounds 5a, 5b and 5f was studied in plasma obtained from non-infected mice, diluted to 80% (v/v) with pH 7.4 isotonic phosphate buffer, at 37°C. These compounds decomposed to corresponding dipeptidyl derivatives 9 in plasma, with first-order rate constants identical to those for the $k_{\text{neut}}$ pathway (Table 1), indicating that plasma enzymes do not catalyse the reaction.

4. Conclusions

Primaquine dipeptide derivatives, 5, bearing an imidazolidin-4-one moiety at the N-terminus are readily hydrolyzed to the parent dipeptide derivatives of primaquine in neutral and basic solutions, with half lives ranging from 0.7 to 31 h at 37°C, depending on the substituents at the imidazolidin-4-one moiety as well as on the C-terminal amino acid directly coupled to primaquine. The most critical factor is the substituent at the imidazolidin-4-one C-2 position, i.e. those derived from the ketone starting material. Imidazolidin-4-ones derived from acetone are generally more stable than their counterparts derived from cyclohexanone or cyclopentanone, while the most reactive prodrug is the one derived from cycloheptanone. Importantly, decomposition is not catalyzed by plasma enzymes. Compounds 5 are capable of reducing the transmission of the infection to mosquitoes as efficiently as primaquine and the corresponding parent dipeptide derivatives. In conclusion, these results herein presented suggest that imidazolidin-4-ones 5 present a stability profile similar to that of imidazolidin-4-ones derived from di- and tripeptides, and thus may be a useful prodrug approach to protect the N-terminal amino acid residue against enzymatic cleavage by aminopeptidases.

5. Experimental protocols

5.1. General

Boc-amino acids were from Bachem, solvents were all of p.a. quality from Merck and were dried over pre-activated 3 Å molecular sieves prior to utilization. All remaining reactants were from...
Sigma–Aldrich. Silica-gel 60 plates (Merck F 254) were used for thin-layer chromatography. Nuclear magnetic resonance (NMR) spectra were acquired on a Bruker AMX 300 spectrometer from solutions of the compounds in deuterated chloroform (CDCl₃) having tetramethylsilane (TMS) as internal reference.¹H and ¹³C chemical shifts are given in parts per million (ppm) and proton–proton coupling constants in Hertz (Hz); ¹H NMR peak multiplicity is indicated by br s (broad singlet), s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (unresolved multiplet). Mass spectra (MS) were acquired by either the matrix-assisted laser desorption ionization – time-of-flight (MALDI-TOF) using either anthracene or 2,5-dihydroxybenzoic acid (DHB) as adjuvant matrices, or the electrospray ionization ion-trap (ESI-IT) techniques, on a Bruker Biflex II or a Finningan LCQ DECA XP MAX spectrometer, respectively.

5.2. Syntheses

5.2.1. Synthesis of compounds 8a–f

The appropriate PQAA¹ [23] (3.1 mmol) was suspended in DCM (30 mL), TEA (4 eq.) was added and the mixture was stirred in an ice-water bath for 30 min. After addition of the Boc₄OH (1.1 eq.) and HOBt (1.2 eq.), the carbodiimide (DCCI, 1.2 eq.) was slowly added to the mixture, which was kept at 0 °C for more 2 h. The reaction was allowed to proceed at room temperature for 2 d with periodic monitoring by TLC. A second stepwise addition of carbodiimide (1.2 eq.) was made, and the reaction prolonged for further 3 d. The solid phase formed was removed by suction filtration and identified as the carbodiimide-derived urea (DCU). The filtrate was evaporated to dryness and the residue dissolved in the minimum amount of warm acetone; the resulting solution was stored overnight at 4 °C and the urea precipitated was again removed by suction filtration. The filtrate was evaporated to dryness and the residue submitted to column chromatography on silica-gel, using DCM/acetone mixtures as eluents. Products were isolated as yellow-orange oils in 50–79% yields and successfully characterized by MS and NMR (see Supporting information).

