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# Inhibitors of Plasmepsin II—potential antimalarial agents

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**Abstract**—In order to overcome the problem of drug resistance in malaria, it appears wise to concentrate drug discovery efforts toward new structural classes and new mechanisms of action. We report our results, targeting Plasmepsin II, a *Plasmodium falciparum* aspartic protease active in hemoglobin degradation, a parasite specific catabolic pathway. The results show that the new structural class is not only inhibiting PMII in vitro but is also active in a *P. falciparum* infected human red blood cell assay.


Due to changing agricultural habits, increased mobility of the population and climate changes, malaria is spreading into formerly unaffected regions. In addition, the rising tide of resistance to most antimalarial drugs has caused an increase in mortality and has complicated disease control. Consequently, malaria is still one of the world's most serious infectious diseases, being the most widespread parasitic disease in man, with 300–500 million people affected, leading to more than 1 million annual deaths, mostly among children.[1]

These facts and a recent publication reporting that organisms with reduced sensitivity to artemisinin derivatives, the last resort against multi-drug resistant *P. falciparum* parasites, have been found in field isolates[2] illustrate the urgent need to discover and develop new antimalarial drugs. In order to successfully overcome the problem with existing drug resistances it appears wise to concentrate drug discovery efforts toward new structural classes with new mechanisms of action. Hemoglobin degradation is a parasite specific catabolic pathway, essential for the survival of *P. falciparum*, the most lethal malaria causing parasite in man. The enzymes involved in this process constitute new and promising drug targets.[3] It has been shown that the parasites are unable to proliferate in human red blood cells in vitro in the presence of inhibitors of aspartic proteases.[4] Plasmepsin II (PMII) was identified as one of at least four parasite specific aspartic proteases involved in hemoglobin degradation inside the acidic food vacuole.[3,5]

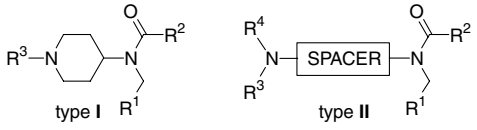


**Figure 1.** General structure of type I and II tertiary amines.

A high-throughput fluorescence resonance energy transfer (FRET) assay was used to measure the enzymatic activity of the isolated enzyme. Screening of a commercial library led to the identification of low μM inhibitors of PMII of types I and II (Fig. 1).

Optimization of type I hits improved the potency by a factor of 250 ($IC_{50}$ PMII from low μM for **1** down to 6 nM for **2**).[6] In parallel, optimization of type II tertiary amines led to a 60-fold increase in potency (from low μM for **3** to 101 nM for **4**).[7†] These compounds

*Keywords*: Malaria; Plasmepsin II.
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† $IC_{50}$ are the mean of several measurements, explaining some differences with the $IC_{50}$ as published earlier.[7]

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were then tested for antimalarial activity in a cell-based assay using *P. falciparum* infected human red blood cells (iRBC assay).[8] Compounds **2** and **4** exhibited a clear antiparasitic activity in this assay, although a shift between the isolated enzyme assay and the cell-based assay of a factor of 10 or more was observed ($IC_{50}$ iRBC/$IC_{50}$ FRET > 10). It was hypothesized that the shift might be due to the difficulty of these rather lipophilic compounds to reach their target in the cellular assay (Fig. 2).

For type II inhibitors, the importance of the 4-*n*-pentyl-benzoyl unit and the length of the spacer for inhibitory activity against PMII had already been reported.[7] Replacing the *n*-pentyl chain by shorter ones as well as the introduction of a heteroatom in the chain led to substantial losses of inhibitory activity toward PMII. It had also been shown that there was a slight preference for the $C_2$ spacer. Until now, little was known of the effect modifications at $R^3$ and $R^4$ would bring to type II PMII inhibitors (see Fig. 1). In order to evaluate the effect of the substituent at the tertiary amine, the synthetic pathway was accommodated as described in Scheme 1 and a series of reductive aminations was conducted using a parallel chemistry approach.

The di-*n*-butyl substitution still resulted in the most active compound of the series as seen in Table 1. No further conclusion could be drawn except that substituents should be quite bulky to retain activity. Additional investigations with respect to the amine substitution are underway.

