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Synthesis and Antimalarial Activities of a Diverse Set of Triazole-Containing Furamidine Analogues

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Four different series of triazole diamidines have been prepared by the Pinner method from the corresponding triazole dinitriles. Copper-catalyzed "click chemistry" was used for the synthesis of 1,4- and 4,5-substituted triazoles, aryl magnesium acetylide reagents for the 1,5-substituted triazoles, with a thermal dipolar addition reaction employed for the 2,4-substituted triazoles. In vitro antimalarial activity against two different PfCRT-modified parasite lines (*Science* **2002**, *298*, 210–213) of *Plasmodium falciparum* and inhibition of hemozoin formation were determined for each compound. Several diamidines with potent nanomolar antimalarial activities were identified, and selected molecules were resynthesized as their diamidoxime triazole prodrugs. One of these prodrugs, OB216, proved to be highly potent in vivo with an ED_{50} value of 5 mg kg^{-1} (po) and an observed 100% cure rate (CD_{100}) of just 10 mg kg^{-1} by oral (po) administration in mice infected with *P. vinckei*.

Introduction

Malaria is a major public health issue with about 50% of the global population at risk.[1] The disease is caused by protozoal parasites of the genus *Plasmodium* with *Plasmodium falciparum* being responsible for about 80% of all malaria cases and about 90% of deaths. In spite of global economic development, malaria is still present in 106 countries, with approximately 250 million new infections and one million deaths per year,[1,2] causing much devastation and a staggering amount of chronic ill health that not only impedes the economic development of these countries, already stricken by poverty, but also impacts on fertility, population growth, worker productivity, and premature mortality.[2,3] In the absence of a vaccine and resistance of *P. falciparum* to the majority of antimalarial drugs in current clinical use, new antimalarial agents are urgently needed to aid the prevention and control of malaria.[4] Cross-resistance is a problem among antimalarial agents due to a lack of chemical diversity. Therefore, the most promising strategy is the discovery of new chemical entities against novel biological targets.[5]

In a program directed at the synthesis of novel analogues of pentamidine, Das and Boykin inserted a furan unit as a linker between the two phenylamidine groups to produce 2,5-bis-(4-amidinophenyl)furan (furamidine; Figure 1), a molecule with potent antitrypanosomal activity.[6,7] Subsequently, the prodrug 2,5-bis(4-methoxyamidinophenyl)furan (pafuramidine) was developed by the same groups for the treatment of human African trypanosomiasis (HAT).[8,9] Pafuramidine reached phase III clinical trials against HAT and *P. jiroveci*,[10,11] although issues with hepatic and renal toxicity of this molecule in humans are likely to preclude its further development. Furamidine has potent antimalarial activity in vitro against chloroquine (CQ)-resistant *P. falciparum* (IC_{50} = 15.5 nM)[12] with low toxicity towards mammalian cells. The amidoxy prodrug pafuramidine has also been tested for the treatment of *P. vivax* and uncomplicated *P. falciparum* infections with a degree of success.[13]

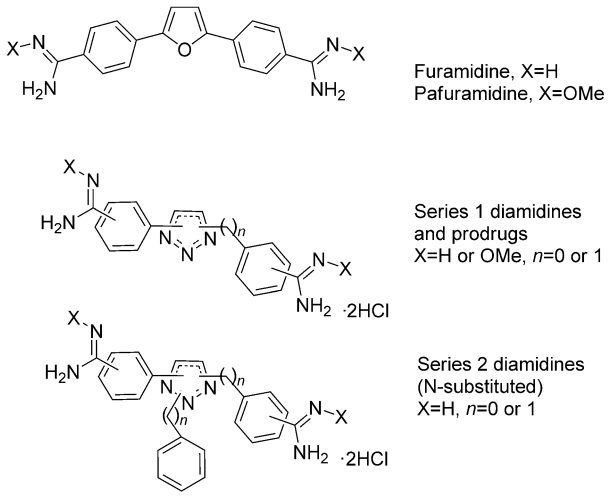

Figure 1. Structures of furamidine and pafuramidine and the general structures of the different triazoles (series 1 and 2) explored in this study.

The use of a triazole functional group as a linker is becoming increasingly popular in medicinal chemistry, and many diverse examples of incorporation of this heterocycle into a vari-

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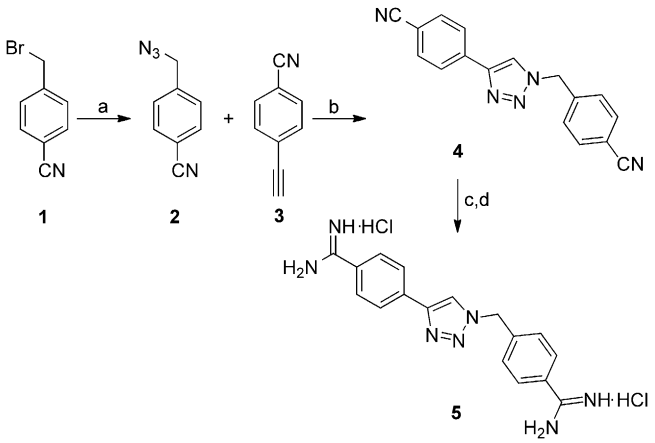
ety of drugs have appeared in the literature.[14–25] Given the structural diversity possible within the triazole ring system, we aimed to produce an array of functionalized triazole-containing diamidines for structure–activity relationship (SAR) analysis. Sixteen different triazole derivatives with different substitution on the triazole and aromatic rings (*para* or *meta*) were prepared (Figure 1): seven 1,4-substituted derivatives, two 1,5-substituted derivatives, two 2,4-substituted derivatives, and six 4,5-substituted derivatives. Our focus was to study the influence of the substitution pattern between the two amidine functions on antimalarial activity, as well as the position of the amidine functions on the aromatic ring. Based on the initial SAR data, we also planned to prepare diamidoxime prodrugs of the most active compounds identified in our in vitro screening, and to evaluate their in vivo performance versus *P. vinckei* by both oral (po) and intraperitoneal (ip) administration.

Diamidine drugs are impermeable to normal human erythrocytes, but are taken up rapidly into *P. falciparum*-infected erythrocytes through the new permeability pathway (NPP) induced by the parasite in the host cell membrane.[25] Once inside the infected cell, it is proposed that this class of drug binds strongly to hematin and kills parasites by inhibition of crystallization/formation of hemozoin in a similar manner to CQ.[26] Based on this potential mechanism of action, we planned to investigate the relationship between antimalarial activity in vitro and the ability to inhibit hemozoin formation for the triazole series described herein.



Scheme 1. *Reagents and conditions*: a) NaN_3, DMF, RT, 4 h; b) $CuSO_4{\cdot}5H_2O$, Na_2CO_3, ascorbic acid, L-proline, H_2O, DMSO, 65 °C, 3 h; c) gaseous HCl, EtOH, RT, 7 d; d) NH_3 2 M in MeOH, EtOH, RT, 24 h.

Results and Discussion

Chemistry

To construct the 1,4-substituted five-membered triazole ring, several methods have been reported with the most suitable being the Huisgen 1,3-dipolar cycloaddition of organic azides to terminal alkynes.[27] This method employs elevated temperatures and generally produces mixtures of 1,4- and 1,5-disubstituted 1,2,3-triazoles.[28,29] Recently, the use of copper(I) salts was shown to exclusively produce 1,4-disubstituted derivatives.[30] Therefore, we decided to use copper(I)-catalyzed azide-alkyne cycloaddition because of the high regioselectivity, mild reaction conditions, and excellent yields.

To synthesize the nonaromatic 1,4-substituted derivative **5** (Scheme 1), azide **2** was generated from the corresponding benzylbromide using sodium azide.[31] The triazole ring was then generated using copper(I)-catalyzed 1,3-dipolar cycloaddition of 4-(azidomethyl)benzonitrile (**2**) to 4-ethynylbenzonitrile (**3**) in a mixture of DMSO and water in 98% yield.[32–34] Finally, diamidine **5** was prepared using a modified Pinner method.[35–37]

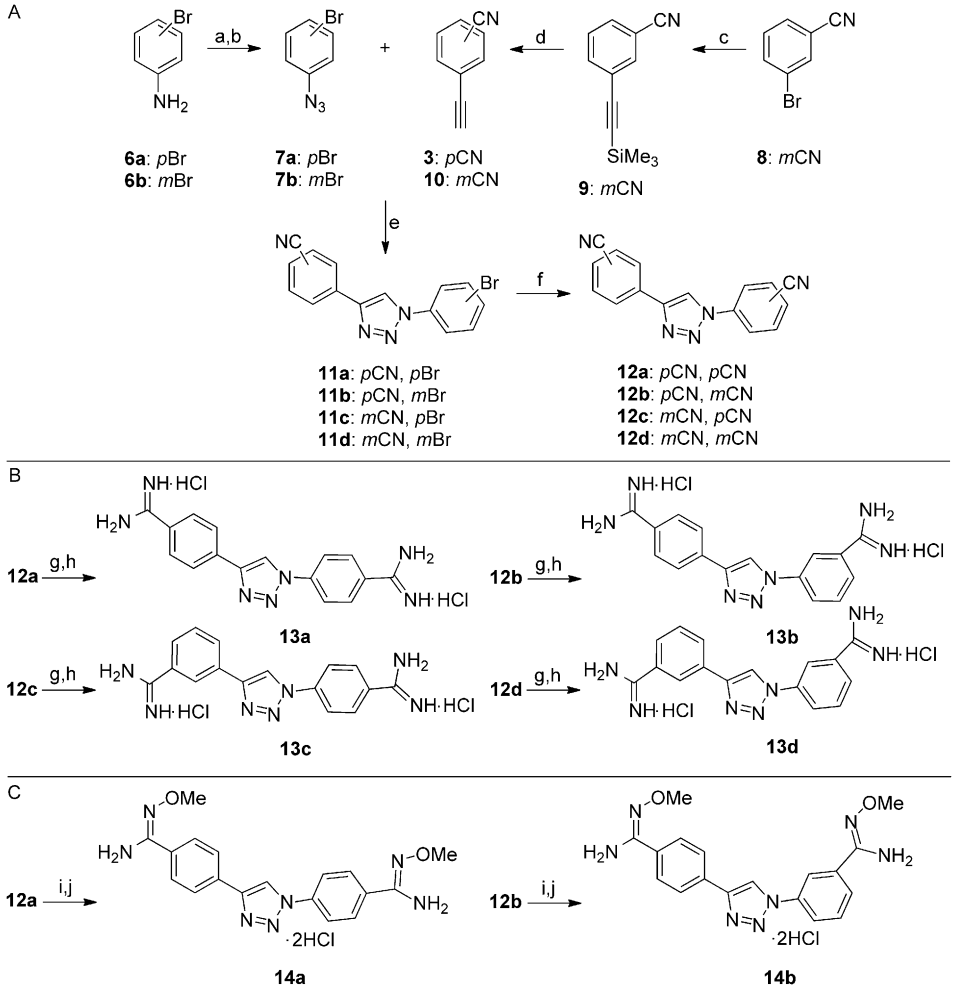
To synthesize the aromatic 1,4-substituted derivatives **13a–d** and **14a–b** (Scheme 2), 1-azido-4-bromobenzene (**7a**) and 1-azido-3-bromobenzene (**7b**) were prepared by diazotization/azidation of the 4- and 3-bromoaniline (**6a** and **6b**, respectively) using first sodium nitrite in dilute hydrochloric acid at 0–5 °C and then sodium azide.[38] The synthesis of alkyne **10** was performed by Sonogashira coupling between 3-bromobenzonitrile (**8**) and ethynyltrimethylsilane in the presence of palladium(0) and copper(I) iodide[39] followed by desilylation using tetra-*n*-butylammonium fluoride (TBAF) to furnish terminal alkyne **10** in 53% yield over two steps.[40] Then, 1,4-disubstituted triazoles **11a–d** were generated using copper(I)-catalyzed 1,3-dipolar cycloaddition in yields ranging from 93 to 97%.[32–34] Diamidines **13a–d** were prepared by initial cyanation of the bromine using copper cyanide in DMF[41] followed by a modified Pinner reaction.[35–37] The two 1,4-disubstituted prodrugs **14a–b** were produced by reacting dinitrile derivatives **12a–b** and hydroxylamine hydrochloride under basic conditions[42] and then alkylating the generated amidoximes using dimethylsulfate.[41,43]

Few methods were available for the preparation of the 1,5-substituted triazole unit from azides and alkynes. These included the Huisgen 1,3-dipolar cycloaddition,[27] ruthenium-catalyzed azide-alkyne cycloaddition,[44] and a method using aryl magnesium acetylides, generated in situ using ethyl magnesium bromide.[45,46] This latter method, developed by Zhang et al., was preferred because the 1,5-disubstituted derivatives were obtained exclusively in good yields, and no catalyst preparation is required.