5.2.2. Synthesis of compounds 9a–f

Compounds 8 were dissolved in a small volume of neat trifluoroacetic acid (TFA, ca. 5 mL) and the deprotection reactions allowed to proceed for 1–2 h at room temperature. Excess TFA was neutralized by dropwise addition of aqueous Na₂CO₃ at 30% until pH 9; the supernatant oily phase formed in this process was extracted six times with 10 mL portions of chloroform and the

Sigma–Aldrich. Silica-gel 60 plates (Merck F₂₅₄₅) were used for thin-layer chromatography. Nuclear magnetic resonance (NMR) spectra were acquired on a Bruker AMX 300 spectrometer from solutions of the compounds in deuterated chloroform (CDCl₃) having

### Table 1

Yields of imidazolidin-4-ones 5 (from precursors 9) and pH-independent pathway rate constant constants, k_{neut}, for the decomposition of neutral prodrugs 5 at 37 °C.

<table>
<thead>
<tr>
<th>Series</th>
<th>Compound R¹</th>
<th>R⁴</th>
<th>Yield, %</th>
<th>k_{neut}/ h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>H</td>
<td>H</td>
<td>41.2</td>
<td>23.5</td>
</tr>
<tr>
<td>b</td>
<td>H</td>
<td>Me</td>
<td>56.5</td>
<td>4.39</td>
</tr>
<tr>
<td>c</td>
<td>H</td>
<td>Bu'</td>
<td>75.2</td>
<td>2.20</td>
</tr>
<tr>
<td>d</td>
<td>H</td>
<td>CH₂Ph</td>
<td>74.8</td>
<td>7.01</td>
</tr>
<tr>
<td>e</td>
<td>H</td>
<td>OEt</td>
<td>69.4</td>
<td>4.52</td>
</tr>
<tr>
<td>f</td>
<td>Me</td>
<td>Me</td>
<td>38.0</td>
<td>68.2</td>
</tr>
<tr>
<td>g</td>
<td>Me</td>
<td>(CH₂)₂SMe</td>
<td>62.0</td>
<td>23.4</td>
</tr>
<tr>
<td>h</td>
<td>Bu'</td>
<td>H</td>
<td>213.7</td>
<td>ND</td>
</tr>
<tr>
<td>i</td>
<td>H</td>
<td>H</td>
<td>73.2</td>
<td>38.6</td>
</tr>
<tr>
<td>j</td>
<td>Me</td>
<td>H</td>
<td>37.0</td>
<td>103.1</td>
</tr>
<tr>
<td>k</td>
<td>Bu'</td>
<td>H</td>
<td>37.9</td>
<td>13.1</td>
</tr>
<tr>
<td>l</td>
<td>CH₂Ph</td>
<td>H</td>
<td>46.1</td>
<td>26.0</td>
</tr>
<tr>
<td>m</td>
<td>H</td>
<td>Me</td>
<td>87.9</td>
<td>8.06</td>
</tr>
<tr>
<td>n</td>
<td>H</td>
<td>CH₂Ph</td>
<td>80.3</td>
<td>7.39</td>
</tr>
<tr>
<td>o</td>
<td>Me</td>
<td>Me</td>
<td>46.4</td>
<td>60.2</td>
</tr>
<tr>
<td>p</td>
<td>Me</td>
<td>(CH₂)₂SMe</td>
<td>80.2</td>
<td>36.7</td>
</tr>
</tbody>
</table>

Notes: a First-order rate constant for the hydrolysis in 80% human plasma in pH 7.4 phosphate buffer at 37 °C.

### Table 2

First-order rate constants, k_{neut}, for the hydrolysis of 5a in acetate and 5a in phosphate buffers at 37 °C; with ionic strength maintained at 0.5 mol dm⁻³ by addition of NaClO₄.