Applying these *SAR*-observations (4-*n*-pentyl-benzoyl unit, biaryl system, $C_2$ spacer, di-*n*-butyl substitution) an attempt to improve the physico-chemical profile of the compounds was made. This was achieved by adding a polar functionality to the preferred biaryl system with the aim to maintain activity against PMII and to increase activity in the iRBC assay. As

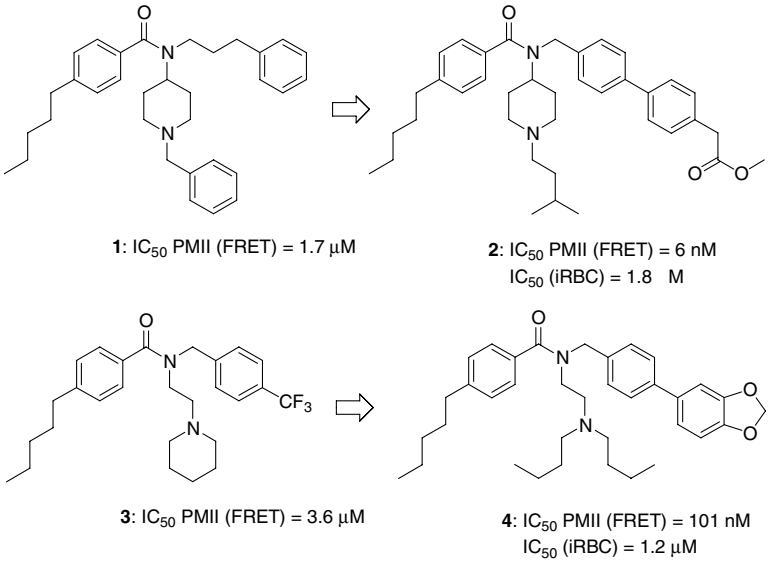
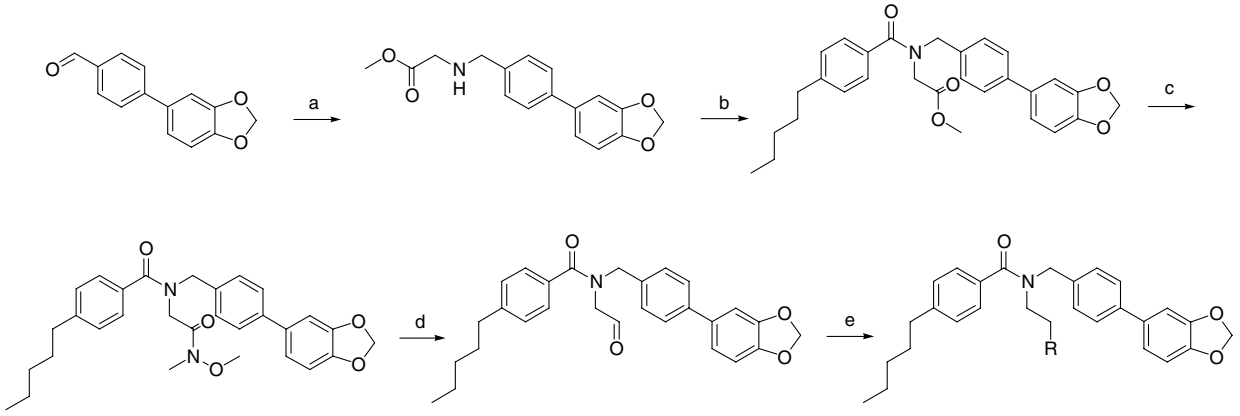
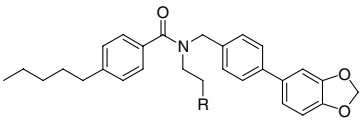


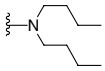
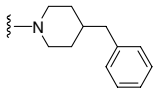
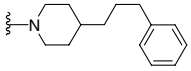
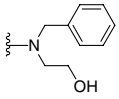
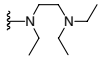

**Figure 2.** First optimization of the lead structures.




**Scheme 1.** Reagents and conditions: (a) i—glycine methyl ester hydrochloride, MeOH, Hünig's base, reflux 4 h; ii—$NaBH_4$, rt, 1 h; (b) 4-*n*-pentyl-benzoyl chloride, $CH_2Cl_2$, Hünig's base, rt; (c) *N*,*O*-dimethyl-hydroxylamine, $AlMe_3$, $CH_2Cl_2$, rt, 47%, three steps; (d) DIBAL-H, THF, −78 °C, 2 h; (e) amine, $CH_3CN$, reflux 4 h; ii—$NaBH_4$, rt, 1 h, 80%, two steps.