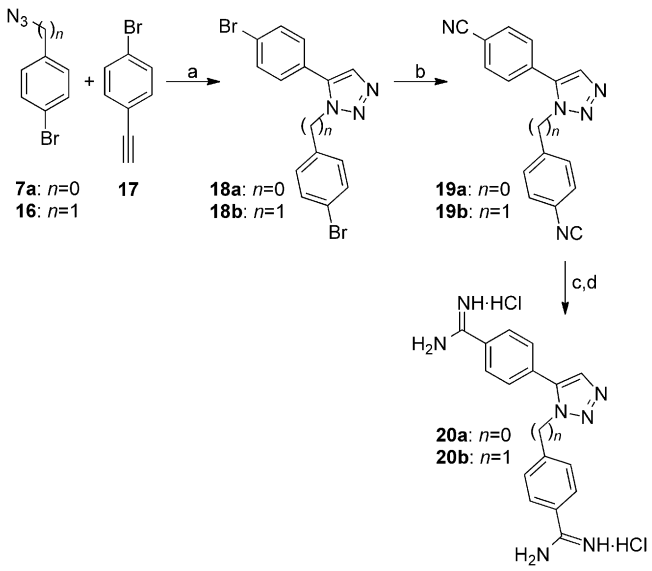
The nonaromatic and aromatic 1,5-disubstituted triazole derivatives **18a** and **18b** were obtained in yields ranging from 89 to 96% using ethyl magnesium bromide in THF.[45,46] Compounds **19a** and **19b** were allowed to react with copper cyanide in DMF to afford dinitrile derivatives **19a** and **19b** in moderate yields.[39] Diamidines **20a** and **20b** were prepared using a modified Pinner method (Scheme 3).[35–37]

To synthesize the 4,5-disubstituted and the 1,4,5- and 2,4,5-trisubstituted triazole units (Scheme 4), 4,4′-dicyanostilbene (**22**) was prepared as an intermediate for both series of compounds using Sonogashira reaction conditions between the 4-bromobenzonitrile (**21**) and the 4-ethynylbenzonitrile (**3**) in the presence of palladium(II), copper(I) iodide and triethylamine.[47]

The 1,4,5-trisubstituted triazole derivatives **25a** and **25b** were prepared using the copper(II)-catalyzed 1,3-dipolar cyclo-



Scheme 2. A) Synthesis of key nitriles **12a**–**d**; B) Preparation of diamidines **13a**–**d**; C) Preparation of prodrugs **14a** and **14b**. *Reagents and conditions*: a) $NaNO_2$, concd HCl, H_2O, 0 °C, 30 min; b) NaN_3, H_2O, RT, 1 h; c) ethynyltrimethylsilane, $Pd(PPh_3)_4$, CuI, Et_3N, 80 °C, 20 h; d) TBAF, −20 °C, 30 min; e) $CuSO_4{\cdot}5H_2O$, Na_2CO_3, ascorbic acid, L-proline, H_2O, DMSO, 65 °C, 3 h; f) CuCN, DMF, 170 °C, 24 h; g) gaseous HCl, EtOH, RT, 7 d; h) NH_3 2 M in MeOH, EtOH, RT, 24 h; i) $NH_2OH{\cdot}HCl$, K_2CO_3, EtOH, 90 °C, 20 h; j) dimethylsulfate, NaOH 2 M, 1,4-dioxane, RT, 20 h.



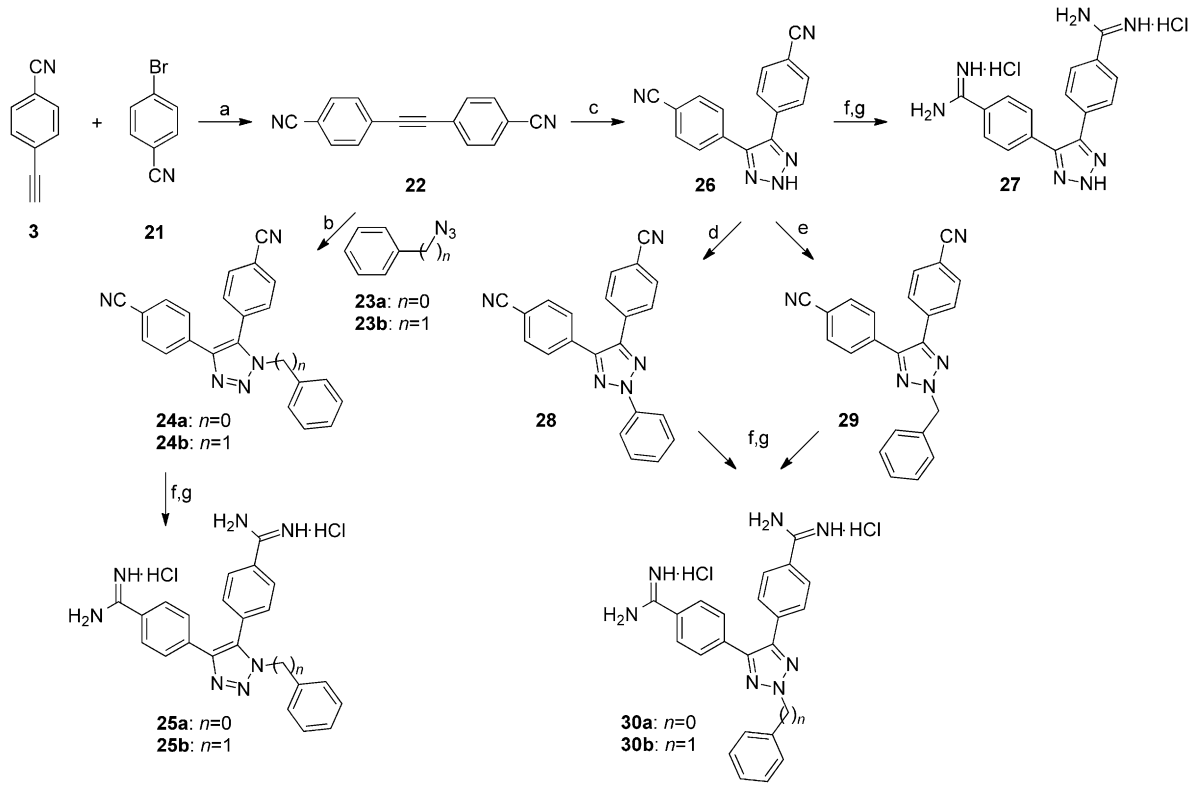
Scheme 3. *Reagents and conditions*: a) EtMgBr, THF, 50 °C, 1 h; b) CuCN, DMF, 170 °C, 24 h; c) gaseous HCl, EtOH, RT, 7 d; d) NH_3 2 M in MeOH, EtOH, RT, 24 h.

addition of azidobenzene **23a** or azidomethylbenzene **23b** to 4,4′-dicyanostilbene (**22**) at 65 °C, but the reaction did not work at this temperature probably due to steric hindrance; therefore, the temperature was increased to 120 °C.[32–34] The Pinner reaction was employed to obtain compounds **25a** and **25b** in yields of 22% and 39%, respectively, over two steps (Scheme 4).[35–37]

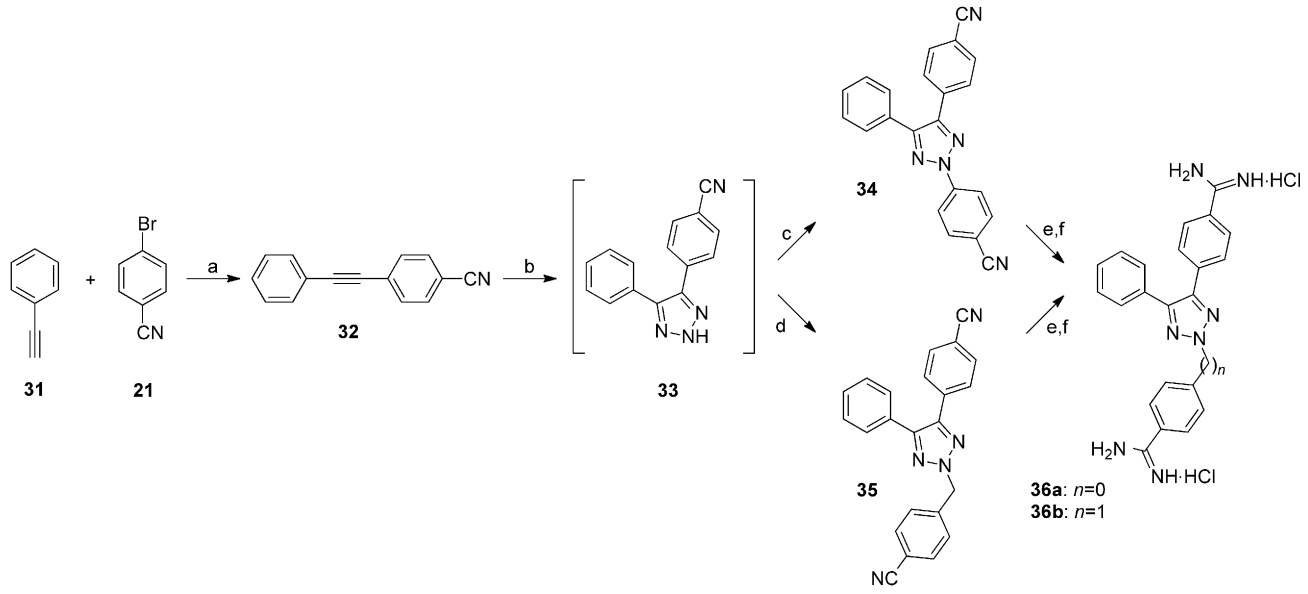
For the synthesis of 4,5-disubstituted derivative **27**, 4,4′-dicyanostilbene (**22**) was allowed to react with sodium azide at reflux in DMF to give the triazole unit in 65% yield (Scheme 4).[48] This reaction was attempted using the copper(I)-catalyzed 1,3-dipolar cycloaddition,[32–34] but failed possibly because the sodium azide was degraded by the catalyst (no reaction with 1.0 equiv of NaN_3, and only 25% product **26** formation with 2.0 equiv). Compound **27** was obtained from derivative **26** in 60% yield using the Pinner reaction.[35–37]

The 2,4,5-trisubstituted triazole derivatives **30a** and **30b** were prepared in two steps (Scheme 4). Compound **28** was prepared in 90% yield by alkylation of triazole derivative **26** with iodobenzene using an iron/copper co-catalyzed Ullmann condensation developed by Taillefer et al.[49] Compound **29** was obtained in 76% yield, again by alkylation of triazole derivative **26**, but with benzylchloride in the presence of potassium carbonate and tetrabutylammonium iodide (TBAI).[48] During this reaction, 19% of 1,4,5-benzyltriazole **24b** was also recovered. The two diamidines **30a** and **30b** were obtained using the Pinner methodology in 71% and 66% yields, respectively.[35–37]

For the 2,4-disubstituted series (Scheme 5), 4-phenylethynylbenzonitrile (**32**) was formed in 98% yield using the Sonogashira methodology between 4-bromobenzonitrile (**21**) and phenylacetylene (**31**) in the presence of palladium(II) and copper(I) iodide in dry triethylamine.[47] The triazole unit was then prepared by reaction between nitrile **32** and sodium azide in DMF.[48] Compound **33** was unstable and so used immediately for the next step. Compound **34** was obtained in 32% yield by alkylation of triazole **33** with 4-bromobenzonitrile using the iron/copper co-catalysed Ullmann condensation developed by Taillefer et al.[49] The low yield can be explained by the instabili-



Scheme 4. *Reagents and conditions*: a) PPh_3, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, 100 °C, 2 h; b) $CuSO_4{\cdot}5H_2O$, Na_2CO_3, ascorbic acid, L-proline, H_2O, DMSO, 120 °C, 15 h; c) NaN_3, DMF, 170 °C, 5 h; d) Cs_2CO_3, CuO, $Fe(acac)_3$, DMF, 90 °C, 30 h; e) K_2CO_3, TBAI, acetone, 70 °C, 20 h; f) gaseous HCl, EtOH, RT, 7 d; g) NH_3 2 M in MeOH, EtOH, RT, 24 h.



Scheme 5. *Reagents and conditions*: a) PPh_3, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, 100 °C, 2 h; b) NaN_3, DMF, 170 °C, 5 h; c) Cs_2CO_3, CuO, $Fe(acac)_3$, DMF, 90 °C, 30 h; d) K_2CO_3, TBAI, acetone, 70 °C, 20 h; e) gaseous HCl, EtOH, RT, 7 d; f) NH_3 2 M in MeOH, EtOH, RT, 24 h.

ty of triazole **33** and also by the duration of the alkylation. Compound **35** was obtained in 74% yield also by alkylation of triazole **33** with 4-bromomethyl benzonitrile in the presence of potassium carbonate and TBAI.[48] The two diamidines **36a** and **36b** were obtained using the Pinner methodology in 63% and 71% yields, respectively.[35–37]

In vitro antimalarial activities

In vitro antimalarial activities were evaluated for each derivative using two different strains of *P. falciparum* with varying CQ sensitivity: $C2^{GCO3}$ is a CQ-sensitive strain, and $C3^{Dd2}$ is a CQ-resistant strain (Table 1).[50] These strains were developed in the Fidock laboratory to probe the role of PfCRT in CQ resistance. The $C2^{GC03}$ line is a recombinant clone generated from the CQ-sensitive GC03 line transfected with its own wild-type allele, and it has been shown to display the full CQ-sensitive phenotype. $C3^{Dd2}$ was produced by allelic modification of the *pfcrt* locus in the GC03 line and expresses the Dd2 mutant CQ-resistant *pfcrt* alleles. Inhibition of hemozoin formation was determined by the ability of the different agents to inhibit the formation of hemozoin according to the method of Stead et al.[51] For each compound, the IC_{50} value was determined and expressed in micromolar (Table 1).

Table 1. Antimalarial activities and heme crystallization of triazole derivatives.