<table>
<thead>
<tr>
<th>Compd</th>
<th>Buffer</th>
<th>[Buffer], mol dm⁻³</th>
<th>pH</th>
<th>k_{neut}/ h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>CH₃CO₂H</td>
<td>0.005</td>
<td>4.45</td>
<td>94.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.010</td>
<td>4.47</td>
<td>95.4</td>
</tr>
<tr>
<td>5s</td>
<td>H₂PO₄</td>
<td>0.050</td>
<td>4.42</td>
<td>95.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.002</td>
<td>6.93</td>
<td>24.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.005</td>
<td>6.95</td>
<td>23.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.010</td>
<td>6.96</td>
<td>25.5</td>
</tr>
</tbody>
</table>

Scheme 3.
organic phases pooled, dried over anhydrous MgSO₄ and evaporated to dryness. Products 9 were thus isolated as yellow-orange oils in 84–99% yields and successfully characterized by MS and NMR (see Supporting Information).

5.2.3. Preparation of the target compounds 5a–s

Compounds 9 were mixed with an excess (2 eq.) of the appropriate ketone (propanone, cyclohexanone, cycloheptanoone and 4-methylcyclohexanone) and TEA (1 eq.) in dry methanol (20 mL) and the mixture refluxed for 3 d in the presence of 4 Å molecular sieves (1 g). The reaction was monitored by TLC and ketone was re-added (1 eq.) once per day. The molecular sieves were removed by decantation and the solution evaporated to dryness. The oily residue was submitted to column chromatography on silica-gel, eluted with appropriate solvents. Fractions containing the chromatographically homogeneous product were pooled and evaporated to dryness, yielding 5a–s as yellow-orange oils (21–88%) that were analyzed by MS and NMR, as detailed below.

3-{7-[(6-Methoxyquinolin-8-yl)amino]-3-aza-2-oxooctyl}-2,2,5-trimethylimidazolidin-4-one, 5b. Yield 56%; δH (CDCl3, 300 MHz) 8.52 (dd, J = 4.24 Hz, J = 1.70 Hz; 1H, Q2); 7.92 (dd, J = 8.28 Hz, J = 1.62 Hz; 1H, Q4); 7.30 (dd, J = 8.27 Hz, J = 4.20 Hz; 1H, Q3); 6.63 (m; 1H, –CH2–NH–CO–CH2–); 6.33 (d, J = 2.43 Hz, 1H, Q5); 6.26 (d, J = 2.38 Hz; 1H, Q7); 5.99 (d, J = 8.10 Hz; 1H, –NH–CH(CH3)–CH2–); 3.89 and 3.88 (d + d, J = 15.28 Hz, J = 15.36 Hz; 1H, –NH–CO–CH(CH3)–CH2–); 3.87 (s; 3H, CH3–O–); 3.68 and 3.66 (d + d, J = 15.37 Hz, J = 15.31 Hz; 1H, –NH–CO–CH(CH3)–NH–); 3.65–3.53 (m; 2H, –CO–CH(CH3)–NH–C–, –NH–CH(CH3)–CH2–); 3.33–3.18 (m; 2H, –CO–CH(CH3)–NH–C–, –NH–CH(CH3)–CH2–); 3.02–2.84 (m; 2H, –CO–CH(CH3)–NH–C–, –NH–CH(CH3)–CH2–); 2.95–2.65 (m; 2H, –CO–CH(CH3)–NH–C–, –NH–CH(CH3)–CH2–); 1.72–1.46 (m; 1H, –(CH2)3–NH–); 1.41–1.16 (m; 1H, –(CH2)3–NH–); 1.38–1.16 (m; 1H, –(CH2)3–NH–); 1.24–0.96 (d + d, J = 6.31 Hz, J = 6.30 Hz; 3H, –(CH2)3–NH–). C22H23N5O3 (413.4247 g mol⁻¹): m/z (MH⁺) = 414.2231.