**Table 1.** Variations in the tertiary amine of type II compounds ($IC_{50}$ in nM)

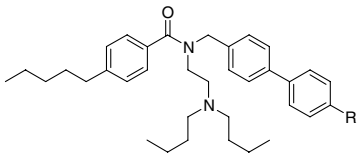


| Compound | R | $IC_{50}$ FRET | Compound | R | $IC_{50}$ FRET |
|---|---|---|---|---|---|
| **4** |  | 101 | **5** |  | 531 |
| **6** |  | 1519 | **7** |  | 1501 |
| **8** |  | 334 | **9** |  | 190 |
| **10** |  | 165 | **11** |  | 2897 |
| **12** |  | 334 | **13** |  | 439 |

described in Table 2, addition of polarity by introduction of an ester (**15**), an acid (**16**) or an alcohol (**17**) did not decrease the shift between the FRET $IC_{50}$ and the iRBC $IC_{50}$ values. Replacement of the ester by an amide (**18**–**23**) resulted in improved activity in the FRET assay but the shift remained and in addition the molecular weight of the compounds was increased.

These results indicated that the physico-chemical properties of the compounds needed to be adjusted by other structural variations. In order to keep the compounds small, the second aryl of the biphenyl moiety was replaced with a heteroaryl or an amide. Using a copper catalyzed coupling according to Buchwald,[9,10] both the aryl–heteroaryl (Table 3, **24** and **25**) and the aryl–amide derivatives (**26**–**29**) were accessible.

**Table 2.** Aryl–amine substituents: comparison of the shift between the FRET and the iRBC assay ($IC_{50}$ in nM)



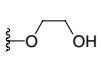
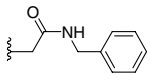
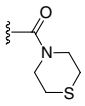
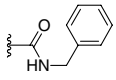
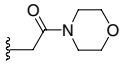
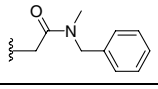
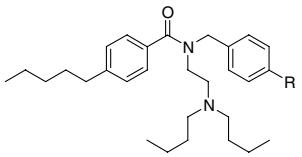
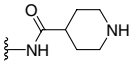
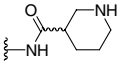
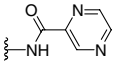
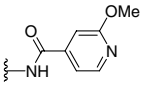
| Compound | R | $IC_{50}$ FRET | $IC_{50}$ iRBC | Compound | R | $IC_{50}$ FRET | $IC_{50}$ iRBC |
|---|---|---|---|---|---|---|---|
| **14** |  | 143 | 2442 | **15** |  | 183 | 2472 |
| **16** |  | 463 | 7152 | **17** |  | 511 | 3658 |
| **18** |  | 46 | 340 | **19** |  | 56 | 751 |
| **20** |  | 72 | 566 | **21** |  | 74 | 885 |
| **22** |  | 77 | 859 | **23** |  | 91 | 725 |

**Table 3.** Aryl–heteroaryl and aryl–amide substituents: comparison of the shift between the FRET and the iRBC assay ($IC_{50}$ in nM)

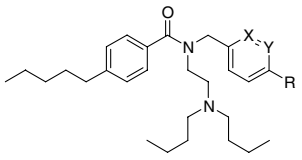


| Compound | R | $IC_{50}$ FRET | $IC_{50}$ iRBC | Compound | R | $IC_{50}$ FRET | $IC_{50}$ iRBC |
|---|---|---|---|---|---|---|---|
| **24** |  | 240 | 825 | **25** |  | 670 | 739 |
| **26** |  | 464 | 550 | **27** |  | 202 | 376 |
| **28** |  | 243 | 605 | **29** |  | 154 | 623 |
| **30** |  | 380 | 1726 | **31** |  | 374 | 273 |
| **32** |  | 212 | 583 | **33** |  | 531 | 2232 |

In comparison to the biphenyl substitution, where a shift of more than 10 between the enzyme FRET assay and the cell-based assay was observed, the shift was significantly reduced by replacement of the terminal aryl of **4** by an indole or amides. The aryl–heteroaryl system was further investigated using a Suzuki coupling in a parallel chemistry setting.

Introduction of a pyridine (Table 3, **30**–**32**) resulted in some activity loss by FRET assay in comparison to **4** or **14** but the assay to assay shift was reduced or even disappeared as shown by **31**. One possible explanation of these findings is that the introduction of polar groups allowed better cell penetration and/or accumulation inside the cells.

