Structure-activity relationships of antiplasmodial pantothenamide analogues reveal a new way by which triazoles mimic amide bonds

Jinming Guan,[a] Erick T. Tjhin,[b] Vanessa M. Howieson,[b] Tanakorn Kittikool,[a] Christina Spry,[b] Kevin J. Saliba,*,[b],[c] Karine Auclair*,[a]

[a] J. Guan, T. Kittikool, Prof. K. Auclair
Department of Chemistry
McGill University
801 Sherbrooke Street West, Montreal, Quebec, Canada H3A 0B8
E-mail: karine.auclair@mcgill.ca

[b] E. T. Tjhin, V. M. Howieson, C. Spry, Assoc. Prof. K. J. Saliba
Research School of Biology
The Australian National University
134 Linnaeus Way, Acton ACT 2601, Australia
E-mail: kevin.saliba@anu.edu.au

[c] Assoc. Prof. K. J. Saliba
Medical School
The Australian National University
134 Linnaeus Way, Acton ACT 2601, Australia
E-mail: kevin.saliba@anu.edu.au

Supporting information for this article is given via a link at the end of the document.

Abstract: Pantothenamides are potent growth inhibitors of the malaria parasite *Plasmodium falciparum*. Their clinical use is, however, hindered due to the ubiquitous presence of pantetheinases in human serum, which rapidly degrade pantothenamides to pantothenate and the corresponding amine. We previously reported that replacement of the labile amide bond with a triazole ring not only imparts stability towards pantetheinases but also improves activity against *P. falciparum*. A small library of new triazole derivatives was synthesized and their use in establishing structure-activity relationships relevant to antiplasmodial activity of this family of compounds is discussed herein. Overall it was observed that 1,4-substitution on the triazole ring and use of an unbranched, one-carbon linker between the pantoyl group and the triazole are optimal for inhibition of intraerythrocytic *P. falciparum* growth. Our results imply that the triazole ring may mimic the amide bond with an orientation different from what was previously suggested for this amide bioisostere.

Introduction

In 2016, the World Health Organization (WHO) reported that nearly half of the world's population was still at risk of malaria.[1] This is aggravated by the fact that *Plasmodium* parasites are increasingly resistant to antimalarial agents.[2] Even resistance to artemisinin—a core component of modern therapy—has been detected in five countries of the Greater Mekong subregion.[1],[3] Novel antimalarial agents are necessary to treat an increasing number of drug resistant malaria cases.[4]
First synthesized by Clifton *et al.* in 1970,[5] pantothenamides (Figure 1a) have attracted interest mostly for their antibacterial activity.[6],[7] In 2013, Spry *et al.* reported that pantothenamides can also inhibit the growth of the malaria parasite *Plasmodium falciparum*.[8] Pantothenamides were later found to be metabolically activated by enzymes of the coenzyme A (CoA) biosynthetic pathway in *P. falciparum*.[9]–[11] This may not only affect CoA biosynthesis but the resulting metabolites are also inferred to inhibit downstream CoA-utilizing pathways, leading to lethal effects on *P. falciparum*,[9],[10] as suggested for bacteria.[12]–[20] Based on their synthetic accessibility, high potency and low cytotoxicity, pantothenamides would be excellent candidates for antimalarial drug development.[21] Unfortunately, however, ubiquitous pantetheinases (also known as vanins) in human serum rapidly hydrolyze pantothenamides into pantothenate and the corresponding amine (Figure 1b),[8],[22] making them unsuitable for therapeutic applications.

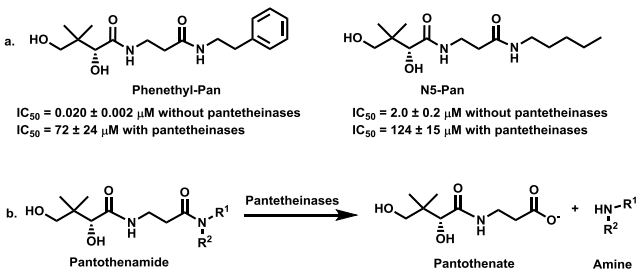


Figure 1. a) Two pantothenamides and their IC_{50} values[8] against *P. falciparum* in the presence or absence of pantetheinases; b) Hydrolysis of pantothenamides by serum pantetheinases. R^1 and R^2 are variable chemical groups.

To overcome the instability of pantothenamides in serum, two strategies have been explored by us and others: 1) the use of pantetheinase inhibitors in combination with pantothenamides;[22],[23] and 2) chemically modifying pantothenamides to prevent pantetheinase action while maintaining potency.[20],[24]–[30] Examples of modifications known to slow down or prevent pantothenamide degradation include alterations at one of the hydroxyl groups,[20],[26] at the geminal-dimethyl group,[24],[26],[28] at the β-alanine moiety,[25],[27] or at the labile amide moiety itself.[29],[30] We have recently reported a series of pantothenamide analogues containing a triazole ring in place of the labile amide bond.[30] The most potent of these were compounds **1** and **2** (Figure 2), which inhibit the growth of the asexual, intraerythrocytic stage of *P. falciparum* at low nanomolar concentrations. This series included only derivatives varied at the triazole *N*-substituent, except for one, less active, compound with a three-carbon linker between the pantoyl group and the triazole ring. We report here a more diversified series of analogues (Figure 2), which allowed us to establish important structure-activity relationships (SARs).