Fig. 1. pH–rate profiles for the hydrolysis of the imidazolidin-4-ones 5a (●) and 5s (▲) in aqueous solutions at 37 °C.
$$J = 2.31\text{ Hz}; 1H, Q5; 6.21 (d, J = 2.20\text{ Hz}; 1H, Q7); 5.94 (d, J = 8.28\text{ Hz}; 1H, –NH–(CH=CH2)3–); 3.89 and 3.88 (d + d, J = 15.47\text{ Hz}; J = 15.45\text{ Hz}; 1H, –CO–CH=CH2–); 3.82 (s; 3H, CH3O–); 3.58(3) and 3.57(9) (d + d, J = 15.61\text{ Hz}, J = 15.53\text{ Hz}; 1H, –CO–CH=CH2–); 3.55–3.52 (m; 1H, –CO–CH=CH(–NH–)); 3.44–3.42 (m; 1H, –NH–CH2(–CH=CH2)3–); 3.27–3.12 (m; 2H, –(CH2)2–CH2–); 2.09–2.05 (m; 1H, –CO–CH(NH–)–CH2–); 1.64–1.49 (m; 4H, –CH2–CH2–CH2–CH2–); 1.33 (s; 3H, –(CH2)3(CH3)–); 1.22(5) (d, J = 6.39\text{ Hz}; 3H, –NH–CH2(–NH–)); 0.96 and 0.95 (d + d, J = 6.74\text{ Hz}; J = 6.84\text{ Hz}; 3H, –CH2(–NH–)3(CH3)); \delta (CDCl3, 75 MHz) 174.83 (–CO–CH2–(–NH–)); 168.72 (–NH–CO–CH2–); 159.16 (QCD3); 144.67 (QCD2); 144.05 (QCD3); 136.45 (QCD4); 134.58 (QCD10); 129.66 (QCD9); 121.65 (QCD7); 96.50 (QCD5); 91.43 (QCD9); 76.02 and 76.01 (–CO–CH2–(–NH–)); 62.50 (–(CH2)2–); 54.98 (CH3O–); 47.51 (–NH–CH2(–NH–)); 44.45 (–CO–CH2–); 39.19 (–(CH2)2–(CH2–CH2–)); 33.77 (–CH2–CH2–); 30.72 (–(CH2)3(CH3)–); 28.95 (–(CH2)2–); 25.57 and 27.56 (–(CH2)3(CH3)–); 26.10 and 26.05 (–CH2–CH2–CH2–); 20.34 (–NH–CH2(–NH–)); 18.97 and 18.95 (–CH2(–NH–)); 16.67 (–(CH2)3(CH3)–); C20H29N5O5 (455.29 g mol–1); m/z (MH+) = 456.40; m/z (MNa+) = 478.40.

Table 3
Effect of imidazolidin-4-ones 5a, 5b, and 5f and primapine on the sporocyst development of Plasmodium berghei ANKA in Anopheles stephensi mosquitoes. Counting of oocysts was carried out at day 10 post-feed.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose, (\mu)mol kg(^{-1})</th>
<th>Mean no. of oocysts per mosquitoes, sSEM(^a)</th>
<th>% Of infected mosquitoes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>12.2 (2.9)</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.2 (0.7)</td>
<td>0.2</td>
</tr>
<tr>
<td>5a</td>
<td>10</td>
<td>4.8 (1.3)</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>3.8 (1.3)</td>
<td>4.4</td>
</tr>
<tr>
<td>5b</td>
<td>10</td>
<td>813 (3.5)(^b)</td>
<td>94.2</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>21 (0.2)</td>
<td>2.4</td>
</tr>
<tr>
<td>5f</td>
<td>10</td>
<td>1.3 (0.2)</td>
<td>1.5</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>86.7 (4.0)</td>
<td>10</td>
</tr>
</tbody>
</table>

\(^a\) Mean standard error.

\(^b\) \(P < 0.05\) versus control, by Student’s t test.