Compd	Series no.	$IC_{50} \pm SD$ [nM][a]		Cytotoxicity IC_{50} [μM][g]	
		$C2^{GC03}$[b]	$C3^{Dd2}$[c]	[μM]	Hemozoin
CQ[a]	NA	11.0 ± 5.3	95.63 ± 27.4	117[e]	18
Pentamidine	NA	81.1 ± 29.5	25.0 ± 8.32	46.6[e]	34
5	1	880.5 ± 64.0	1090 ± 84	–	45
13a (OB076)	1	87.2 ± 7.8	14.2 ± 5.4 (2.0)[d][52]	8.10[e] 27.5[f]	9.0
13b (OB125)	1	276.5 ± 24.0	79.5 ± 14.0 (4.0)[d][52]	86.0[e] 29.2[f]	61
13c	1	440.0 ± 54.3	145 ± 24.1 (3.0)[d][52]	8.6[e]	18
13d	1	866.0 ± 27.5	300 ± 26.1 (21.0)[d][52]	219.0[e]	6.3
14a (OB216)	1	> 1000	> 1000	55.2[f]	> 100
14b (OB135)	1	> 1000	> 1000	57.1[f]	> 100
20a	2	199 150 ± 728	75 920 ± 191	ND	9.0
20b	2	28 000 ± 574	7615 ± 167	ND	41
25a	2	4400 ± 89	1896 ± 64	ND	26
25b	2	129 500 ± 373	30 490 ± 676	ND	10
27	1	571 200 ± 345	165 010 ± 403	ND	706
30a	2	808 ± 56	731.3 ± 42	ND	ND
30b	2	1856 ± 95	738.2 ± 21.1	ND	5.0
36a	2	750 ± 22	331 ± 88	ND	51
36b	2	1040 ± 43	2200 ± 38	ND	115

[a] Chloroquine (CQ). [b] *P. falciparum* (CQ-sensitive). [c] *P. falciparum* (CQ-resistant). $C2^{GC03}$ and $C3^{Dd2}$ were produced by allelic modification of the *Pfcrt* locus in the GC03 line and express the GC03 wild-type CQ-sensitive sensitive and the Dd2 mutant CQ-resistant *pfcrt* alleles, respectively; $n = 3$ for IC_{50} data. [d] K1 strain. [e] Against L6 rat myoblast cells; values are the mean of duplicate determinations; values taken from Ref. [52]. [f] Against human HL60 cell lines; average of two determinations. [g] Hemozoin formation inhibition. ND = not determined. NA = not applicable.

The aim of this work was to build on the previous studies of Tidwell et al.[53] and compare additional analogues within the triazole diamidine family with the best hits identified in the previous study. In addition, we were also keen to: 1) explore the potential of the most promising analogues in their prodrug amidoxime form; and 2) to investigate potential correlations between hematin dimerization inhibition and inherent antiplasmodial activity versus both CQ-sensitive and CQ-resistant strains of the parasite.

Against the CQ-sensitive $C2^{GC03}$ strain, the 1,4-diaryl-substituted triazoles **13a**, **13b**, and **13c** exhibit good antiplasmodial activity, with IC_{50} values of 87, 276, and 440 nM, respectively, while a weak antiplasmodial activity was observed for compounds **5** and **13d** (880 nM and > 866 nM, respectively). As expected, the best three triazole-containing antimalarial agents expressed better activity versus the CQ-resistant strain by between three- and five-fold, with **13a** expressing excellent activity ($IC_{50} = 14.2$ nM). Indeed, this pattern of antimalarial potency by diamidines versus CQ-sensitive and CQ-resistant parasites has been noted previously in the literature.[51] By using the $C2^{GC03}$ and $C3^{Dd2}$ clones, we demonstrate higher potency for diamidines against the CQ-resistant strain, and furthermore, our results point to a key role in the membrane transporter protein PfCRT mediating sensitivity to dicationic drugs.[50]

Notably in previous work, the IC_{50} values[3] for compounds **13a–c** were in the single-digit nanomolar region[53]—the reason for the discrepancy may be due to the fact that, in our assays, the total incubation time was 48 h, whereas 72 h was used previously. This observation suggests a time-dependent mechanism of action, which may be due to stage-specific target presentation. We are currently performing experiments to further investigate this observation.

The results show that for compounds **13a–d**, IC_{50} values are influenced by several factors, with the position of the amidine function on the phenyl ring significantly influencing antimalarial activity. Substitution at the *para* position in both aromatic rings provided the most potent activity, with *meta*/*para* combinations less effective. Remarkably, insertion of a methylene spacer between the heterocyclic ring system and the aromatic ring causes a large decrease in activity (**5** vs **13a**). The 2,4-substituted analogues **36a** and **36b** exhibit weak antiplasmodial activities with IC_{50} values of 750 and > 1 μM for **20a** and **20b**, respectively, against CQ-sensitive parasites.

As observed for **13a–d**, compound **36a** exhibits greater potency against CQ-resistant parasites with a twofold increase in activity. Compounds **20a**, **20b**, and **36b** exhibit no antiplasmodial activity ($IC_{50} > 1000$ nM). Unfortunately, the 4,5-substituted analogues also exhibit poor antiplasmodial activities. Compound **30a** exhibited an IC_{50} value of 808 nM, and only weak antiplasmodial activity was observed for derivatives **27**, **30b**, **25a**, and **25b**. Analogues **30a** and **30b** also exhibit poor antiplasmodial activity against the CQ-resistant strain with IC_{50} values of 731 and 738 nM, respectively. Compounds **25a**, **25b**, and **27** also exhibit poor antiplasmodial activity (> 1000 nM). Thus, a general trend, observed regardless of the actual substitution pattern, was that an α-atom linkage through the central triazole heterocycle, either on nitrogen or carbon, resulted in reduced in vitro activity.

For the more potent analogues, cytotoxicity data against L6 rat myoblast and HL60 cell lines are given (Table 1). For **13a**, **13b**, and prodrugs **14a**, and **14b** low cytotoxicity was observed with good overall in vitro therapeutic indices (> 500).

Previous work has shown that pentamidine accumulation and activity can be blocked by inhibitors of hemoglobin digestion, suggesting that pentamidine activity relies on the release of ferriprotoporphyrin IX (FPIX) during hemoglobin proteolysis.[25] This phenomena is not restricted to pentamidine, and it was clearly shown by Stead et al.[25] that antimalarial activity and accumulation of propamidine, stilbamidine, and berenil were also antagonized by protease enzyme inhibitors, reinforcing the proposal that FPIX liberated from hemoglobin is a key target for this class of drug. From Table 1, it is clear that all of the triazole analogues are quite active at inhibiting hemozoin formation in this assay, and several are more effective than pentamidine. However, there is no correlation between the measured IC_{50} value for hemozoin inhibition and the measured antimalarial activity. This is particularly striking for **20a** and **25b**, both of which are more effective than pentamidine in this assay yet are effectively inactive as antimalarial agents up to 1000 nM. Possible explanations for this observation could be that these molecules have poor penetration through the NPP pathway, or triazole diamidines have additional parasite targets apart from FPIX.[54–58]

In vivo antimalarial activities

Based on the in vitro activity profiles, the 1,4-bisaryl analogues emerged as the most promising subset, and amidoxime prodrugs **14a** and **14b**, prepared as described in Scheme 2, were selected for evaluation in vivo against the *P. vinckei petteri* (279 BY) strain that infects mice.[59] Both drugs **13a** and **13b** showed potent antimalarial activity with complete clearance of blood parasitemia in infected mice and complete cure without recrudescence after ip administration. The doses able to reduce parasitemia by 50% (ED_{50} values) were 0.41 mg kg^{-1} and 0.94 mg kg^{-1} for **13a** (OB076) and **13b** (OB125), respectively (see Figure 2a). These activities are of the same order of magnitude as that of CQ, which has an ED_{50} value (ip) of 1.1 mg kg^{-1}. In addition, compounds **13a** and **13b** achieved a total cure of *P. vinckei*-infected mice (without recrudescence) at doses of just 0.8 mg kg^{-1} and 2 mg kg^{-1} by ip route. Notably, we observed a large difference between the ED_{50} values (ip) of

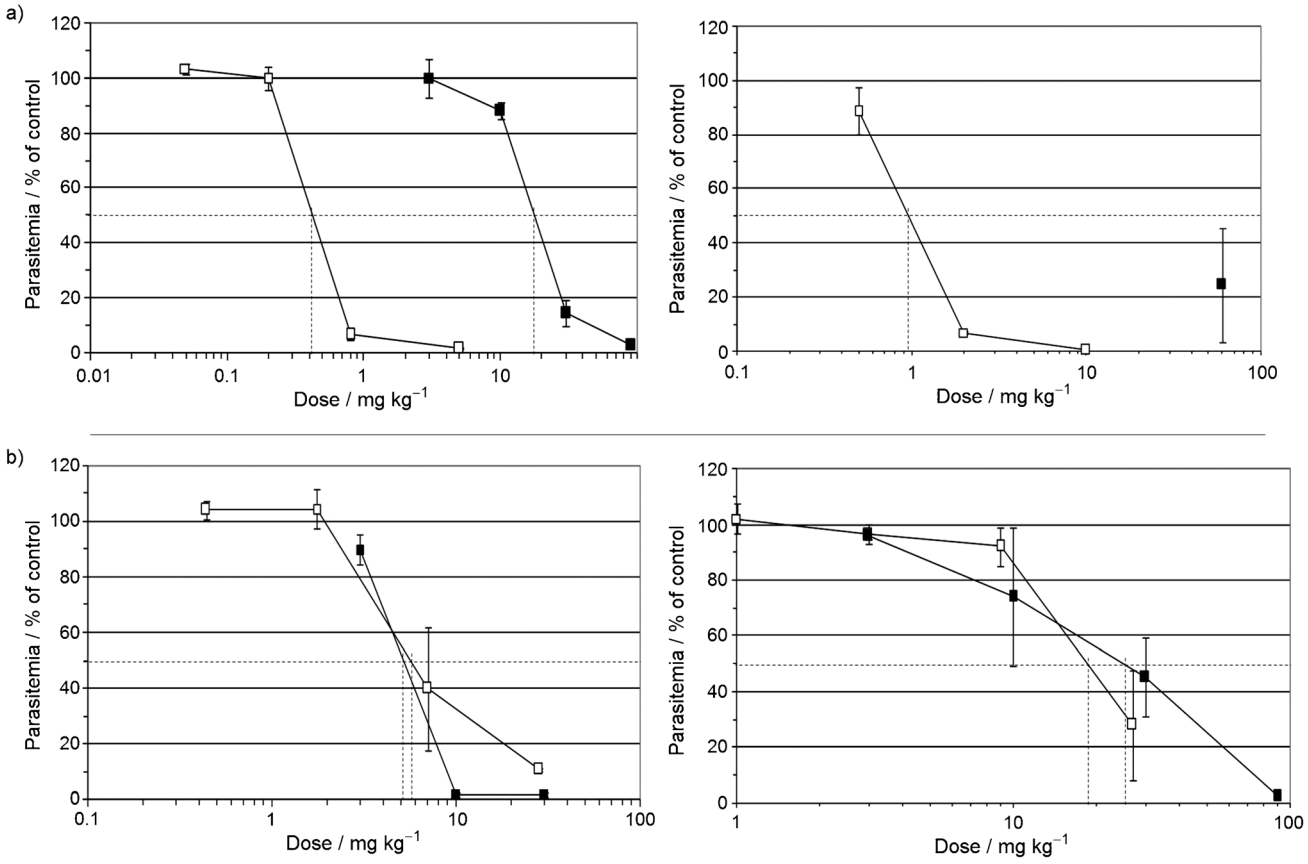


Figure 2. a) In vivo antimalarial properties of **13a** (OB076; left) and **13b** (OB125; right) at different doses against *P. vinckei*-infected mice. Treatment consisted of one daily ip injection (□) or po (■) for four consecutive days. Parasitemia were monitored at on day 5. Results shown are the mean of at least three mice per dosage ± SEM. ED_{50} (ip) values are 0.41 mg kg^{-1} and 0.94 mg kg^{-1} for **13a** and **13b**, respectively, and ED_{50} (po) values are 18 mg kg^{-1} and < 60 mg kg^{-1}, respectively. b) In vivo antimalarial properties of the bioprecursors **14a** (OB216; left) and **14b** (OB135; right) at different doses against *P. vinckei*-infected mice. Treatment consisted in one daily ip (□) or po (■) injection for four consecutive days. Parasitemia were monitored at on day 5. Results shown are the mean of at least three mice per dosage ± SEM. ED_{50} (ip) values are 5.8 mg kg^{-1} and 18 mg kg^{-1} for **14a** and **14b**, respectively, and ED_{50} (po) values are 5.1 mg kg^{-1} and 23 mg kg^{-1}, respectively.

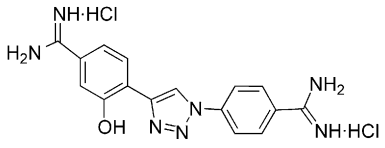
prodrugs compared to the ED_{50} values (ip) of the parent drugs, that is, **13a** versus **14a**, and **13b** versus **14b**. Using ip administration, the prodrugs are 14- to 19-fold less active than the parent drugs (see Figure 2b).

After po administration of 60 mg kg^{-1}, drug **13b** is less active than drug **13a** with a decrease in parasitemia of 76% and 100%, respectively. Further experiments showed that drug **13a**, the most potent drug in vitro and in vivo, has an ED_{50} value by oral (po) route of 18 mg kg^{-1} and causes a complete cure at 30 mg kg^{-1}.

Prodrug **14b** exhibited good activity when administered orally, with an ED_{50} value of 23 mg kg^{-1}, similar to the ED_{50} value observed with ip administration of 18 mg kg^{-1}. The ip/po ratio, which is an indicator of the oral bioavailability, is 78%, indicating a likely high absorption of the prodrug when administered by the oral route. Prodrug **14a** showed even better activity, with similar ED_{50} values of 5.8 mg kg^{-1} and 5.1 mg kg^{-1} for ip and po, respectively. The ip/po ratio of this prodrug is about 100%, which might indicate similar absorption of the prodrug when administered by either the ip and oral route.