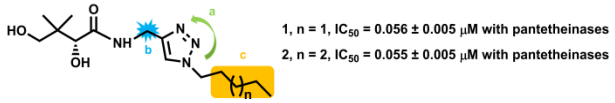
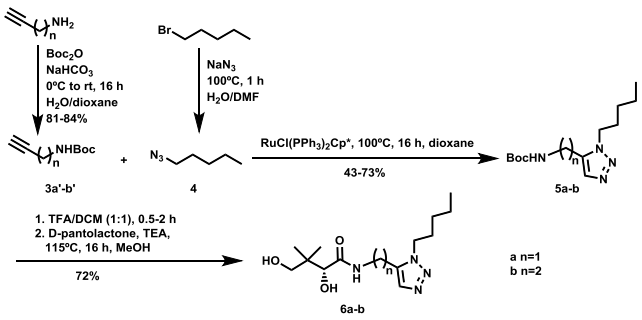


Figure 2. Structure of previously reported compounds **1** and **2** with their respective inhibitory activities[30] on the growth of intraerythrocytic *P. falciparum* in the presence of pantetheinases, and design of the molecules reported herein. The synthetic targets were designed based on compounds **1** and **2**, which were altered as follows: a) the relative positions of the two substituents on the triazole ring were changed (green); b) the pantoyl-triazole linker was varied (blue); and c) the effect of the triazole *N*-substituent was explored further (yellow).

It is worth noting that although the bioactivation pathway of pantothenamides in *P. falciparum* is known, their actual targets in the parasite are not fully elucidated and may include several CoA-utilizing enzymes. As such, docking studies are impractical. Since 1,2,3-triazoles are known to be good amide bioisosteres,[31],[32] and mechanistic studies suggest that compound **1** may inhibit the growth of *P. falciparum* by the same mechanism as pantothenamides,[9] the new derivatives presented here were designed with inspiration from the SARs reported for pantothenamides.

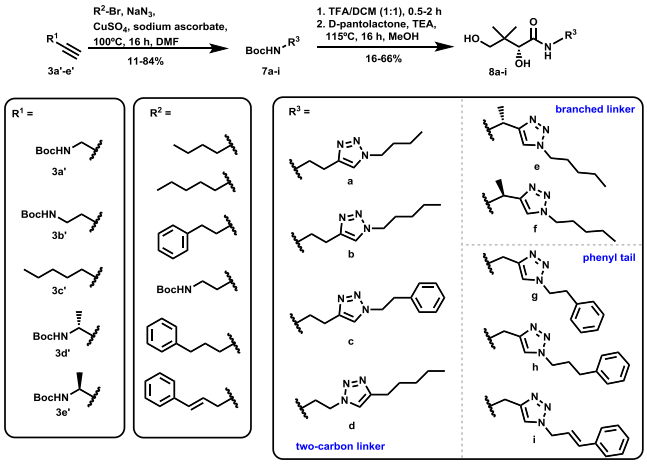
Results

Tron *et al.* have suggested that 1,4-disubstituted-1,2,3-triazoles and *Z*-amide bonds may be isosteric, while 1,5-substituted-1,2,3-triazoles may mimic *E*-amide bonds.[32] To determine the optimal triazole configuration for antiplasmodial activity, we synthesized the 1,5-substituted triazole **6a**, as shown in Scheme 1 (**6b** is discussed later). To this end, Boc-protected **3a′** was synthesized from commercially available propargylamine, and the pentyl azide **4** was synthesized from 1-bromopentane. For the subsequent cycloaddition step to produce **5a**, two different ruthenium catalysts, RuCl(cod)Cp* and $RuCl(PPh_3)_2Cp^*$, were tested.[33] With the former, no reaction was observed at room temperature and only very little conversion was detected at 100°C. In contrast, $RuCl(PPh_3)_2Cp^*$ gave a reasonable yield at 100°C. Finally, the desired product **6a** was generated after Boc-deprotection of **5a** and subsequent condensation with D-pantolactone. Compound **6a** was then tested for its inhibition of *P. falciparum* growth in erythrocytes (Table 1). Its IC_{50} value was 36 ± 10 μM, suggesting that the 1,4-substituted triazole is favored over the 1,5-substituted one.



Scheme 1. Synthetic method for the preparation of 1,5-substituted triazoles **6a**–**b**.

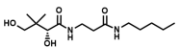
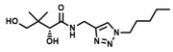
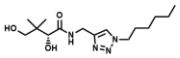
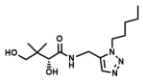
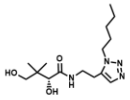
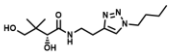
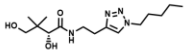
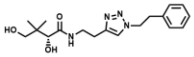
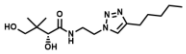
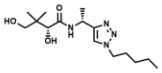
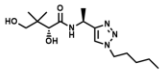
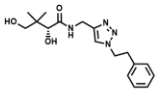
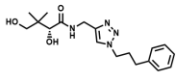
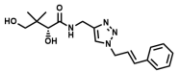
It was next envisaged to study the linker between the pantoyl and the triazole moieties. So far only triazole derivatives with one- or three-carbon linkers have been reported as pantothenamide mimics. Moreover, according to the current theory for how triazoles act as amide bioisosteres,[32] compounds **1** and **2** would be short by one carbon in the linker compared to their analog, N5-Pan. It was therefore hypothesized that 1,4-substituted triazoles with a two-carbon linker might be optimal, as in target compounds **8a**–**d** (Scheme 2). These four compounds were prepared by Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) to afford **7a**–**d**, before Boc-deprotection and reaction with D-pantolactone as before. Compounds **8a**–**c** were prepared from **3b′** and different commercial alkyl bromides in the presence of sodium azide, while compound **8d** was synthesized from 2-(Boc-amino)ethyl bromide and 1-heptyne (**3c′**) in the presence of sodium azide.