Fig. 2. Correlation of decomposition rates, \(k_{\text{rate}}\), of imidazolidin-4-ones 5e,n,q,r with those of their encephalin counterparts 11.
3-[6-Methoxyquinolin-8-yl]amino-3-aza-1-methyl-2-oxoocetyl]-S-[(2-methylthio)ethyl]-2,2-dimethylimidazolidin-4-one, 5g. Yield 62%; $\delta_1$ (CDCl$_3$, 300 MHz) 8.05(4) and 8.50(9) (dd + dd, J = 7.2 Hz, J = 1.2 Hz), 7.40–7.05 (m), 6.92 (br s, 2H, –CH$_2$–NH$_2$); 5.78 (d, J = 8.26 Hz, J = 1.60 Hz); 1H, Q4); 7.66–7.58 (m, 1H, –(CH$_2$)$_3$–NH$_2$); 7.28 (dd, J = 8.27 Hz, J = 4.20 Hz; 1H, Q3); 6.31 (d, J = 2.42 Hz; 1H, Q5); 6.25 and 6.24 (d + d, J = 3.01 Hz and J = 2.82 Hz; 1H, Q7); 5.98 (d, J = 6.91 Hz; 1H, –NH–CH$(\cdots)$–CH$_2$–); 3.86(5) and 3.86(1) (s + s; 3H; CH$_3$–O); 3.76–3.72 (m, 1H, –CO–CH$_2$–); 3.68–3.58 (m, 2H, –NH–CH$_2$–CH$_2$–CH$_2$–CO–CH$_2$–PH–); 3.36–3.26 (m; 1H, –(CH$_2$)$_3$–CH$_2$–); 3.21–3.10 (m; 1H, –(CH$_2$)$_2$–CH$_2$–); 2.61–2.51 (m; 2H, –CH$_2$–CH$_2$–S–); and 2.04 and 2.01 (s + s; 3H, –CH$_3$–S–CH$_2$); 1.98 (bs; 1H, –CO–CH$_2$–NH$\_2$–); 1.85–1.65 (m; 4H, –CH$_2$–CH$_2$–CH$_2$–); 1.63 and 1.60 (s + s; 6H, –N–(CH$_2$)$_2$–NH$_2$–); and 1.37 and 1.31 (bs + bs; 3H, –CO–CH$_2$–(CH$_3$)$_2$–); 1.28(1) and 1.27(8) (d + d, J = 6.32 Hz; J = 6.34 Hz; 3H, –NH–CH$(\cdots)$–CH$_2$–). $\delta_2$ (CDCl$_3$, 75 MHz) 176.13 and 176.07 (–CO–(CH$_2$)$_2$–NH$\_2$–); 172.59 and 172.25 (–NH–CO–CH$_2$–(CH$_3$)$_2$–); 159.81 (Q4), 145.33 (Q2); 144.73 (Q8); 135.76 and 135.73 (Q4); 135.19 (Q10); 130.32 and 130.30 (Q9); 122.28 (Q3); 97.12 and 97.07 (Q7); 92.08 (Q5); 78.21 (–CH$(\cdots)$–NH$\_2$–); 50.74 and 52.77 (–NCH$(\cdots)$–NH$_2$–); 55.79 and 57.72 (–CO–CH$_2$–(CH$_3$)$_2$–); 55.64 and 55.63 (CH$_3$–O–); 48.20 and 48.16 (–NH–CH$_2$–CH$_2$–); 39.77 and 39.72 (–CH$_2$–CO–); 34.39 (–CH$(\cdots)$–CH$_2$–); 31.02 and 31.01 (–CH$_2$–S–CH$_2$–S–); 30.79 and 30.76 (–CH$_2$–CH$_2$–S–); 28.80 and 28.73 (–CH$_3$–CH$_2$–CH$_2$–NH$_2$–); 26.66 and 26.64 (–CH$_2$–CH$_2$–CH$_2$–); 26.11 and 26.03 (–CH$_3$–CH$_2$–CH$_2$–NH$_2$–); 20.95 and 20.91 (–NH–CH$_2$–CH$_2$–CH$_2$–); 16.02 (–S–CH$_2$–); 15.64 and 15.62 (–CO–CH$_2$–CH$_2$–); and 15.18 and 15.17 (–CH$_2$–CO–CH$_2$–O–). C$_{23}$H$_{33}$N$_5$O$_3$ (501.28 g mol$^{-1}$); m/z (M$^+$) = 524.90.