The in vivo results clearly demonstrate that of the 1,4-biaryl-substituted analogues, those with amidine groups in the *para* position of the A-ring and *meta* position of C-ring (i.e., **13b**) are less active than their *para*-position counterparts (i.e., **13a**). The same result was observed for the prodrugs, indicating that the position of the proamidine moiety is also important for inherent potency. The difference in activity between the *meta*-substituted C-ring functionalization and *para*-substituted C-ring is also found in vitro for drugs **13a** and **13b** against $C2^{GC03}$ and $C3^{Dd2}$ strains, and this trend was also seen in previous work by Tidwell et al.[53] Interestingly, against a CQ-sensitive Nigerian strain, similar IC_{50} values were observed for **13a** and **13b**, demonstrating that strain-dependent sensitivity to this class of compound is apparent.

The curative dose (CD_{100}) values for prodrugs **14a** and **14b** are 10 mg kg^{-1} and 30 mg kg^{-1}, respectively, in this experiment. Notably, when administered subcutaneously at 30 mg kg^{-1} daily for four days, therelated triazole **37**, with an in vitro activity of 0.6 nM against K1 *P. falciparum*, did not reduce parasitemia in a mouse model of *P. berghei*.[53] We previously demonstrated that membrane-impermeable diamidines gain access into the infected red blood cell through an induced pathway termed the NPP. This pathway is absent in *P. berghei*-infected blood cells, and as such, diamidines are inactive in this in vivo model, underlining that alternative murine species of *Plasmodia* should be considered for the appropriate assessment of for cationic drugs as potential antimalarial agents.



37 (IC_{50}=0.6 nM; K1 strain)

Conclusions

Sixteen different triazole derivatives with different substitutions across both the triazole ring system and on the aromatic ring (amidine functional group *para* or *meta* in A/C rings) were prepared to probe further SAR in the triazole diamidine family of potential antimalarial agents. Two compounds in this series, **13a** (OB076) and **13b** (OB125), have good in vitro activity against human *P. falciparum* parasites. Moreover, data generated in the $C2^{GC03}$ and $C3^{Dd2}$ clones demonstrate a role for PfCRT in mediating sensitivity to diamidine-based drugs, and this is the subject of additional research in our laboratories. We have established for the first time the potent in vivo activity of these compounds against the rodent *P. vinckei* parasite with ip administration. Interestingly, after a four-day treatment regime, these two compounds were able to completely cure mice of malaria infection. Prodrugs **14a** (OB216) and **14b** (OB135) are only weakly active in vitro indicating that the prodrug/drug conversion does not occur in vitro, however, both of these prodrugs are capable of achieving a complete cure at po doses lower than 30 mg kg^{-1}, with **14a** representing a lead compound for further studies.

Experimental Section

Chemistry

General: All reactions requiring anhydrous conditions were conducted using oven-dried glassware with magnetic stirring under N^2, unless otherwise noted. Needles for the transfer of reagents were dried at 120 °C and allowed to cool in desiccators over P_2O_5 before use. THF was distilled from benzophenone and CH_2Cl_2 from CaH_2. Other solvents and reagents were used as obtained from supplier, unless otherwise noted. Reactions were monitored by TLC using precoated silica gel 60 plates (Merck), or precoated silica gel 60 RP-18 F_{254s} plates (Merck). Reaction components were visualized initially under UV (254 nm), and then by treatment with acidic *p*-anisaldehyde stain or ninhydrin, followed by gentle heating. Organic extracts were dried using $MgSO_4$, unless stated otherwise. Column chromatography was carried out on silica gel pore size 60 Å (40–63 μm) or on Polygoprep 100–50 C18 (Macherey–Nagel). Melting points (mp) were measured on a Gallenkamp capillary melting point apparatus and are uncorrected.

^{1}H NMR and ^{13}C NMR spectra were recorded at 305 K with a Brüker AV400 spectrometer. Samples were prepared in $CDCl_3$ with the residual $CHCl_3$ peak used as an internal reference (fixed at 7.26 ppm), or in [D_6]DMSO with the residual DMSO peak used as an internal reference (fixed at 2.50 ppm). The ^{1}H NMR spectra are reported as follows: chemical shift (δ) in parts per million (ppm) (multiplicity, relative integral, coupling constant(s) (J) in Hertz (Hz), assignment), where multiplicity is defined as: m = multiplet, s = singlet, d = doublet, t = triplet, q = quadruplet or a combination thereof). ^{13}C NMR spectra were conducted using a J-modulated sequence. Samples were prepared in $CDCl_3$, with the residual solvent central peak of the triplet used as an internal reference (77.00 ppm), or in [D_6]DMSO with the residual solvent central peak of the septuplet used as an internal reference (39.50 ppm). Mass spectra (MS) were recorded on a VG analytical 7070E machine and Fisons TRIO spectrometers using electrospray (ESI) and chemical ionization (CI). Analytical HPLC chromatographs were recorded on a GIBSON appara-

tus using a Genesis C18 column (4.6 mm×300 mm, 4.0 μm) and UV photoiode array detection at 230 and 254 nm. Mobile phases consisted of mixtures of MeCN (10–85%) and water, with both solvents containing formic acid (80 mM), ammonium formate (20 mM), and Et_3N (15 mM). Flow rates were maintained at 1.5 mL min^{-1}. For every derivative, MeCN was increased linearly from 10 to 85% over 10 min before re-equilibration.

4-Azidomethylbenzonitrile (2): A mixture of DMF (60 mL) and benzonitrile **1** (5.88 g, 30.0 mmol) was treated slowly with NaN_3 (3.9 g, 60.0 mmol). After 4 h of stirring at RT, the content of the flask was poured into water (100 mL). The mixture was extracted with Et_2O (3×100 mL). The combined organic layers were washed with water (5×300 mL) and brine (1×300 mL), dried over $MgSO_4$, filtered and concentrated in vacuo to afford **2** as a pale-yellow oil (4.63 g, 98%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.66 (d, 2H, J=8.3 Hz, ArH), 7.45 (d, 2H, J=8.3 Hz, ArH), 4.46 ppm (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ=141.2, 132.7 (2C), 129.2 (2C), 118.9, 112.5, 54.4 ppm; MS (ESI+): m/z (%): 159 $[M+H]^+$ (100); HRMS (ESI+): m/z $[M+H]^+$ calcd for $C_8H_7N_4$: 159.0592, found: 159.0595.

1-(4-Cyanobenzyl)-4-(4-cyanophenyl)-[1,2,3]triazole (4): A solution of benzonitrile **2** (500 mg, 3.16 mmol) and benzonitrile **3** (402 mg, 3.16 mmol), $CuSO_4{\cdot}5H_2O$ (1.5 mg, 0.0057 mmol), ascorbic acid (2.0 mg, 0.0114 mmol), L-proline (3.0 mg, 0.0227 mmol), and Na_2CO_3 (2.5 mg, 0.0227 mmol) in DMSO/water (2 mL, 9:1) was stirred at 65 °C overnight. After completion, the mixture was cooled before adding saturated aq NH_4Cl (40 mL), the precipitate obtained was filtered and washed with distilled H_2O (100 mL) and dried in vacuo. The crude product was purified by column chromatography on silica gel ($CHCl_3$) to give **4** as a white powder (890 mg, 99%): mp: 156–157 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.93 (d, 2H, J=8.6 Hz, ArH), 7.84 (s, 1H, CH triazole), 7.71 (d, 2H, J=8.6 Hz, ArH), 7.70 (d, 2H, J=8.6 Hz, ArH), 7.41 (d, 2H, J=8.6 Hz, ArH), 5.68 ppm (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ=147.2, 139.8, 134.9, 133.4 (2C), 133.2 (2C), 128.9 (2C), 126.5 (2C), 121.2, 119.0, 118.4, 113.5, 112.3, 54.1 ppm; MS (CI+): m/z (%): 286 $[M+H]^+$ (100); HRMS (CI+): m/z $[M+H]^+$ calcd for $C_{17}H_{12}N_5$: 286.1014, found: 286.1009.

1-(4-Amidinobenzyl)-4-(4-amidinophenyl)-[1,2,3]triazole dihydrochloride (5): Triazole **4** (1 g, 3.5 mmol) in dry EtOH (40 mL) was added to a flask, and the mixture was cooled in an ice-water bath. To saturate the reaction medium with HCl, gaseous HCl was bubbled through the solution for 20 min before the flask was sealed. The mixture was stirred at RT for one week. The reaction mixture was diluted with dry Et_2O (100 mL), and the precipitate was filtered off under a stream of N_2, rinsed with anhyd Et_2O and dried in vacuo to give an unstable yellow powder. The imidate intermediate was suspended in a mixture of dry NH_3 (2 M in EtOH, 25 mL) and dry EtOH (25 mL), and stirred under N_2 at RT for 24 h. After evaporating the solvent, the crude material was solubilized in aq NaOH (1 M, 20 mL) and MeCN (20 mL) to generate the free base. After stirring for 1 h, the formed precipitate was washed with MeCN, water and Et_2O. A mixture of MeOH and concd HCl (50:50) was used to give the desired HCl salt as a white solid (790 mg, 55%): mp: >360 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=9.53 (s, 4H, NH_2 or NH·HCl), 9.36 (s, 4H, NH_2 or NH·HCl), 9.00 (s, 1H, CH triazole), 8.09 (d, 2H, J=8.0 Hz, ArH), 7.97 (d, 2H, J=8.0 Hz, ArH), 7.89 (d, 2H, J=8.0 Hz, ArH), 7.58 (d, 2H, J=8.0 Hz, ArH), 5.84 ppm (s, 2H, CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=165.7, 165.5, 145.7, 141.9, 136.0, 129.4 (2C), 129.1 (2C), 128.7 (2C), 128.2, 127.3, 125.6 (2C), 123.9, 52.9 ppm; MS (CI+): m/z (%): 286 $[M-2NH_2]^+$ (100); HRMS (CI+): m/z $[M-2NH_2]^+$ calcd for $C_{17}H_{13}N_5$: 286.1327, found: 286.1321; HPLC: t_R=3.89 min (100% area).

1-Azido-4-bromobenzene (7a): A mixture of 4-bromoaniline **6a** (3 g, 17.44 mmol), water (50 mL), and concd HCl (50 mL) was stirred at 0–5 °C for 30 min. The amine hydrochloride was then deazotized by the dropwise addition of aq $NaNO_2$ (1.44 g, 20.93 mmol, in 21 mL H_2O). The solution was stirred at 0–5 °C and treated with aq NaN_3 (2.27 g, 34.88 mmol, in 21 mL H_2O). After stirring for 1 h, the reaction mixture was extracted with CH_2Cl_2 (3× 150 mL). The combined organic layers were washed with water and brine, dried over $MgSO_4$, filtered and and concentrated in vacuo to afford the azide as a yellow oil (3.41 g, 98%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.39 (d, 2H, J=8.7 Hz, ArH), 6.83 ppm (d, 2H, J=8.7 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ=139.6, 133.2 (2C), 121.1 (2C), 118.2 ppm; MS (EI+): m/z (%): 199 $[M+H]^+$ (15), 197 (15), 171 $[M+H-N_2]^+$ (56), 169 (51); HRMS (ESI+): m/z $[M+H]^+$ calcd for $C_6H_5BrN_3$: 198.9589, found: 198.9594.

1-Azido-3-bromobenzene (7b): Product **7b** was prepared from **6b** (3 g, 17.44 mmol) using the same protocol as described for **7a**. Azide **7b** was obtained as a yellow oil (3.34 g, 97%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.25 (dd, 1H, J=2.1, 8.1 Hz, ArH), 7.19 (t, 1H, J=8.1 Hz, ArH), 7.16 (t, 1H, J=2.1 Hz, ArH), 6.94 ppm (dd, 1H, J=2.1, 8.1 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ=143.2, 132.5, 129.6, 124.9, 123.8, 119.3 ppm; MS (ESI+): m/z (%): 199 $[M+H]^+$ (100); HRMS (ESI+): m/z $[M+H]^+$ calcd for $C_6H_5BrN_3$: 198.9589, found: 198.9594.

3-Trimethylsilanylethynylbenzonitrile (9): A mixture of **8** (18.0 g, 99.00 mmol), $Pd(PPh_3)_4$ (1.39 g, 1.98 mmol), and CuI (754 mg, 3.96 mmol) was slowly flushed with N_2 followed by the addition of dry Et_3N (250 mL). The stirring mixture was treated with ethynyltrimethylsilane (20.55 mL, 148.00 mmol), and the solution turned dark brown. The mixture was heated to 80 °C under N_2 overnight. The mixture was cooled, concentrated in vacuo, and Et_2O (250 mL) was added to the residue. The mixture was then filtered and concentrated, and the brown crude product was purified by chromatography on silica gel (hexane/EtOAc, 98:2) to afford **9** as a white solid (17.0 g, 86%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.63 (t, 1H, J=1.4 Hz, ArH), 7.55 (ddd, 1H, J=1.3, 1.4, 7.9 Hz, ArH), 7.47 (ddd, 1H, J=1.3, 1.4, 7.9 Hz, ArH), 7.31 (t, 1H, J=7.9 Hz, ArH), 0.15 ppm (s, 9H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=136.4, 135.6, 131.7, 128.8,123.0, 116.5, 112.1, 103.2, 99.8, 0.0 ppm (3C); MS (CI+): m/z (%): 199 $[M+H]^+$ (100); HRMS (CI+): m/z $[M]^+$ calcd for $C_{12}H_{13}NSi$: 199.0817, found: 199.0821.