Scheme 2. General synthetic method for preparation of 1,4-substituted triazoles **8a**–**i**.

The IC_{50} values measured for the antiplasmodial activity of **8a**–**d** were all substantially higher than those of compounds **1** and **2** (Table 1), demonstrating that a one-carbon linker between the pantoyl and the triazole moieties is optimal. Interestingly, comparing the activity of compound **8b** in the presence of pantetheinases to that of N5-Pan in the absence of pantetheinases, reveals similar IC_{50} values, suggesting that the triazole moiety in **8b** may mimic the amide bond of N5-Pan well. On the other hand, the higher potency of compound **1** is consistent with the formation of additional interactions not accessible to N5-Pan or **8b**. It is also worth noting that compounds **8b** and **8d** were designed to have the ring substituents switched relative to one another. The slightly higher potency of **8b** over that of **8d** is consistent with a nitrogen being preferred over carbon at the attachment point of the alkyl chain.

Table 1. Effect of compounds **6a**–**b** and **8a**–**i** on the proliferation of *P. falciparum* in the presence of pantetheinases (unless otherwise noted), reported as the 50% inhibitory concentration (IC_{50}), and predicted physicochemical parameters of compounds **1, 2, 6a**−**b**, **8a**−**i**.

	Compound	IC_{50}[a] (μM)	LogP	MR (cm^3/mol)	tPSA	LogS
N5-Pan		2.0 ± 0.2[b]	0.24	77.22	98.66	-1.102
1		0.056 ± 0.005[c]	1.19	83.04	97.52	-1.693
2		0.055 ± 0.005[c]	1.61	87.64	97.52	-2.111
6a		36 ± 10	0.82	83.06	97.52	-1.461
6b		>100	0.93	87.66	97.52	-1.627
8a		5.3 ± 1.7	0.88	83.04	97.52	-1.44
8b		3.3 ± 0.9	1.3	87.64	97.52	-1.857
8c		5.4 ± 0.8	1.65	98.14	97.52	-2.398
8d		8.5 ± 3.0	1.61	87.64	97.52	-1.995
8e		>100	1.51	87.73	97.52	-2.094
8f		>100	1.51	87.73	97.52	-2.094
8g		25 ± 9	1.54	93.54	97.52	-2.232
8h		16 ± 4	1.96	98.14	97.52	-2.506
8i		55 ± 10	1.78	99.51	97.52	-2.644

[a] Values are mean ± SEM from three independent experiments, each carried out in triplicate; [b] measured in the absence of pantetheinases; data from reference 8; [c] data from reference 30.

A two-carbon linker variant of compound **6a**, the 1,5-substituted triazole **6b**, was also synthesized using the same method as described for compound **6a** (Scheme 1). The combination of the two suboptimal features, 1,5-substitution and two-carbon linker, was found to be highly detrimental to antiplasmodial activity, as concluded from the measured IC_{50} value >100 μM (Table 1).

The effect of branching in the linker was next investigated with a methyl group added to the one-carbon linker (compounds **8e**,**f**, Scheme 2). As for compounds **8a**–**d**, they were synthesized by CuAAC and reaction with D-pantolactone, but from

3d′,**e′** and pentyl bromide in the presence of sodium azide. Compound **3d′** was prepared from Boc-D-alaninol via Dess-Martin periodinane (DMP) oxidation to generate the desired aldehyde, before conversion to alkyne **3d′** with the use of dimethyl (1-diazo-2-oxopropyl)phosphonate (DDOP or Bestmann-Ohira reagent) (Scheme 3). Compound **3e′** was similarly synthesized but directly from commercial Boc-L-alaninal (Scheme 3). Antiplasmodial activity measurement (Table 1) revealed IC_{50} values above 100 μM for both **8e** and **8f**, implying that branching of the linker is detrimental to activity, at least with a one-carbon linker. This contrasts with the beneficial effect reported for alpha-methylation of the two-carbon linker in the pantothenamide series.[27]

The most potent antiplasmodial pantothenamide reported so far is phenethyl-Pan (Figure 1), which is not stable in serum as demonstrated by its IC_{50} value increasing 3,600-fold in the presence of pantetheinases.[8] Compared to its analog, N5-Pan, phenethyl-Pan is 100-fold more active in the absence of pantetheinases.[8] Inspired by this, a small series of 1,4-substituted triazole-containing compounds including a phenyl group in the triazole *N*-substituent, as in compounds **8c**,**g–i**, were prepared starting from **3a′** or **3b′**, and the corresponding alkyl bromides in the presence of sodium azide (Scheme 2). Unexpectedly, none of them had a superior antiplasmodial activity when compared to compound **1** or **2**, or even N5-Pan (Table 1).