**Table 4.** Increasing hydrophilicity / polarity: comparison of the shift between the FRET and the iRBC assay ($IC_{50}$ in nM)



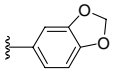
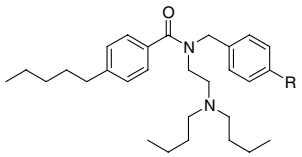
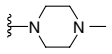
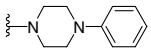
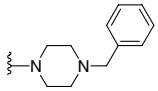
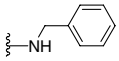
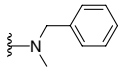
| Compound | R | X | Y | $IC_{50}$ PMII FRET | $IC_{50}$ iRBC |
|---|---|---|---|---|---|
| **34** |  | C | N | 589 | 609 |
| **35** | | N | C | 2345 | 693 |
| **36** |  | C | N | 437 | 677 |
| **37** | | N | C | 1912 | 698 |
| **38** |  | C | N | 736 | 411 |
| **39** |  | C | N | 587 | 458 |
| **40** | | N | C | 5266 | 465 |
| **41** |  | C | N | 642 | 457 |
| **42** | | N | C | 4553 | 630 |
| **43** |  | C | N | 2229 | 560 |
| **44** | | N | C | 8530 | 639 |

**Table 5.** Aryl–amine substituents: comparison of the shift between the FRET and the iRBC essay ($IC_{50}$ in nM)



| Compound | R | $IC_{50}$ FRET | $IC_{50}$ iRBC | Compound | R | $IC_{50}$ FRET | $IC_{50}$ iRBC |
|---|---|---|---|---|---|---|---|
| **45** |  | 1491 | 550 | **46** |  | 369 | 628 |
| **47** |  | 188 | 1757 | **48** |  | 2549 | 431 |
| **49** |  | 200 | 1278 | **50** |  | 646 | 790 |
| **51** |  | 429 | 686 | **52** |  | 289 | 609 |
| **53** |  | 224 | 539 | **54** |  | 574 | 617 |
| **55** |  | 1816 | 207 | **56** |  | 3015 | 453 |

The results depicted in Table 3 encouraged the study of further replacements of phenyl-rings by heteroaryl-systems as summarized in Table 4.

A distinct *SAR* can be derived from Table 4: in the pyridine–aryl system **34** and **36** some activity was lost in the FRET assay compared to **14** and **4** but the assay to assay shift was significantly reduced. For **35** and **37**, a 10-fold loss of activity was observed in the FRET assay compared to **14** and **4** but an inverted shift ($IC_{50}$ iRBC/$IC_{50}$ FRET < 1) was observed, the activity in the iRBC was better than on the isolated enzyme. This was also true for the bis-pyridines **38** to **42**: there was no shift when Y = N and an inverted shift was observed when X = N. The same was true for the pyridine–pyrimidine system (**43** and **44**). This inverted shift could be due to off target activity, better cell penetration and/or intracellular accumulation.

Having shown that an introduction of polarity by means of replacing CH-groups in the biaryl system by N-atoms resulted in reduced $IC_{50}$ shift between the assays, the effect of replacing the biaryl system by an aryl–amine group was examined. Applying the Buchwald–Hartwig aryl-amination protocol[11] in a parallel chemistry setting, the compounds depicted in Table 5 were synthesized.

These results demonstrated that the introduction of polarity via an aryl–amine system resulted in reduced or inverted $IC_{50}$ shift between the assays. Compounds down to 207 nM in the iRBC assay could be obtained (see **55**) but the *SAR* was not as clear as with the biaryl system.

In conclusion, it was shown that type II compounds are promising leads to provide PMII inhibitors. The problem of the $IC_{50}$ shift between the isolated enzyme assay (FRET) and the cell-based assay (iRBC) could be solved by the introduction of polar groups without significantly increasing the molecular weight. The bis-heteroaryl moiety was a preferred pattern where hydrophilicity / polarity could be introduced. The pyridine–pyridine biaryl replacement showed activity in the iRBC assay below 300 nM with a clear *SAR*. Further work toward the optimization of the biaryl class by replacement of the pyridine by small heterocycles is ongoing. It was also demonstrated that the aryl–amide unit pattern as well as the aryl–amine are beneficial, resulting in compound **55** with an $IC_{50}$ of 207 nM in the iRBC essay.

## Acknowledgments

The authors thank Mr. Christian Scheurer of the STI for carrying out the iRBC assays, Prof. H. Ramuz and Prof. J.-M. Bourgeois for stimulating discussions, and Dr. T. Weller and Dr. W. Fischli for constantly supporting the project.

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