3-Ethynylbenzonitrile (10): Benzonitrile **9** (16.5 g, 82.80 mmol) was dissolved in dry THF (200 mL). This solution was then cooled to −20 °C using an acetone/dry ice bath. TBAI (91.06 mL, 1 M in THF, 91.06 mmol) was added, and the mixture was stirred at −20 °C for 30 min. Water (250 mL) was added, and the mixture was extracted with Et_2O (3×250 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by chromatography on silica gel (hexane/EtOAc, 95:5) to give **10** as a white solid (6.5 g, 62%): mp: 42–44 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.76 (t, 1H, J=1.4 Hz, ArH), 7.70 (ddd, 1H, J=1.4, 1.4, 7.9 Hz, ArH), 7.63 (ddd, 1H, J=1.4, 1.4, 7.9 Hz, ArH), 7.50–7.55 (m, 3H, ArH), 7.45 (t, 1H, J=7.9 Hz, ArH), 3.20 ppm (s, 1H, CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ=136.6, 135.9, 132.5, 129.7, 124.1, 118.3, 113.3, 81.6, 80.2 ppm; MS (EI+): m/z (%): 127 $[M]^+$ (100); HRMS (CI+): m/z $[M]^+$ calcd for 127.0422, found: 127.0423.

1-(4-Bromophenyl)-4-(4-cyanophenyl)-[1,2,3]triazole (11a): The same protocol used for compound **4** was followed to generate product **11a**, using bromobenzene **7a** (2.34 g, 11.80 mmol) and benzonitrile **3** (1.5 g, 11.80 mmol). Compound **11a** was obtained as a pale-yellow solid (3.6 g, 94%): mp: 190–192 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.28 (s, 1H, CH triazole), 8.02 (d, 2H, J = 8.6 Hz, ArH), 7.76 (d, 2H, J = 8.6 Hz, ArH), 7.70 ppm (m, 4H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 147.2, 136.1, 134.8, 133.5 (2C), 133.3 (2C), 126.7 (2C), 123.3, 122.4 (2C), 119.0, 119.0, 112.4 ppm; MS (CI+): *m/z* (%): 327 (37), 325 $[M]^+$ (38), 247 $[M+H-Br]^+$ (100); HRMS (CI+): *m/z* $[M]^+$ calcd for $C_{15}H_{10}N_4Br$: 325.0089, found: 325.0081.

1-(3-Bromophenyl)-4-(4-cyanophenyl)-[1,2,3]triazole (11b): The same protocol as described for compound **4** was used to generate **11b** from bromobenzene **7b** (1.56 g, 7.87 mmol) and benzonitrile **3** (1 g, 7.87 mmol). The crude product was purified by column chromatography on silica gel (CH_2Cl_2) to give **11b** as a pale-yellow powder (2.47 g, 97%): mp: 176–177 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.56 (s, 1H, CH triazole), 8.19 (t, 1H, J = 2.0 Hz, ArH), 8.11 (d, 2H, J = 8.6 Hz, ArH), 8.01 (ddd, 1H, J = 2.0, 2.1, 8.1 Hz, ArH), 7.98 (d, 2H, J = 8.6 Hz, ArH), 7.74 (ddd, 1H, J = 2.0, 2.1, 8.1 Hz, ArH), 7.61 ppm (t, 1H, J = 8.1 Hz, ArH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 145.7, 137.4, 134.4, 133.1 (2C), 131.9, 131.6, 125.8 (2C), 122.5, 122.4, 121.5, 119.0, 118.7, 110.5 ppm; MS (ESI+): *m/z* (%): 327 $[M+H]^+$ (100), 325 $[M+H]^+$ (92); HRMS (ESI+): *m/z* $[M]^+$ calcd for $C_{15}H_{10}N_4Br$: 325.0089, found: 325.0074.

1-(4-Bromophenyl)-4-(3-cyanophenyl)-[1,2,3]triazole (11c): The same protocol as described for compound **4** was used to generate **11c** from bromobenzene **7a** (3.9 g, 19.70 mmol) and benzonitrile **10** (2.5 g, 19.70 mmol). The crude product was purified by column chromatography on silica gel (CH_2Cl_2) to give **11c** as a white solid (5.95 g, 93%): mp: 164–167 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.26 (s, 1H, CH triazole), 8.18 (t, 1H, J = 1.4 Hz, ArH), 8.17 (ddd, 1H, J = 1.4, 1.5, 7.9 Hz, ArH), 7.70 (m, 4H, ArH), 7.66 (ddd, 1H, J = 1.4, 1.5, 7.9 Hz, ArH), 7.59 ppm (t, 1H, J = 7.9 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 147.0, 136.1, 133.5 (2C), 132.3, 131.8, 130.4, 130.3, 129.7, 123.3, 122.4 (2C), 118.8, 118.5, 113.7 ppm; MS (ESI+): *m/z* (%): 327 (100), 325 $[M+H]^+$ (97), 247 $[M+H-Br]^+$ (90); HRMS (ESI+): *m/z* $[M]^+$ calcd for $C_{15}H_{10}N_4Br$: 325.0089, found: 325.0080.

1-(3-Bromophenyl)-4-(3-cyanophenyl)-[1,2,3]triazole (11d): The same protocol as described for compound **4** was used to generate **11d** from bromobenzene **7b** (3.9 g, 19.70 mmol) and benzonitrile **10** (2.5 g, 19.70 mmol). The crude was purified by column chromatography on silica gel (CH_2Cl_2) to give the product as a white solid (6.13 g, 96%): mp: 162–165 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.29 (s, 1H, CH triazole), 8.17–8.19 (m, 1H, ArH), 8.17 (ddd, 1H, J = 1.4, 1.6, 7.8 Hz, ArH), 8.00 (t, 1H, J = 2.0 Hz, ArH), 7.76 (ddd, 1H, J = 1.9, 2.0, 8.2 Hz, ArH), 7.66 (ddd, 1H, J = 1.4, 1.6, 7.8 Hz, ArH), 7.62 (ddd, 1H, J = 1.9, 2.0, 8.2 Hz, ArH), 7.60 (dt, 1H, J = 1.6, 7.8 Hz, ArH), 7.45 ppm (t, 1H, J = 8.2 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 146.9, 138.1, 132.6, 132.3, 131.8, 131.7, 130.4, 130.3, 129.7, 124.0, 123.9, 119.4, 118.8, 118.6, 113.7 ppm; MS (ESI+): *m/z* (%): 327 (100), 325 $[M+H]^+$ (98); HRMS (ESI+): *m/z* $[M]^+$ calcd for $C_{15}H_{10}N_4Br$: 325.0089, found: 325.0079.

1,4-Bis-(4-cyanophenyl)-[1,2,3]triazole (12a): A suspension of triazole **11a** (1.3 g, 4.0 mmol) and CuCN (716 mg, 8.0 mmol) in dry DMF (35 mL) was heated to reflux under N_2 for 24 h. The cooled reaction was poured into a mixture of water (50 mL) and saturated aq NH_4Cl (30 mL). The suspension was filtered, washed with water, and dried in vacuo. The crude product was purified by column chromatography on silica gel (CH_2Cl_2) to give **12a** as a pale-yellow solid (810 mg, 75%): mp: 250–252 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.64 (s, 1H, CH triazole), 8.17 (m, 4H, ArH), 8.12 (d, 2H, J = 8.2 Hz, ArH), 7.99 ppm (d, 2H, J = 8.2 Hz, ArH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 146.4, 139.7, 134.8 (2C), 134.7, 133.5 (2C), 126.3 (2C), 121.9, 120.9 (2C), 119.1, 118.4, 111.7, 111.1 ppm; MS (CI+): *m/z* (%): 272 $[M+H]^+$ (100); HRMS (CI+): *m/z* $[M+H]^+$ calcd for $C_{16}H_{10}N_5$: 272.0936, found: 272.0932.

1-(3-Cyanophenyl)-4-(4-cyanophenyl)-[1,2,3]triazole (12b): The same protocol described for compound **12a** was used to generate **12b** from triazole **11b** (2.32 g, 7.13 mmol). The crude product was purified by column chromatography on silica gel (9:1 CH_2Cl_2/EtOAc) to give **12b** as a pale-yellow solid (1.47 g, 76%): mp: 214–215 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.60 (s, 1H, CH triazole), 8.46 (s, 1H, ArH), 8.33 (d, 1H, J = 8.2 Hz, ArH), 8.10 (d, 2H, J = 8.2 Hz, ArH), 8.02 (d, 1H, J = 8.2 Hz, ArH), 8.00 (d, 2H, J = 8.2 Hz, ArH), 7.87 ppm (t, 1H, J = 8.2 Hz, ArH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 146.3, 137.2, 134.8, 133.6 (2C), 132.9, 131.8, 126.2 (2C), 125.1, 123.9, 122.0, 119.1, 118.1, 113.2, 111.1 ppm; MS (ESI+): *m/z* (%): 272 $[M+H]^+$ (100); HRMS (ESI+): *m/z* $[M+H]^+$ calcd for $C_{16}H_{10}N_5$: 272.0936, found: 272.0937.

1-(4-Cyanophenyl)-4-(3-cyanophenyl)-[1,2,3]triazole (12c): The same protocol as described for compound **12a** was used to generate **12c** from triazole **11c** (5.5 g, 16.91 mmol). The crude product was purified by column chromatography on silica gel (9:1 CH_2Cl_2/EtOAc) to give **12c** as a white solid (3.64 g, 79%): mp: 202–205 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.60 (t, 1H, J = 1.8 Hz, CH triazole), 8.33 (t, 1H, J = 1.6 Hz, ArH), 8.27 (d, 1H, J = 7.8 Hz, ArH), 8.16 (m, 4H, ArH), 7.88 (d, 1H, J = 7.8 Hz, ArH), 7.74 ppm (t, 1H, J = 7.8 Hz, ArH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 146.2, 139.7, 134.8 (2C), 132.3, 131.5, 130.9, 130.1, 129.1, 121.4, 120.8 (2C), 118.8, 118.4, 112.6, 111.7 ppm; MS (CI+): *m/z* (%): 272 $[M+H]^+$ (100); HRMS (CI+): *m/z* $[M+H]^+$ calcd for $C_{16}H_{10}N_5$: 272.0936, found: 272.0930.

1,4-Bis-(3-cyanophenyl)-[1,2,3]triazole (12d): The same protocol as described for compound **12a** was used to generate **12d** from triazole **11d** (5.5 g, 16.91 mmol). The crude product was purified by column chromatography on silica gel (9:1 CH_2Cl_2/EtOAc) to give **12d** as a white solid (3.45 g, 75%): mp: 198–200 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.54 (t, 1H, J = 2.0 Hz, CH triazole), 8.42 (t, 1H, J = 1.5 Hz, ArH), 8.30 (dd, 1H, J = 1.2, 7.7 Hz, ArH), 8.29 (t, 1H, J = 1.2 Hz, ArH), 8.24 (dd, 1H, J = 1.5, 8.0 Hz, ArH), 8.00 (dd, 1H, J = 1.2, 7.7 Hz, ArH), 7.86 (dd, 1H, J = 1.5, 8.0 Hz, ArH), 7.85 (dt, 1H, J = 1.5, 7.9 Hz, ArH), 7.73 ppm (dt, 1H, J = 1.2, 7.9 Hz, ArH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 146.0, 137.2, 132.8, 132.2, 131.8, 131.5, 130.9, 130.0, 129.0, 124.9, 123.7, 121.4, 118.8, 118.1, 113.2, 112.6 ppm; MS (ESI+): *m/z* (%): 272 $[M+H]^+$ (100); HRMS (ESI+): *m/z* $[M+H]^+$ calcd for $C_{16}H_{10}N_5$: 272.0936, found: 272.0930.

1,4-Bis-(4-benzamidine)-1H-[1,2,3]triazole dihydrochloride (13a):[53] The same protocol as described for the synthesis of compound **5** was used to generate amidine **13a** from triazole **12a** (1 g, 3.69 mmol). A mixture of MeOH and and concd HCl (1:1) was used to give the desired HCl salt as a white solid (1.23 g, 84%): mp: 357–359 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.84 (s, 1H, CH triazole), 9.59 (s, 4H, NH·HCl or NH_2), 9.45 (s, 4H, NH·HCl or NH_2), 8.25 (d, 2H, J = 8.2 Hz, ArH), 8.18 (d, 2H, J = 8.9 Hz, ArH), 8.15 (d, 2H, J = 8.9 Hz, ArH), 8.03 ppm (d, 2H, J = 8.2 Hz, ArH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 165.5, 165.0, 146.6, 140.2, 135.3, 130.7 (2C), 129.5 (2C), 128.2, 127.7, 125.8 (2C), 122.0, 120.2 ppm (2C); MS (ESI+): *m/z* (%): 306 $[M+H]^+$ (100), 153.5 $[M+H]^{2+}$ (55); HRMS

(ESI+): m/z $[M+H]^+$ calcd for $C_{16}H_{16}N_7$: 306.1467, found: 306.1475; HPLC: $t_R = 5.03$ min (98.1% area).