Previous studies have concluded that compounds **1** and **2** are stable in the presence of pantetheinases and in human serum based on the similar IC_{50} values obtained in the presence and absence of pantetheinases.[30] To further demonstrate the stability of triazole derivative **8b** under the conditions of our assay, compound **8b** was incubated in Albumax II (a highly-purified BSA preparation used as a serum replacement to grow *P. falciparum in vitro*) for 4 days. N5-Pan was used as an example of an unstable compound. As shown in Table 2, N5-Pan was fully degraded after 4 days in the presence of Albumax II and/or BSA (both of which contain pantetheinases), while compound **8b** was stable under all the conditions tested.

Table 2. Stability of N5-Pan and **8b** under different conditions, as measured by LCMS. Values are mean ± SEM from two independent experiments.

Components in K*P*$_i$ buffer	Remaining after 4 days (± SEM, %)
N5-Pan	90 ± 29
N5-Pan + Albumax II	0
N5-Pan + BSA	0
N5-Pan + BSA + Albumax II	0
8b + BSA	84 ± 5
8b + BSA + Albumax II	100 ± 16

Discussions

It can be difficult to draw SARs with pantothenamides or their analogs,[5],[8],[10],[16],[18],[24],[25],[30] because their antimicrobial activity is affected by multiple factors such as the participation of more than one enzyme during bioactivation, the possible involvement of multiple different targets in the mode of action, as expected for pantothenate antimetabolites, and of course, cell permeability. Our results with triazole derivatives mimicking pantothenamides clearly imply that the 1,4-substitution pattern on the triazole ring, with a simple methylene linker between the triazole and the pantoyl moieties, can overcome all the mechanistic barriers and display antiplasmodial activity at low nanomolar concentrations.

As shown in Table 1, the antiplasmodial activity of the triazole derivatives is not correlated to predicted lipophilicity (LogP), molar refractivity (MR), topological polar surface area (tPSA) or aqueous solubility (LogS). The results presented herein are consistent with the triazole ring of compound **8b** being a good mimic of the amide bond of N5-Pan, while the use of a shorter linker, as in compounds **1** and **2**, may lead to a different binding mode to the target(s) and/or activating enzymes, with enhanced antiplasmodial activity. Furthermore, we believe that this shorter linker has serendipitously rendered the triazole in compound **1** a bioisostere of the flipped amide in compound **9** (Figure 3), a pantothenamide mimic with high nanomolar antiplasmodial activity and stability to pantetheinases.[29] We propose that the triazole N-2 of compound **1** may mimic the carbonyl oxygen of the flipped amide bond in compound **9** more closely than the triazole N-3, while the triazole N-3 of **8b** is a better mimic of the carbonyl oxygen of the (original orientation) amide bond in N5-Pan (Figure 3). The 10-fold improved activity of **1** over that of **9**, may be explained by extra interactions possible via N-1 and/or N-3 of **1**. That N-1 may be involved in additional favorable interactions is implied from comparing compounds **8b** and **8d**, which suggests that a nitrogen atom is preferred over a carbon atom at the site of attachment of the alkyl group. Overall, our proposed arrangement of the triazole to mimic an amide bond is consistent with the similar IC_{50} values observed for compounds **1** and **9**, for compounds **8b** and N5-Pan, and also for the previously reported compounds **10**[25] and **11**[30] (Figure 3).

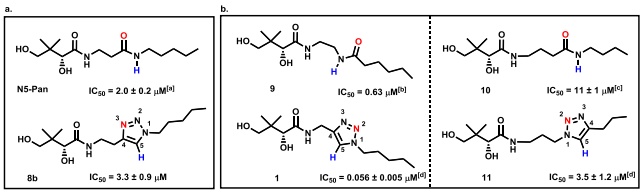


Figure 3. a) In compound **8b**, the triazole ring is designed to mimic the amide bond of N5-Pan, as proposed by Tron *et al.* for 1,4-substituted triazoles.[32] Thus N-3 of the triazole (red) may mimic the H bond acceptor (C=O) of the amide (red), and the triazole hydrogen substituent (blue) may mimic the amide H bond donor (blue). b) In contrast, we propose an additional way in which triazoles may mimic amide bonds, where the triazole N-2, instead of N-3, mimics the C=O H bond accepting capabilities of the amide. Unless otherwise noted all IC_{50} values were measured in the presence of pantetheinases. Except for N5-Pan, all compounds shown in this figure are not substrates of pantetheinases. [a] Measured in the absence of pantetheinases; data from reference 8; [b] data from reference 29; [c] data from reference 25; [d] data from reference 30.

Conclusions

In conclusion, we propose that the triazole ring can mimic amide bonds in different ways, which should all be considered when optimizing compound structure. It has been suggested that 1,4-substituted 1,2,3-triazole rings display structural similarity to *Z*-amides, with the lone pair of the triazole N-3 mimicking the carbonyl oxygen of the amide bond, the triazole H-5 acting as a H bond donor, like the amide NH, and the electrophilic and polarized triazole C-4 being electronically similar to the carbonyl carbon.[32] Our results suggest that in some cases the triazole N-2 (instead of N-3) may mimic the carbonyl oxygen of the amide bond. This is consistent with the fact that in 1,2,3-triazoles, both N-2 and N-3 are known to react with electrophiles.[34]

The 1,2,3-triazole ring is among the most commonly used scaffolds in drugs.[35],[36] The triazole derivatives **1** and **2** are stable in serum, show excellent antiplasmodial activity, are not toxic to human cells[30] and have a new mode of action compared to clinical drugs,[9]–[11] making them attractive hits for the development of novel antimalarials.