3-[6-Methoxyquinolin-8-yl]amino-3-aza-1-isobutyl-2-oxoocetyl]-2,2-dimethylimidazolidin-4-one, 5h. Yield 21%; $\delta_1$ (CDCl$_3$, 300 MHz) 8.54–8.52 (m; 1H, Q2); 7.92 (d, J = 8.12 Hz; 1H, Q4); 7.72–7.28 (m; 1H, Q3); 6.33 (d, J = 1.65 Hz; 1H, Q5); 6.27 (d, J = 1.58 Hz; 1H, Q7); 6.02–5.92 (m; 1H, –NH–CH$(\cdots)$–CH$_2$–); 4.42–4.40 (m; 1H, –CH$(\cdots)$–NH$_2$–); 3.89 (2s; 3H, CH$_3$–O–); 3.89–3.86 (m; 1H, –CH$(\cdots)$–CO–); 3.66–3.53 (m; 2H, –NH–CH$(\cdots)$–CH$_2$–CH$_2$–CH$_2$–); 3.30–3.20 (m; 2H, –(CH$_2$)$_2$–CH$_2$–); 1.71–1.54 (m; 9H, –CH$_2$–CH$_2$–CH$_2$–CO–NH–CH$_2$–CH$_2$–CH$_2$–); 1.30–1.13 (m; 9H, –N–CO–(CH$_2$)$_2$–NH–CH$_2$–CH$_2$–); 0.94–0.81 (m; 6H, –CH$_3$–CH$_2$–CH$_2$–). C$_{23}$H$_{33}$N$_5$O$_3$ (501.28 g mol$^{-1}$); m/z (M$^+$) = 524.90.

3-[6-Methoxyquinolin-8-yl]amino-3-aza-1-methyl-2-oxoocetyl]-1,4-diazaspiro[4.5]decane-2-one, 5i. Yield 38%; $\delta_1$ (CDCl$_3$, 300 MHz) 8.52 (dd, J = 4.22 Hz, J = 1.63 Hz; 1H, Q2); 792 (dd, J = 8.26 Hz, J = 1.53 Hz; 1H, Q4); 7.30 (d, J = 8.25 Hz, J = 4.20 Hz; 1H, Q3); 6.69 (bs, 1H, –(CH$_2$)$_3$–NH$\_2$–); 6.33 (d, J = 2.51 Hz; 1H, Q5); 6.26 (d, J = 2.46 Hz; 1H, Q7); 5.98 (d, J = 7.55 Hz; 1H, –NH–CH$_2$–CH$_2$–NH–); 5.93–3.89 (m; 4s + 4H, –CH$_2$–CH$_2$–CH$_2$–CH$_2$–); 3.72–3.53 (m; 2H, –CO–CH$_2$–CH$_2$–); 2.87 (d, J = 8.12 Hz; 1H, –CO–CH$_2$–CH$_2$–); 2.34 (t, J = 6.52 Hz; 1H, –CO–CH$_2$–CH$_2$–NH–); 1.69–1.62 (m; 17H, –CH$_2$–CH$_2$–CH$_2$–NH–CH$_2$–CH$_2$–CH$_2$–); 1.29 (d, J = 6.29 Hz; 3H, –NH–CH$_2$–CH$_2$–); 0.94 and 0.92 (d + d, J = 5.41 Hz; 1H, Q1); 5.15 Hz; 6H, –CH$_2$–CH$_2$–CH$_2$–). C$_{23}$H$_{33}$N$_5$O$_3$ (501.28 g mol$^{-1}$); m/z (M$^+$) = 524.90.
J = 4.82 Hz; 1H, -(CH2)2-NH-); 6.33 (d, J = 2.48 Hz; 1H, Q5); 6.26 (d, J = 2.43 Hz; 1H, Q7); 5.99 (bs; 1H, -NH-(CH2)2-CH2-); 3.89 (bs; 4H, CH2-O-, -NH-(CO-CH2)-); 3.68-3.60 (m; 2H, -NH-CO-CH2-NH-); 3.51 (q, J = 6.88 Hz; -CO-(CH2)2-NH-); 3.21-3.16 (m; 2H, -(CH2)2-CH2-NH-); 1.83 (d, J = 4.34 Hz; 1H, -CO-(CH2)2-CH2-); 1.71-1.57 (m; 10H, -(CH2)2-); 1.52-1.46 (m; 4H, -(CH2)2-CH2-); 1.33 and 1.30 (d, J = 7.05 Hz, J = 7.01 Hz; 3H, -CO-(CH2)-CH2-); 129 (d, J = 6.35 Hz; 3H, -NH-(CH2)2-CH2-); δC (CDCl3, 75 MHz) 177.48 (-N-(CO-CH2)-); 169.66 (-N-(CO-CH2)-); 159.82-92.12 (ArC); 79.12 (-N-(C-)-); 55.64 (CH2-O-); 53.80 (NH-(CH2)2-); 48.20 (-CO-(CH2)2-NH-); 45.32 (-NH-(CH2)2-); 44.20 (-CO-(CH2)2-CH2-); 39.73 (-CH2-); 37.58 (-CH2-); 34.43 (-CH-(CH2)2-); 27.31 (-CH2-); 26.61 (-CH2-CH2-CH2-); 24.95 (-NH-(CH2)-CH2-); 23.00 (-CH2-); 22.46 (-CH2-); 20.99 (-NH-(CH2)-CH2-); 18.10 and 18.07 (-CH-(CH2)-NH-). C32H41N5O3 (543.3209 g mol⁻¹); m/z (MH⁺) = 542.87; m/z (MNa⁺) = 564.87.