1-(3-Amidinophenyl)-4-(4-amidinophenyl)-[1,2,3]triazole dihydrochloride (13b).[53] The same protocol as described for the synthesis of compound **5** was used to generate amidine **13b** from triazole **12b** (900 mg, 3.32 mmol). Purification by column chromatography on reversed phase silica gel (water) gave the product as a white solid (1.01 g, 73%): mp: 330–332 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 9.89$ (s, 1H, CH triazole), 9.76 (s, 2H, NH·HCl or NH_2), 9.52 (s, 4H, NH·HCl or NH_2), 9.32 (s, 2H, NH·HCl or NH_2), 8.62 (s, 1H, ArH), 8.36 (d, 1H, $J = 8.0$ Hz, ArH), 8.18 (d, 2H, $J = 8.4$ Hz, ArH), 8.03 (d, 2H, $J = 8.4$ Hz, ArH), 8.01 (d, 1H, $J = 8.0$ Hz, ArH), 7.91 ppm (t, 1H, $J = 8.0$ Hz, ArH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 165.5$, 165.0, 146.5, 136.9, 135.4, 131.2, 130.0, 129.5 (2C), 128.8, 127.8, 125.8 (2C), 125.2, 122.1, 120.4 ppm; MS (ESI+): m/z (%): 306 $[M+H]^+$ (100); HRMS (ESI+): m/z $[M+H]^+$ calcd for $C_{16}H_{16}N_7$: 306.1467, found: 306.1455; HPLC: $t_R = 4.66$ min (100% area).

1-(4-Amidinophenyl)-4-(3-amidinophenyl)-[1,2,3]triazole dihydrochloride (13c):[53] The same protocol as described for the synthesis of compound **5** was used to generate imidate **13c** from triazole **12c** (1 g, 3.69 mmol). Purification by column chromatography on reversed phase silica gel (water) gave the product as a white solid (1.08 g, 65%): mp: 266–268 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 9.83$ (s, 1H, CH triazole), 9.66 (s, 4H, NH_2 and NH·HCl), 9.42 (s, 4H, NH_2 and NH·HCl), 8.56 (s, 1H, ArH), 8.30 (d, 1H, $J = 7.8$ Hz, ArH), 8.25 (d, 2H, $J = 8.7$ Hz, ArH), 8.16 (d, 2H, $J = 8.7$ Hz, ArH), 7.89 (d, 1H, $J = 7.8$ Hz, ArH), 7.78 ppm (t, 1H, $J = 7.8$ Hz, ArH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 165.9$, 165.0, 146.8, 140.3, 131.0, 130.7 (2C), 130.6, 130.3, 129.3, 128.3 (2C), 125.4, 121.4, 120.2 ppm (2C); MS (ESI+): m/z (%): 306 $[M+H]^+$ (100), 153.5 $[M+H]^{2+}$ (20); HRMS (ESI+): m/z $[M+H]^+$ calcd for $C_{16}H_{16}N_7$: 306.1467, found: 306.1470; HPLC: $t_R = 4.74$ min (100% area).

1,4-Bis-(3-amidinophenyl)-[1,2,3]triazole dihydrochloride (13d):[53] The same protocol as described for the synthesis of compound **5** was used to generate imidate **13d** from triazole **12d** (1 g, 3.69 mmol). Purification by column chromatography on reversed phase silica gel (water) gave the product as a white solid (1.15 g, 72%): mp: >360 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 9.76$ (t, 1H, $J = 1.2$ Hz, CH triazole), 9.51 (s, 8H, NH_2 and NH·HCl), 8.54 (t, 1H, $J = 1.2$ Hz, ArH), 8.47 (t, 1H, $J = 1.2$ Hz, ArH), 8.34 (d, 1H, $J = 7.9$ Hz, ArH), 8.27 (dd, 1H, $J = 1.2$, 7.8 Hz, ArH), 7.99 (d, 1H, $J = 7.9$ Hz, ArH), 7.91 (t, 1H, $J = 7.9$ Hz, ArH), 7.85 (dd, 1H, $J = 1.2$, 7.8 Hz, ArH), 7.78 ppm (t, 1H, $J = 7.8$ Hz, ArH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 165.9$, 165.0, 146.7, 136.9, 131.3, 131.1, 130.6, 130.3, 130.2, 129.5, 128.8, 128.3, 125.2, 125.1, 121.4, 120.3 ppm; MS (ESI+): m/z (%): 306 $[M+H]^+$ (100), 153.5 $[M+H]^{2+}$ (14); HRMS (ESI+): m/z $[M+H]^+$ calcd for $C_{16}H_{16}N_7$: 306.1467, found: 306.1475; HPLC: $t_R = 4.72$ min (99.7% area).

1,4-Bis-(4-*N*-methoxyamidinophenyl)-[1,2,3]triazole dihydrochloride (14a): NH_2OH·HCl (1.57 g, 22.56 mmol) was suspended in abs EtOH (50 mL), K_2CO_3 (2.08 g, 15.04 mmol) was added, and the solution was stirred at RT for 1 h. Triazole **12a** (2.04 g, 7.52 mmol) was added to the solution, and the reaction mixture was stirred at reflux (90 °C) for 24 h. After cooling, the mixture was filtered, and the filtrate was concentrated in vacuo. The solid obtained was washed with water and Et_2O. The crude product was then dissolved in 1,4-dioxane (80 mL), and the solution was cooled to 0 °C. A solution of 2 N NaOH (150 mL) was added slowly, followed by dropwise addition of Me_2SO_4 (5.7 g, 45.12 mmol) in 1,4-dioxane (40 mL). After addition of the reagents, the ice bath was removed, and the mixture was stirred at RT overnight. Then, the solvent was evaporated in vacuo, and the crude product was washed with water and Et_2O. The residue was purified by column chromatography on silica gel (hexane/EtOAc, 6:4→5:5) to afford the free amidoxime as a white solid. This solid was solubilized in MeOH/concd HCl (1:1) to generate the dihydrochloride salt as a white solid (1.34 g, 35% over two steps): mp: 210–213 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 9.73$ (s, 1H, CH triazole), 8.15 (m, 4H, ArH), 8.04 (d, 2H, $J = 8.4$ Hz, ArH), 7.95 (d, 2H, $J = 8.4$ Hz, ArH), 3.89 (s, 3H, CH_3), 3.87 ppm (s, 3H, CH_3); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 157.6$ (2C), 146.7, 139.0, 138.9, 134.5, 129.5 (2C), 129.1 (2C), 125.8 (2C), 121.5 (2C), 120.1 (2C), 63.6, 62.9 ppm; MS (ESI+): m/z (%): 366 $[M+H]^+$ (100); HRMS (ESI+): m/z $[M+H]^+$ calcd for $C_{18}H_{20}N_7O_2$: 366.1678, found: 366.1678; HPLC: $t_R = 8.48$ min (100% area).

1-(3-*N*-Methoxyamidinophenyl)-4-(4-*N*-methoxyamidinophenyl)-[1,2,3]triazole dihydrochloride (14b): The same protocol as described for compound **14a** was used to generate product **14b** from triazole **12b** (1.70 g, 6.27 mmol). This solid was solubilised in MeOH/concd HCl (1:1) to generate the dihydrochloride salt as a white solid (1.28 g, 40% over two steps): mp: 180–183 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 9.82$ (s, 1H, CH triazole), 8.50 (s, 1H, ArH), 8.24 (d, 1H, $J = 7.8$ Hz, ArH), 8.16 (d, 2H, $J = 8.0$ Hz, ArH), 7.98 (d, 2H, $J = 8.0$ Hz, ArH), 7.95 (d, 1H, $J = 7.8$ Hz, ArH), 7.82 (t, 1H, $J = 8.0$ Hz, ArH), 3.91 (s, 3H, CH_3), 3.90 ppm (s, 3H, CH_3); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 156.4$, 153.6, 144.5, 134.8, 132.9, 128.9, 127.7, 127.4, 126.1 (2C), 123.8, 123.5 (2C), 121.7, 119.9, 117.6, 62.0, 61.2 ppm; MS (ESI+): m/z (%): 366 $[M+H]^+$ (100); HRMS (ESI+): m/z $[M+H]^+$ calcd for $C_{18}H_{20}N_7O_2$: 366.1678, found: 366.1674; HPLC: $t_R = 8.99$ min (98.8% area).

1-Azidomethyl-4-bromobenzene (16): The same protocol as described for compound **2** was used to generate product **16** from **15** (2 g, 8.0 mmol). The combined organic extracts after the workup were concentrated in vacuo to afford the product as a pale-yellow oil (1.64 g, 97%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.49$ (d, 2H, $J = 8.4$ Hz, ArH), 7.17 (d, 2H, $J = 8.4$ Hz, ArH), 4.28 ppm (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 134.8$, 132.4 (2C), 130.3 (2C), 122.8, 54.5 ppm; MS (ESI+): m/z (%): 213 $[M+H]^+$ (100); HRMS (ESI+): m/z $[M+H]^+$ calcd for $C_7H_7BrN_3$: 212.9745, found: 212.9738.

1,5-Bis-(4-bromophenyl)-1*H*-[1,2,3]triazole (18a): A solution of **17** (0.9 g, 4.97 mmol) in dry THF (3 mL) was treated with EtMgBr (1 M in THF, 4.97 mL, 4.97 mmol) at RT under N_2. The reaction mixture was heated to 50 °C for 15 min. After cooling to RT, a solution of **7a** (1.0 g, 4.97 mmol) in dry THF (3 mL) was added. The resulting solution was stirred at RT for 30 min, and then heated to 50 °C for 1 h before quenching with saturated aq NH_4Cl (20 mL). The layers were separated, and the aqueous layer was extracted CH_2Cl_2 (3× 30 mL). The combined organic layers were washed with water and brine, dried over $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography on silica gel (24:1 CH_2Cl_2/EtOAc) gave the product as a yellow solid (1.81 g, 96%): mp: 142–144 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.86$ (s, 1H, CH triazole), 7.59 (d, 2H, $J = 8.5$ Hz, ArH), 7.52 (d, 2H, $J = 8.4$ Hz, ArH), 7.24 (d, 2H, $J = 8.5$ Hz, ArH), 7.10 ppm (d, 2H, $J = 8.4$ Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 137.1$, 135.7, 134.1, 133.2 (2C), 132.8 (2C), 130.5 (2C), 126.9 (2C), 125.8, 124.4, 123.9 ppm; MS (ESI+): m/z (%): 400 (45), 402 $[M+Na]^+$ (100), 404 (40), 434 $[M+Na+MeOH]^+$ (26); HRMS (ESI+): m/z $[M+Na]^+$ calcd for $C_{14}H_9Br_2NaN_3$: 401.9040, found: 401.9029.

1-(4-Bromobenzyl)-5-(4-bromophenyl)-[1,2,3]triazole (18b): The same protocol as described for compound **18a** was used to gener-

ate product **18b** from **17** (0.9 g, 4.97 mmol) and 4-bromobenzene **16** (1.05 g, 4.97 mmol). Purification by column chromatography on silica gel (CH_2Cl_2/hexane, 9:1, then 100% CH_2Cl_2) gave the product as a yellow solid (1.73 g, 89%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.73 (s, 1H, CH triazole), 7.57 (d, 2H, J = 8.2 Hz, ArH), 7.42 (d, 2H, J = 8.4 Hz, ArH), 7.10 (d, 2H, J = 8.4 Hz, ArH), 6.95 (d, 2H, J = 8.2 Hz, ArH), 5.48 ppm (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 137.5, 134.6, 133.9, 132.8 (2C), 132.5 (2C), 130.8 (2C), 129.2 (2C), 126.0, 124.6, 122.9, 51.7 ppm; MS (ESI+): *m/z* (%): 416 $[M+Na]^+$ (100); HRMS (ESI+): *m/z* $[M+Na]^+$ calcd for $C_{15}H_{11}Br_2NaN_3$: 414.9197, found: 414.9209.

1,5-Bis-(4-cyanophenyl)-1*H*-[1,2,3]triazole (19a): The same protocol as described for compound **12a** was used to generate product **19a** from triazole **18a** (1.65 g, 4.35 mmol). For this reaction, 4.0 equiv of CuCN (1.56 g, 17.4 mmol) were used. Purification by column chromatography on silica gel (8:2 CH_2Cl_2/EtOAc) gave the product as a pale-yellow solid (560 mg, 45%): mp: 176–178 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.96 (s, 1H, CH triazole), 7.79 (d, 2H, J = 8.6 Hz, ArH), 7.72 (d, 2H, J = 8.3 Hz, ArH), 7.51 (d, 2H, J = 8.6 Hz, ArH), 7.37 ppm (d, 2H, J = 8.3 Hz, ArH),); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 139.7, 136.5, 135.1, 134.1 (2C), 133.4 (2C), 131.0, 129.6 (2C), 125.8 (2C), 118.1, 117.7, 114.2, 114.1 ppm; MS (ESI+): *m/z* (%): 272 $[M+H]^+$ (100); HRMS (ESI+): *m/z* $[M+H]^+$ calcd for $C_{16}H_{10}N_5$: 272.0936, found: 272.0928.

1-(4-Cyanobenzyl)-5-(4-cyanophenyl)-[1,2,3]triazole (19b): The same protocol as described for compound **12a** was used to generate product **19b** from triazole **18b** (1.64 g, 4.17 mmol). For this reaction, 4.0 equiv of CuCN were used. Purification by column chromatography on silica gel (CH_2Cl_2/EtOAc, 10:0→9:1→8:2) gave the product as a pale-yellow solid (700 mg, 62%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.14 (s, 1H, CH triazole), 7.96 (d, 2H, J = 8.0 Hz, ArH), 7.78 (d, 2H, J = 8.0 Hz, ArH), 7.69 (d, 2H, J = 8.0 Hz, ArH), 7.18 (d, 2H, J = 8.0 Hz, ArH), 5.88 ppm (s, 2H, CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 141.5, 136.8, 134.6, 133.3 (2C), 133.1 (2C), 131.2, 129.7 (2C), 128.3 (2C), 118.8, 118.7, 112.4, 111.1, 51.4 ppm; MS (ESI+): *m/z* (%): 286 $[M+H]^+$ (100); HRMS (ESI+): *m/z* $[M+H]^+$ calcd for $C_{17}H_{12}N_5$: 286.1014, found: 286.1021.