Experimental Section

Chemistry

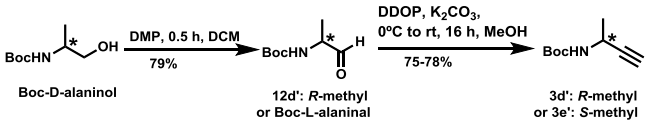
Materials and methods

All reagents were purchased either from Chem Impex International Inc. or Sigma-Aldrich Canada. Reagents and solvents were used without further purification. Dry solvents were obtained from an Innovative Tech Pure Solve MD-7 Solvent purification system. MilliQ-quality water was used whenever water is mentioned. Flash chromatography was performed on RediSep Rf Gold Silica Flash Chromatography Columns from Teledyne ISCO. TLC analysis (F-254) was performed with 60 Å silica gel TLC plates from Silicycle. HRMS spectra were acquired at the McGill University Mass Spectral Facility on an EXACTIVE instrument in orbitrap mode. The NMR chemical shifts (δ) are reported in parts per million (ppm) and are referenced to the internal standard TMS. Compound purity was determined by reverse phase HPLC analyses using an Agilent 1100 modular system. An UltiMate 3000 UHPLC System from Thermo Fisher Scientific coupled to a MaXis Impact HD Mass Spectrometer from Bruker were used for the stability study. Unless otherwise mentioned, all the reactions were performed under nitrogen atmosphere.

General protocol 1 for synthesis of compounds 3a′–b′

Compounds **3a′–b′** were prepared using a previously reported method with small modifications as detailed below.[30] In a round-bottom flask (100 mL), the desired alkynyl amine hydrochloride (4.74 mmol, 1 equiv.) was dissolved in H_2O/dioxane (2:1, 30 mL), before addition of sodium bicarbonate (9.47 mmol, 2 equiv.). The solution was cooled on ice for 10 min. A solution of di-*tert*-butyl dicarbonate (6.16 mmol, 1.3 equiv.) in dioxane (10 mL) was next added slowly. The mixture was allowed to warm to room temperature and stir for 16 hours. Next, water (50 mL) was added to dilute the reaction mixture. The resulting aqueous solution was extracted with ethyl acetate (4 × 15 mL). The combined organic layers were dried over anhydrous sodium sulfate and the solvent was evaporated *in vacuo* to give the crude product, which was purified by flash chromatography on silica gel using a gradient of 0–30% ethyl acetate/hexanes.

General protocol 2 for synthesis of compounds 3d′–e′



Scheme 3. Synthetic method for preparation of compounds **3d′–e′**.

Compounds **3d′–e′** were prepared using a previously reported method with small modifications as detailed below.[37] To an ice-cooled methanol solution (3 mL) of Boc-alanine aldehyde (1.73 mmol, 1 equiv.) and dimethyl (1-diazo-2-oxopropyl)phosphonate (1.99 mmol, 1.15 equiv.) was added K_2CO_3 (2.60 mmol, 1.5 equiv.). The mixture was stirred for 16 hours while allowed to warm to room temperature. The mixture was next filtered through Celite and concentrated *in vacuo*. The crude residue was purified by flash chromatography on silica gel using a gradient of 0–30% ethyl acetate/hexanes.

General protocol 3 for synthesis of Boc-protected 1,5-substituted triazoles (5a–b)

In a microwave vial (5 mL), sodium azide (0.97–2.07 mmol, 1.75 equiv.) dissolved in water (0.4 mL) and 1-bromopentane (0.96–1.77 mmol, 1.5 equiv.) in DMF (2.1 mL) were mixed and then heated to 100°C for 1 hour in a microwave reactor to complete the bromo-azide exchange. After cooling down, the crude reaction mixture was diluted with DCM (5 mL) and then dried over anhydrous sodium sulfate. The partially hydrated sodium sulfate was removed by filtration and the filtrate was concentrated to around 1.5 mL *in vacuo* to yield the crude pentyl azide.

In a glove box, $RuCl(PPh_3)_2Cp^*$ (0.02–0.08 mmol, 0.07 equiv.) dissolved in anhydrous dioxane (0.5 mL) was added to a pressure vessel (5 mL), before addition of the desired alkyne (0.50–1.18 mmol, 1 equiv.) pre-dissolved in dioxane (1 mL) and the crude azide also dissolved in dioxane (1.5 mL). The resulting mixture was allowed to react at 100°C for 16 hours. The crude product was purified by flash chromatography on silica gel using a gradient of 0-100% ethyl acetate/hexanes.