3-[(6-Methoxyquinolin-8-yl)amino]-3-aza-2-oxoctyl-3-benzyl-1,4-diazaspiro[4.5]decane-2-one, 5q. Yield 62%; δC (CDCl3, 300 MHz) 8.52 (dd, J = 4.21 Hz, J = 1.61 Hz; 1H, Q2); 7.90 and 7.89 (dd = dd, J = 8.26 Hz, J = 1.77 Hz and J = 1.78 Hz; 1H, Q4); 7.31-7.29 (m; 6H, Q3); 7.00-6.98 (m; 3H, -NH-(CH2)-CH2-); 2.44 (H, -C(CH3)-CH2-); 1.70 (d, J = 2.44 Hz; 1H, Q7); 6.02-5.97 (m; 2H, -N-(CH2)2-CH2-); 31.47 (-CH2-CH2-S-); 30.76 and 30.73 (-CH2-CH3-); 26.70 and 26.64 (-CH2-CH2-CH2-); 23.35 (-CH2-CH2-CH2-); 22.51 (-CH2-CH2-CH2-); 20.91 (-CH2-CH2-); 16.29 (-CH2-S-CH3); 15.67 (-CO-(CH2)-); C32H41N5O3 (543.3209 g mol⁻¹); m/z (MH⁺) = 542.87; m/z (MNa⁺) = 564.87.
5.3. Kinetics of hydrolysis

The kinetics of hydrolysis of imidazolin-4-ones 5 were studied by HPLC using a Merck Hitachi L-7110 pump with a L-7400 UV detector set at 254 nm, a manual sample injection module equipped by HPLC using a Merck Hitachi L-7110 pump with a L-7400 UV detector set at 254 nm, a manual sample injection module equipped by HPLC using a Merck Hitachi L-7110 pump with a L-7400 UV detector set at 254 nm. The kinetics of hydrolysis of imidazolin-4-ones 5 were studied at 37.0 ± 0.1 °C, in aqueous buffers with ionic strength kept at 0.5 M by the addition of NaClO4. The buffers used were acetate (pH 3.0–5.9), phosphate (pH 6.0–7.5), and borate (pH 8.0–11.0). Sodium hydroxide and hydrochloric acid were added to acetonitrile to quench the reaction and precipitate plasma substrate. These samples were centrifuged and the supernatant was analyzed by the HPLC method described above for the presence of substrate and products.

5.5. In vivo gametocytocidal activity

BalbC mice were infected by intrauterine 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 intrauterine administration with one single dose of each compound 5 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 A.stephensi female mosquitoes, which were allowed to feed for 2 h. After the blood meal, unfed female 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. [18].

Appendix. Supporting information

Supporting information associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2009.01.018.

References


[34] (a) For Gly-Gly: The Index Merck, eleventh ed. Merck, Rahway, 1989;