1,5-Bis-(4-amidinophenyl)-1H-[1,2,3]triazole dihydrochloride (20a): The same protocol as described for the synthesis of compound **5** was used to generate amidine **20a** from triazole **19a** (650 mg, 2.40 mmol). Purification by column chromatography on reversed phase silica gel (water) gave the product as a white solid (520 mg, 55%): mp: 278–281 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.60 (s, 4H, NH·HCl and NH_2), 9.43 (s, 4H, NH·HCl and NH_2), 8.33 (t, 1H, J = 0.8 Hz, CH triazole), 8.04 (d, 2H, J = 8.3 Hz, ArH), 7.93 (d, 2H, J = 8.1 Hz, ArH), 7.72 (d, 2H, J = 8.1 Hz, ArH), 7.56 ppm (d, 1H, J = 8.3 Hz, ArH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 165.1, 165.1, 140.2, 137.1, 134.8, 131.5, 130.2 (2C), 129.5, 129.4 (2C), 129.1 (2C), 128.9, 126.5 ppm (2C); MS (ESI+): *m/z* (%): 306 $[M+H]^+$ (100); HRMS (ESI+): *m/z* $[M+H]^+$ calcd for $C_{16}H_{16}N_7$: 306.1467, found: 306.1456; HPLC: t_R = 3.07 min (100% area).

1-(4-Amidinobenzyl)-5-(4-amidinophenyl)-[1,2,3]triazole dihydrochloride (20b): The same protocol as described for the synthesis of compound **5** was used to generate imidate **20b** from triazole **19b** (450 mg, 1.66 mmol). Purification by column chromatography on reversed phase silica gel (water) gave the product as a white solid (370 mg, 46%): mp: 252–255 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.46 (s, 8H, NH_2 and NH·HCl), 8.18 (s, 1H, CH triazole), 7.98 (d, 2H, J = 8.1 Hz, ArH), 7.79 (d, 4H, J = 8.1 Hz, ArH), 7.21 (d, 2H, J = 8.1 Hz, ArH), 5.93 ppm (s, 2H, CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 165.5, 165.2, 141.8, 136.8, 134.6, 131.6, 129.3 (2C), 129.0 (2C), 129.0 (2C), 128.9, 127.9, 127.6 (2C), 51.5 ppm; MS (ESI+): *m/z* (%): 382 $[M+Cu]^+$ (100), 320 $[M+H]^+$ (14), 160.5 $[M+2H]^{2+}$ (24); HRMS (ESI+): *m/z* $[M+H+Cu]^+$ calcd for $C_{17}H_{17}N_7Cu$: 382.0841, found: 382.0826; HPLC: t_R = 3.12 min (99.3% area).

4,4′-Dicyanostilbene (22): A flask was charged with compounds **3** (1 g, 7.87 mmol) and **21** (1.43 g, 7.87 mmol), PPh_3 (41 mg, 0.16 mmol), $Pd(PPh_3)_2Cl_2$ (110 mg, 0.16 mmol), and dry Et_3N (30 mL). The mixture was stirred for 10 min with Ar bubbling through the solution, and then CuI (60 mg, 0.31 mmol) was added. The mixture was then heated to reflux with stirring under Ar for 2 h. Following the removal of Et_3N in vacuo, $CHCl_3$ was added, and the solution was filtered, washed with 15% aq K_2CO_3, water, and brine, dried over $MgSO_4$, filtered, and the $CHCl_3$ was removed. The crude product was purified by column chromatography on silica gel (hexane/CH_2Cl_2, 6:4→5:5→4:6→3:7) to give the product as a white solid (1.63 g, 91%): mp: 249–251 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.68 (d, 4H, J = 8.3 Hz, ArH), 7.64 ppm (d, 4H, J = 8.3 Hz, ArH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 132.7 (4C), 132.6 (4C), 127.5 (2C), 118.7 (2C), 112.8 (2C), 92.0 ppm (2C); MS (EI+): *m/z* (%): 228 $[M]^+$ (100); HRMS (EI+): *m/z* $[M]^+$ calcd for $C_{16}H_8N_2$: 228.0687, found: 228.0695.

Azidobenzene (23a): The same protocol as described for compound **7a** was used to generate product **23a** from aniline (5 g, 53.70 mmol). The product was obtained after concentrating the organic extracts in vacuo as an orange oil (5.85 g, 91.5%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33 (t, 2H, J = 8.2 Hz, ArH), 7.12 (t, 1H, J = 8.2 Hz, ArH), 7.01 ppm (d, 2H, J = 8.2 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 140.5, 130.2 (2C), 125.3, 119.5 ppm (2C).

Azidomethylbenzene (23b): The same protocol as described for compound **2** was used to generate product **23b** from benzylbromide (2 g, 11.7 mmol). The product was obtained as a pale-yellow oil after concentrating the organic extracts in vacuo. (1.48 g, 95%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.29–7.41 (m, 5H, ArH), 4.32 ppm (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 135.8, 129.3 (2C), 128.7, 128.7 (2C), 55.2 ppm.

1-Phenyl-4,5-bis-(4-cyanophenyl)-[1,2,3]triazole (24a): A solution of **23a** (522 mg, 4.38 mmol), **22** (1 g, 4.38 mmol), $CuSO_4 \cdot 5H_2O$ (55 mg, 0.22 mmol), ascorbic acid (77 mg, 0.44 mmol), L-proline (101 mg, 0.88 mmol) and Na_2CO_3 (93 mg, 0.88 mmol) in DMSO/water (9:1, 8 mL) was stirred at 120 °C overnight (15 h). The mixture was cooled, and a saturated aq NH_4Cl was added (100 mL). The suspension was filtered, washed with water, and dried in vacuo. The crude product was purified by column chromatography on silica gel (CH_2Cl_2/hexane, 9:1, then 100% CH_2Cl_2) to give the product as a brown pale solid (1.18 g, 77%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.70 (d, 2H, J = 8.4 Hz, ArH), 7.67 (d, 2H, J = 8.8 Hz, ArH), 7.64 (d, 2H, J = 8.8 Hz, ArH), 7.43–7.49 (m, 3H, ArH), 7.33 (d, 2H, J = 8.4 Hz, ArH), 7.27 ppm (dt, 2H, J = 1.8, 6.7 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 144.0, 136.0, 135.0, 133.5 (2C), 133.3, 133.0 (2C), 132.2, 131.2 (2C), 130.3, 130.1 (2C), 128.2 (2C), 125.6 (2C), 118.9, 118.1, 114.4, 112.4 ppm; MS (ESI+): *m/z* (%): 370 $[M+Na]^+$ (100); HRMS (ESI+): *m/z* $[M+Na]^+$ calcd for $C_{22}H_{13}N_5Na$: 370.1069, found: 370.1062.

1-Benzyl-4,5-bis-(4-cyanophenyl)-[1,2,3]triazole (24b): The same protocol as described for compound **24a** was used to generate product **24b** from **23b** (583 mg, 4.38 mmol) and **22** (1 g, 4.38 mmol). Purification by column chromatography on silica gel (CH_2Cl_2) gave the product as a pale-yellow solid (950 mg, 60%):

mp: 178–180 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.74 (d, 2H, J = 8.2 Hz, ArH), 7.60 (d, 2H, J = 8.5 Hz, ArH), 7.56 (d, 2H, J = 8.5 Hz, ArH), 7.23–7.32 (m, 5H, ArH), 6.98 (d, 2H, J = 8.2 Hz, ArH), 5.45 ppm (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 143.9, 135.1, 134.8, 133.5 (2C), 132.9 (2C), 132.4, 131.2 (2C), 129.4 (2C), 129.1 (2C), 127.7 (2C), 127.5 (2C), 118.9, 118.1, 114.7, 112.1, 53.1 ppm; MS (ESI+): *m/z* (%): 362 $[M+H]^+$ (100); HRMS (ESI+): *m/z* $[M+H]^+$ calcd for $C_{23}H_{16}N_5$: 362.1406, found: 362.1409.

1-Phenyl-4,5-bis-(4-amidinophenyl)-[1,2,3]triazole dihydrochloride (25a): The same protocol as described for the synthesis of compound **5** was used to generate amidine **25a** from triazole **24a** (1 g, 2.86 mmol). Purification by column chromatography on reversed phase silica gel (water) gave the product as a white solid (716 mg, 53%): mp: 245–250 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.47 (s, 8H, NH_2 and NH·HCl), 7.95 (d, 2H, J = 8.4 Hz, ArH), 7.89 (d, 2H, J = 8.4 Hz, ArH), 7.69 (d, 4H, J = 8.4 Hz, ArH), 7.46–7.53 ppm (m, 5H, ArH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 165.5, 165.2, 143.2, 135.9, 135.7, 134.7, 132.5, 131.4 (2C), 130.4, 129.9 (2C), 129.5, 129.2 (2C), 129.2 (2C), 127.8, 127.2 (2C), 126.4 ppm (2C); MS (ESI+): *m/z* (%): 382 $[M+H]^+$ (16), 191.5 $[M+H]^{2+}$ (100); HRMS (ESI+): *m/z* $[M+H]^+$ calcd for $C_{22}H_{20}N_7$: 382.1780, found: 382.1769; HPLC: t_R = 6.57 min (100% area).

1-Benzyl-4,5-bis-(4-amidinophenyl)-[1,2,3]triazole dihydrochloride (25b): The same protocol as described for the synthesis of compound **5** was used to generate amidine **25b** from triazole **24b** (860 mg, 2.38 mmol). Purification by column chromatography on reversed phase silica gel (water) gave the product as a white solid (430 mg, 36%): mp: 218–220 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.60 (s, 2H, NH_2 or NH·HCl), 9.42 (s, 2H, NH_2 or NH·HCl), 9.36 (s, 2H, NH_2 or NH·HCl), 9.18 (s, 2H, NH_2 or NH·HCl), 8.01 (d, 2H, J = 8.1 Hz, ArH), 7.81 (d, 2H, J = 8.1 Hz, ArH), 7.67 (d, 2H, J = 8.1 Hz, ArH), 7.63 (d, 2H, J = 8.1 Hz, ArH), 7.29 (m, 3H, ArH), 7.01 (m, 2H, ArH), 5.53 ppm (s, 2H, CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 165.4, 165.2, 143.0, 135.9, 135.5, 134.3, 132.4, 131.1 (2C), 129.7, 129.4 (2C), 129.1 (2C), 129.0 (2C), 128.5, 127.7 (2C), 127.6, 127.0 (2C), 52.0 ppm; MS (ESI+): *m/z* (%): 396 $[M+H]^+$ (30), 198.6 $[M+H]^{2+}$ (100); HRMS (ESI+): *m/z* $[M+H]^+$ calcd for $C_{23}H_{22}N_7$: 396.1937, found: 396.1938; HPLC: t_R = 6.82 min (98.4% area).

4,5-Bis-(4-cyanophenyl)-[1,2,3]triazole (26): NaN_3 (1.14 g, 17.52 mmol) was added slowly to a mixture of DMF (40 mL) and **22** (2 g, 8.76 mmol). After stirring at reflux for 6 h, the solvent was evaporated. The crude product was purified by column chromatography on silica gel (CH_2Cl_2/EtOAc, 9:1→8:2) to afford the product as a yellow solid (1.55 g, 65%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.89 (s, 1H, NH), 7.84 (d, 4H, J = 8.2 Hz, ArH), 7.63 ppm (d, 4H, J = 8.2 Hz, ArH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 163.2 (2C), 134.9 (2C), 133.2 (4C), 129.1 (4C), 118.9 (2C), 111.5 ppm (2C); MS (ESI+): *m/z* (%): 272 $[M+H]^+$ (100); HRMS (ESI+): *m/z* $[M+H]^+$ calcd for $C_{16}H_{10}N_5$: 272.0936, found: 272.0939.

4,5-Bis-(4-amidinophenyl)-[1,2,3]triazole dihydrochloride (27): The same protocol as described for the synthesis of compound **5** was used to generate amidine **27** from triazole **26** (900 mg, 3.32 mmol). Purification by column chromatography on reversed phase silica gel (water) gave the product as a white solid (790 mg, 60%): mp: 310–312 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.54 (s, 4H, NH_2 or NH·HCl), 9.31 (s, 4H, NH_2 or NH·HCl), 7.94 (d, 4H, J = 8.3 Hz, ArH), 7.74 ppm (d, 4H, J = 8.3 Hz, ArH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 165.4 (2C), 144.5 (2C), 129.1 (4C), 128.7 (2C), 128.6 (4C), 128.2 ppm (2C); MS (ESI+): *m/z* (%): 306 $[M+H]^+$ (100), 153.5 $[M+2H]^{2+}$ (26); HRMS (ESI+): *m/z* $[M+H]^+$ calcd for $C_{16}H_{16}N_7$: 306.1467, found: 306.1452; HPLC: t_R = 3.45 min (100% area).