General protocol 4 for synthesis of Boc-protected 1,4-substituted triazoles (7a–i)

Compounds **7a–i** were prepared using a previously reported method with small modifications as detailed below.[30] The desired alkyl bromide (0.89–1.93 mmol, 1.5 equiv.) in DMF (4 mL), the alkyne (0.59–1.29 mmol, 1 equiv.) in DMF (3 mL), sodium azide (0.89–1.93 mmol, 1.5 equiv.) in water (1 mL), sodium ascorbate (0.24–0.52 mmol, 0.4 equiv.) in water (1 mL) and copper sulfate (0.1 equiv.) in water (3 mL) were individually added to a pressure vessel (20 mL). The reaction was heated to 100°C in an oil bath for 16 hours. Once the mixture had cooled down, 1 M aqueous ammonium hydroxide (28 mL) was added. The product was extracted in ethyl acetate (4 × 20 mL). The combined organic layers were dried over anhydrous sodium sulfate. After filtration to remove the solid, and evaporation of the solvent *in vacuo*, the crude product was loaded on silica gel and purified with flash chromatography using a gradient of 0–100% ethyl acetate/hexanes.

General protocol 5 for synthesis of final products (6a–b, 8a–i)

The above-mentioned Boc-protected 1,4-substituted triazole or 1,5-subsituted triazole (0.19–0.79 mmol, 1 equiv.) was dissolved in a 1:1 mixture of DCM and TFA (2–4 mL). The reaction was stirred at room temperature for 0.5–2 hours, or until completion of the deprotection as judged by TLC. Next, 1 M aqueous NaOH (10–20 mL) was used to quench the reaction, making sure that the final pH was higher than 9. The product was next extracted in ethyl acetate (4 × 10 mL). The combined organic layers were dried over anhydrous sodium sulfate. The solid was removed by filtration and the solvent was evaporated *in vacuo* to afford the crude free amine, which was used directly in the next step.

In a pressure vessel (5 mL), the free amine dissolved in anhydrous methanol or ethanol (2 mL) was mixed with D-pantolactone (0.75–3.16 mmol, 4 equiv.) and triethylamine (0.75–3.16 mmol, 4 equiv.). The reaction was then heated to 115°C for 16 hours in an oil bath. The reaction mixture was directly loaded on a silica gel column and purification was achieved with a gradient of 0–100% ethyl acetate/hexanes, followed by 0–50% methanol/ethyl acetate.

Synthesis of compound 12d′

Compound **12d′** was prepared using a previously reported method with small modifications as detailed below.[38] Boc-D-alaninol (5.71 mmol, 1 equiv.) in wet DCM (10 mL) was added to DMP (2.86 mmol, 0.5 equiv.). The mixture was stirred at room temperature for 15 min, before another batch of DMP (2.86 mmol, 0.5 equiv.) was added and the mixture was stirred for another 15 min. The reaction mixture was diluted in ethyl acetate (30 mL) and washed with aqueous $Na_2S_2O_3$/$NaHCO_3$ (6 g $Na_2S_2O_3$ dissolved in 20 mL of aqueous saturated $NaHCO_3$ solution). The ethyl acetate layer was subsequently dried over anhydrous sodium sulfate and concentrated *in vacuo*. Purification was achieved on silica gel using a gradient of 0–50% ethyl acetate/hexanes.

Biology

In vitro antiplasmodial activity assays

P. falciparum parasite (3D7 strain) culture and *in vitro* antiplasmodial activity assays were performed exactly as previously reported.[30]

Stability study

Briefly, to a solution of potassium phosphate buffer (5 mM, pH 7.5), Albumax II (at a final concentration of 5%), dithiothreitol (DTT, at a final concentration of 0.5 mM), bovine serum albumin (at a final concentration of 0.01%), and the tested compound (at a final concentration 1−5 μM) were added. The system was incubated for 4 days at 37°C. Immediately (time = 0) and after 96 h, 100 μL of the reaction mixture was removed, diluted 3−4 fold in cold methanol (−20°C), and vortexed for 5 min to terminate the reaction. Following removal of the precipitated protein by centrifugation (20 000 × *g*, 10 min), 1−20 μL of the supernatant was injected into the LCMS for analysis. N5-Pan was selected-ion-monitored at *m/z* = 289.2000 and 311.1943 ± 0.1, and compound **8b** was selected-ion-monitored at *m/z* = 313.2234 and 335.2054 ± 0.1. The elution conditions are listed in Table 3.

Table 3. LCMS conditions used for the stability study.

Flow rate: 0.3 mL/min; **Temperature**: 30°C		
Column: Luna 5μm C5 50 X 200 mm from Phenomenex		
Time (min)	**0.1% formic acid water solution (%)**	**MeOH (%)**
0	75	25
1	75	25
8	0	100
10	0	100
10.5	75%	25

Funding Sources

This work was financially supported by grants from the Australian National Health and Medical Research Council (to K.J.S. and K.A.; APP1129843) and the Canadian Institute of Health Research (to K.A.).

Acknowledgements

The China Scholarship Council is thanked for financial support to J.G. The Australian Government provided scholarships for E.T.T. and V.M.H. We are grateful to the Canberra branch of the Australian Red Cross Blood Service for providing red blood cells.

Keywords: Amide bioisostere • Pantothenamide • *Plasmodium falciparum* • Triazole • Vanin

References:

[1] World Health Organization. World malaria report. **2016**.
[2] B. K. Verlinden, A. Louw, L. Birkholtz. Resisting resistance: is there a solution for malaria? *Expert Opin. Drug Discov.* **2016**, *11*, 395–406.
[3] World Health Organization. Status report on artemisinin and ACT resistance. **2017**.
[4] T. N. C. Wells, R. H. van Huijsduijnen, W. C. Van Voorhis. Malaria medicines: a glass half full? *Nat. Rev. Drug Discov.* **2015**, *14*, 424–442.
[5] G. Clifton, S. R. Bryant, C. G. Skinner. *N*′-(substituted) pantothenamides, antimetabolites of pantothenic acid. *Arch. Biochem. Biophys.* **1970**, *137*, 523–528.
[6] C. Spry, K. Kirk, K. J. Saliba. Coenzyme A biosynthesis: an antimicrobial drug target. *FEMS Microbiol Rev.* **2008**, *32*, 56–106.
[7] W. J. Moolman, M. de Villiers, E. Strauss. Recent advances in targeting coenzyme A biosynthesis and utilization for antimicrobial drug development. *Biochem. Soc. Trans.* **2014**, *42*, 1080–1086.
[8] C. Spry, C. Macuamule, Z. Lin, K. G. Virga, R. E. Lee, E. Strauss, K. J. Saliba. Pantothenamides are potent, on-target inhibitors of *Plasmodium falciparum* growth when serum pantetheinase is inactivated. *PLoS One* **2013**, *8*, e54974.
[9] E. T. Tjhin, C. Spry, A. L. Sewell, A. Hoegl, L. Barnard, A. E. Sexton, V. M. Howieson, A. G. Maier, D. J. Creek, E. Strauss, R. Marquez, K. Auclair, K. J. Saliba. Mutations in the pantothenate kinase of the malaria parasite *P. falciparum* confer resistance or hypersensitivity to diverse pantothenate analogues. *PLoS Pathog.* **2018**, *14*, e1006918.
[10] M. de Villiers, C. Spry, C. J. Macuamule, L. Barnard, G. Wells, K. J. Saliba, E. Strauss. Antiplasmodial mode of action of pantothenamides: pantothenate kinase serves as a metabolic activator not as a target. *ACS Infect. Dis.* **2017**, *3*, 527–541.

[11] J. E. Chiu, J. Thekkiniath, J. Choi, B. A. Perrin, L. Lawres, M. Plummer, A. Z. Virji, A. Abraham, J. Y. Toh, M. V. Zandt, A. S. I. Aly, D. R. Voelker, C. B. Mamoun. The antimalarial activity of the pantothenamide α-PanAm is via inhibition of pantothenate phosphorylation. *Sci. Rep.* **2017**, *7*, 14234.

[12] E. Strauss, T. P. Begley. The antibiotic activity of *N*-pentylpantothenamide results from its conversion to ethyldethia-coenzyme A, a coenzyme A antimetabolite. *J. Biol. Chem.* **2002**, *277*, 48205–48209.

[13] Y. Zhang, M. W. Frank, K. G. Virga, R. E. Lee, C. O. Rock, S. Jackowski. Acyl carrier protein is a cellular target for the antibacterial action of the pantothenamide class of pantothenate antimetabolites. *J. Biol. Chem.* **2004**, *279*, 50969–50975.

[14] R. Leonardi, S. Chohnan, Y. Zhang, K. G. Virga, R. E. Lee, C. O. Rock, S. Jackowski. A pantothenate kinase from *Staphylococcus aureus* refractory to feedback regulation by coenzyme A. *J. Biol. Chem.* **2005**, *280*, 3314–3322.

[15] J. Tomas, J. E. Cronan. Antibacterial activity of *N*-pentylpantothenamide is due to inhibition of coenzyme A synthesis. *Antimicrob. Agents Chemother.* **2010**, *54*, 1374–1377.

[16] M. de Villiers, L. Barnard, L. Koekemoer, J. L. Snoep, E. Strauss. Variation in pantothenate kinase type determines the pantothenamide mode of action and impacts on coenzyme A salvage biosynthesis. *FEBS J.* **2014**, *281*, 4731–4753.

[17] S. J. Hughes, T. Antoshchenko, K. P. Kim, D. Smil, H. Park. Structural characterization of a new *N*-substituted pantothenamide bound to pantothenate kinase from *Klebsiella pneumoniae* and *Staphylococcus aureus*. *Proteins* **2014**, *82*, 1542–1548.

[18] S. J. Hughes, L. Barnard, K. Mottaghi, W. Tempel, T. Antoshchenko, B. S. Hong, A. Allali-Hassani, D. Smil, M. Vedadi, E. Strauss, H. Park. Discovery of potent pantothenamide inhibitors of *Staphylococcus aureus* pantothenate kinase through a minimal SAR study: inhibition is due to trapping of the product. *ACS Infect. Dis.* **2016**, *2*, 627–641.

[19] Z. L. P. Arnott, S. Nozaki, D. C. F. Monteiro, H. E. Morgan, A. R. Pearson, H. Niki, M. E. Webb. The mechanism of regulation of pantothenate biosynthesis by the PanD-PanZ·AcCoA complex reveals an additional mode of action for the antimetabolite *N*-pentyl pantothenamide (N5-Pan). *Biochemistry* **2017**, *56*, 4931–4939.

[20] L. Barnard, K. J. Mostert, W. A. L. van Otterlo, E. Strauss. Developing pantetheinase-resistant pantothenamide antibacterials: structural modification impacts on PanK interaction and mode of action. *ACS Infect. Dis.* **2018**, *4*, 736–743.

[21] K. J. Saliba, C. Spry. Exploiting the coenzyme A biosynthesis pathway for the identification of new antimalarial agents: the case for pantothenamides. *Biochem. Soc. Trans.* **2014**, *42*, 1087–1093.

[22] H. E. Pett, P. A. M. Jansen, P. H. H. Hermkens, P. N. M. Botman, C. A. Beuckens-Schortinqhuis, R. H. Blaauw, W. Graumans, M. van de Vegte-Bolmer, K. M. J. Koolen, F. P. J. T. Rutjes, K. J. Dechering, R. W. Sauerwein, J. Schalkwijk. Novel pantothenate derivatives for anti-malarial chemotherapy. *Malar. J.* **2015**, *14*, 169.

[23] P. A. M. Jansen, P. H. H. Hermkens, P. L. J. M. Zeeuwen, P. N. M. Botman, R. H. Blaauw, P. Burghout, P. M. van Galen, J. W. Mouton, F. P. J. T. Rutjes, J. Schalkwijk. Combination of pantothenamides with vanin inhibitors as a novel antibiotic strategy against gram-positive bacteria. *Antimicrob. Agents Chemother.* **2013**, *57*, 4794–4800.

[24] T. O. Akinnusi, K. Vong, K. Auclair. Geminal dialkyl derivatives of *N*-substituted pantothenamides: synthesis and antibacterial activity. *Bioorg. Med. Chem.* **2011**, *19*, 2696–2706.

[25] M. de Villiers, C. Macuamule, C. Spry, Y. M. Hyun, E. Strauss, K. J. Saliba. Structural modification of pantothenamides counteracts degradation by pantetheinase and improves antiplasmodial activity. *ACS Med. Chem. Lett.* **2013**, *4*, 784–789.

[26] A. Hoegl, H. Darabi, E. Tran, E. Awuah, E. S. C. Kerdo, E. Habib, K. J. Saliba, K. Auclair. Stereochemical modification of geminal dialkyl substituents on pantothenamides alters antimicrobial activity. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 3274–3277.

[27] C. J. Macuamule, E. T. Tjhin, C. E. Jana, L. Barnard, L. Koekemoer, M. de Villiers, K. J. Saliba, E. Strauss. A pantetheinase-resistant pantothenamide with potent, on-target, and selective antiplasmodial activity. *Antimicrob. Agents Chemother.* **2015**, *59*, 3666–3668.

[28] J. Guan, M. Hachey, L. Puri, V. Howieson, K. J. Saliba, K. Auclair. A cross-metathesis approach to novel pantothenamide derivatives. *Beilstein J. Org. Chem.* **2016**, *12*, 963–968.

[29] P. H. H. Hermkens, J. Schalkwijk, P. A. M. Jansen, P. Botman. Pantothenamide analogues. WO 2016/072854 A2. **2016**.

[30] V. M. Howieson, E. Tran, A. Hoegl, H. L. Fam, J. Fu, K. Sivonen, X. X. Li, K. Auclair, K. J. Saliba. Triazole substitution of a labile amide bond stabilizes pantothenamides and improves their antiplasmodial potency. *Antimicrob. Agents Chemother.* **2016**, *60*, 7146–7152.

[31] E. Bonandi, M. S. Christodoulou, G. Fumagalli, D. Perdicchia, G. Rastelli, D. Passarella. The 1,2,3-triazole ring as a bioisostere in medicinal chemistry. *Drug Discov. Today* **2017**, *22*, 1572–1581.

[32] G. C. Tron, T. Pirali, R. A. Billington, P. L. Canonico, G. Sorba, A. A. Genazzani. Click chemistry reactions in medicinal chemistry: applications of the 1,3-dipolar cycloaddition between azides and alkynes. *Med. Res. Rev.* **2008**, *28*, 278–308.

[33] B. C. Boren, S. Narayan, L. K. Rasmussen, L. Zhang, H. Zhao, Z. Lin, G. Jia, V. V. Fokin. Ruthenium-catalyzed azide-alkyne cycloaddition: scope and mechanism. *J. Am. Chem. Soc.* **2008**, *130*, 8923–8930.

[34] J. Hou, X. Liu, J. Shen, G. Zhao, P. G. Wang. The impact of click chemistry in medicinal chemistry. *Expert Opin. Drug Discov.* **2012**, *7*, 489–501.

[35] R. D. Taylor, M. MacCoss, A. D. G. Lawson. Rings in drugs. *J. Med. Chem.* **2014**, *57*, 5845–5859.

[36] S. Zhang, Z. Xu, C. Gao, Q. Ren, L. Chang, Z. Lv, L. Feng. Triazole derivatives and their anti-tubercular activity. *Eur. J. Med. Chem.* **2017**, *138*, 501–513.

[37] O. Krenk, J. Kratochvíl, M. Špulák, V. Buchta, J. Kuneš, L. Nováková, M. Ghavre, M. Pour. Methodology for synthesis of enantiopure 3,5-disubstituted pyrrol-2-ones. *Eur. J. Org. Chem.* **2015**, 5414–5423.

[38] X. Yan, T. O. Akinnusi, A. T. Larsen, K. Auclair. Synthesis of 4′-aminopantetheine and derivatives to probe aminoglycoside *N*-6′-acetyltransferase. *Org. Biomol. Chem.* **2011**, *9*, 1538–1546.