2-Phenyl-4,5-bis-(4-cyanophenyl)-[1,2,3]triazole (28): A flask was charged with $Fe(acac)_3$ (453 mg, 1.28 mmol), CuO (61 mg, 0.43 mmol), triazole **26** (1.16 g, 4.28 mmol), iodobenzene (1.31 g, 6.41 mmol), and Cs_2CO_3 (2.79 g, 8.55 mmol). Then, dry DMF (12 mL) was added, and the flask was closed and heated to 90 °C for 30 h. After cooling the mixture to RT, dilution with CH_2Cl_2 and subsequent filtration followed. The filtrate was washed with water (5× 20 mL), and the organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography on silica gel (CH_2Cl_2/hexane, 5:5→6:4→7:3→8:2) gave the product as a white powder (1.33 g, 90%): mp: 169–170 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.17 (dd, 2H, J = 1.2, 8.7 Hz, ArH), 7.74 (m, 8H, ArH), 7.54 (t, 2H, J = 7.3 Hz, ArH), 7.43 ppm (tt, 1H, J = 1.2, 7.3 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 144.9 (2C), 139.6, 135.1 (2C), 133.1 (4C), 129.9 (2C), 129.3 (4C), 128.8 (2C), 119.4 (2C), 118.8, 113.2 ppm (2C); MS (CI+): *m/z* (%): 370 $[M+Na]^+$ (100); HRMS (CI+): *m/z* $[M+Na]^+$ calcd for $C_{22}H_{13}N_5Na$: 370.1069, found: 370.1065.

2-Benzyl-4,5-(cyanophenyl)-[1,2,3]triazole (29): A solution of triazole **26** (750 mg, 2.76 mmol) in dry acetone (75 mL) was treated with K_2CO_3 (1.91 g, 13.8 mmol), BnCl (1.75 g, 13.8 mmol), and TBAI (102 mg, 10% mol). The reaction mixture was heated to reflux under N_2 overnight. Thereafter, the reaction mixture was concentrated in vacuo. Purification by column chromatography on silica gel (CH_2Cl_2/hexane, 7:3→8:2→9:1,then 100% CH_2Cl_2) gave the product as a white solid (760 mg, 76%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.67 (d, 4H, J = 8.3 Hz, ArH), 7.63 (d, 4H, J = 8.3 Hz, ArH), 7.46 (dd, 2H, J = 1.3, 7.3 Hz, ArH), 7.40 (t, 1H, J = 7.3 Hz, ArH), 7.38 (t, 2H, J = 7.3 Hz, ArH), 5.66 ppm (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 143.9 (2C), 135.4 (2C), 134.8, 133.3 (4C), 129.4 (2C), 129.2 (5C), 128.8 (2C), 118.8 (2C), 112.9 (2C), 59.7 ppm; MS (ESI+): *m/z* (%): 384 $[M+Na]^+$ (100); HRMS (ESI+): *m/z* $[M+Na]^+$ calcd for $C_{23}H_{15}N_5Na$: 384.1225, found: 384.1222.

2-Phenyl-4,5-bis-(4-amidinophenyl)-[1,2,3]triazole dihydrochloride (30a): The same protocol as described for the synthesis of compound **5** was used to generate amidine **30a** from triazole **28** (1 g, 2.88 mmol). Purification by column chromatography on reversed phase silica gel (water) gave the product as a white solid (1.2 g, 74%): mp: 298–300 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.63 (s, 4H, NH_2 and NH·HCl), 9.41 (s, 4H, NH_2 and NH·HCl), 8.17 (d, 2H, J = 7.8 Hz, ArH), 8.01 (d, 4H, J = 8.4 Hz, ArH), 7.83 (d, 4H, J = 8.4 Hz, ArH), 7.65 (t, 2H, J = 7.8 Hz, ArH), 7.53 ppm (t, 1H, J = 7.8 Hz, ArH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 164.9 (2C), 144.7 (2C), 138.6, 134.7 (2C), 129.8 (2C), 128.7 (4C), 128.5 (5C), 128.3 (2C), 118.7 ppm (2C); MS (ESI+): *m/z* (%): 382 $[M+H]^+$ (30), 191.5 $[M+H]^{2+}$ (65); HRMS (ESI+): *m/z* $[M+H]^+$ calcd for $C_{22}H_{20}N_7$: 382.1780, found: 382.1785; HPLC: t_R = 7.72 min (99.7% area).

2-Benzyl-4,5-bis-(4-amidinophenyl)-[1,2,3]triazole dihydrochloride (30b): The same protocol as described for the synthesis of compound **5** was used to generate amidine **30b** from triazole **29** (840 mg, 2.32 mmol). Purification by column chromatography on reversed phase silica gel (water) gave the product as a pale-yellow solid (830 mg, 66%): mp: 230–234 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.44 (s, 8H, NH_2 and NH·HCl), 7.94 (d, 4H, J = 8.4 Hz, ArH), 7.70 (d, 4H, J = 8.4 Hz, ArH), 7.34–7.48 (m, 5H, ArH), 5.80 ppm (s, 2H, CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 165.4 (2C), 143.6 (2C), 135.6 (2C), 135.6, 129.2 (2C), 129.1 (4C), 128.7 (6C), 128.6 (2C), 128.4, 79.6 ppm; MS (ESI+): *m/z* (%): 396 $[M+H]^+$ (28), 198.5 [*M*+

2H]$^{2+}$ (100); HRMS (ESI+): *m/z* $[M+H]^+$ calcd for $C_{23}H_{22}N_7$: 396.1937, found: 396.1926; HPLC: $t_R = 5.90$ min (98.9% area).

4-Phenylethynylbenzonitrile (32): The same protocol as described for compound **22** was used to generate **32** from **31** (2 g, 19.58 mmol) and **21** (3.56 g, 19.58 mmol). Purification by column chromatography on silica gel (hexane/CH_2Cl_2, 5:5→4:6) gave the product as a white solid (3.91 g, 98%): mp: 95–97 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.64$ (d, 2H, $J = 8.4$ Hz, ArH), 7.60 (d, 2H, $J = 8.4$ Hz, ArH), 7.55 (d, 1H, $J = 7.5$ Hz, ArH), 7.54 (d, 1H, $J = 7.5$ Hz, ArH), 7.37–7.41 ppm (m, 3H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 132.5 (2C), 132.5 (2C), 132.2 (2C), 129.6, 128.9 (2C), 128.7, 122.6, 119.0, 111.9, 94.2, 88.1 ppm; MS (ESI+): *m/z* (%): 226 $[M+Na]^+$ (100); HRMS (ESI+): *m/z* $[M+Na]^+$ calcd for $C_{15}H_9NNa$: 226.0633, found: 226.0622.

4-(4-Cyanophenyl)-5-phenyl-[1,2,3]triazole (33): A solution of **32** (1.9 g, 9.35 mmol) in DMF (30 mL) was treated slowly with NaN_3 (1.22 g, 18.70 mmol). After 6 h at reflux with stirring, water (100 mL) was added to the reaction flask. The mixture was extracted with CH_2Cl_2 (3×100 mL). The combined organic layers were washed with water (300 mL) and brine, dried over $MgSO_4$, filtered and concentrated in vacuo to afford the product as an orange solid. This product was unstable, and so used in the subsequent reaction without further purification.

2,4-Bis-(4-cyanophenyl)-5-phenyl-[1,2,3]triazole (34): The same protocol as described for compound **28** was used to generate product **34** from benzonitrile **33** (1.10 g, 4.47 mmol) and **21** (1.63 g, 8.93 mmol). Purification by column chromatography on silica gel (CH_2Cl_2/hexane, 5:5→6:4→7:3→8:2) gave the product as a white powder (490 mg, 32% over two steps): mp: 126–128 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.32$ (d, 2H, $J = 8.8$ Hz, ArH), 7.83 (d, 2H, $J = 8.8$ Hz, ArH), 7.78 (d, 2H, $J = 8.5$ Hz, ArH), 7.70 (d, 2H, $J =$ 8.5 Hz, ArH), 7.59 (d, 1H, $J = 7.2$ Hz, ArH), 7.58 (d, 1H, $J = 7.8$ Hz, ArH), 7.46–7.50 ppm (m, 3H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 148.3, 145.7, 142.4, 135.0, 134.0 (2C), 132.9 (2C), 130.1, 129.8, 129.4 (2C), 129.2 (2C), 129.0 (2C), 119.5 (2C), 118.9, 118.6, 113.1, 111.6 ppm; MS (ESI+): *m/z* (%): 370 $[M+Na]^+$ (100); HRMS (ESI+): *m/z* $[M+Na]^+$ calcd for $C_{22}H_{13}N_5Na$: 370.1069, found: 370.1055.

2-(4-Cyanobenzyl)-4-(4-cyanophenyl)-5-phenyl-[1,2,3]triazole (35): A solution of triazole **33** (1.1 g, 4.47 mmol) in dry acetone (120 mL) was treated with K_2CO_3 (3.09 g, 22.33 mmol), 4-(bromomethyl)benzonitrile (4.38 g, 22.33 mmol), and TBAI (165 mg, 10% mol). The reaction mixture was heated to reflux under N_2 overnight. Thereafter, the reaction mixture was concentrated in vacuo. Purification by column chromatography on silica gel (CH_2Cl_2/hexane, 5:5→6:4→7:3→8:2) gave the product as a white solid (1.2 g, 74% over two steps): mp: 49–51 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.69$ (d, 2H, $J = 8.4$ Hz, ArH), 7.68 (d, 2H, $J = 8.6$ Hz, ArH), 7.63 (d, 2H, $J = 8.6$ Hz, ArH), 7.52 (d, 2H, $J = 8.4$ Hz, ArH), 7.47–7.50 (m, 2H, ArH), 7.40–7.44 (m, 3H, ArH), 5.71 ppm (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 146.5$, 143.8, 140.2, 135.6, 133.2 (2C), 132.8 (2C), 130.4, 129.6, 129.3 (2C), 129.2 (2C), 129.0 (2C), 128.8 (2C), 119.0, 118.8, 113.0, 112.5, 59.7 ppm; MS (ESI+): *m/z* (%): 384 $[M+Na]^+$ (100); HRMS (ESI+): *m/z* $[M+Na]^+$ calcd for $C_{23}H_{15}N_5Na$: 384.1225, found: 384.1241.

2,4-Bis-(4-amidinophenyl)-5-phenyl-[1,2,3]triazole dihydrochloride (36a): The same protocol as described for the synthesis of compound **5** was used to generate amidine **36a** from triazole **34** (450 mg, 1.30 mmol). Purification by column chromatography on reversed phase silica gel (water) gave the product as a white solid (390 mg, 63%): mp: 248–252 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta =$ 9.40 (s, 8H, NH·HCl and NH_2), 8.34 (d, 2H, $J = 8.2$ Hz, ArH), 8.10 (d, 2H, $J = 8.2$ Hz, ArH), 7.94 (d, 2H, $J = 7.9$ Hz, ArH), 7.82 (d, 2H, $J =$ 7.9 Hz, ArH), 7.59–7.63 (m, 2H, ArH), 7.50–7.55 ppm (m, 3H, ArH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 165.4$, 165.0, 147.7, 145.6, 142.4, 135.0, 130.6 (2C), 130.0, 129.6, 129.4 (2C), 129.1 (2C), 128.9, 128.8 (3C), 128.8 (2C), 127.6, 118.9 ppm; MS (ESI+): *m/z* (%): 382 $[M+H]^+$ (18), 191.5 $[M+2H]^{2+}$ (100); HRMS (ESI+): $[M+H]^+$ calcd for $C_{22}H_{20}N_7$: 382.1780, found: 382.1785; HPLC: $t_R = 7.45$ min (99.3% area).

2-(4-Amidinobenzyl)-4-(4-amidinophenyl)-5-phenyl-[1,2,3]triazole dihydrochloride (36b): The same protocol as described for the synthesis of compound **5** was used to generate amidine **36b** from triazole **35** (1 g, 2.77 mmol). Purification by column chromatography on reversed phase silica gel (water) gave the product as a white solid (920 mg, 71%): mp: 215–220 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 9.47$ (s, 4H, NH·HCl and NH_2), 9.37 (s, 4H, NH·HCl and NH_2), 7.90 (d, 2H, $J = 8.7$ Hz, ArH), 7.88 (d, 2H, $J = 8.2$ Hz, ArH), 7.68 (d, 2H, $J = 8.7$ Hz, ArH), 7.63 (d, 2H, $J = 8.2$ Hz, ArH), 7.43–7.51 (m, 5H, ArH), 5.92 ppm (s, 2H, CH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 165.8$, 165.5, 145.4, 143.1, 141.5, 135.8, 130.4, 129.3, 129.3 (2C), 129.0 (4C), 128.9 (2C), 128.5 (2C), 128.4 (2C), 128.3, 128.1, 57.9 ppm; MS (ESI+): *m/z* (%): 396 $[M+H]^+$ (30), 198.5 $[M+2H]^{2+}$ (100); HRMS (ESI+): $[M+H]^+$ and $[M+H]^{2+}$ calcd for $C_{23}H_{22}N_7$: 396.1937, found: 396.1939; HPLC: $t_R = 7.04$ min (99.2% area).

Biology

In vitro growth inhibition assay of P. falciparum *(GC03 and Dd2)*: *P. falciparum* cultures consisted of a 2% suspension of O+ erythrocytes in RPMI-1640 medium (R8758, glutamine, and $NaHCO_3$) supplemented with 10% pooled human AB+ serum, 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) at pH 7.4, and 20 μM gentamicin sulfate. Cultures were grown under a gaseous headspace of 4% O_2, 3% CO_2 in N_2 at 37 °C. Parasite growth was synchronized by treatment with sorbitol. Drug sensitivity experiments were performed as described by Smilkstein et al.[59] IC_{50} values were calculated by using the four-parameter logistic method (Grafit program; Erithacus Software, UK).

In vitro inhibition of hemozoin formation: For each product, the IC_{50} value was determined and expressed in micromolar. To measure this, NaOAc (0.5 M, pH 5.2), ghost membranes, a 3 mM solution of iron(III) FPIX (dissolved in 0.1 M NaOH), a 0.1 M solution of HCl, and the different drug dilutions (1 mM, 500 μM, 100 μM, 50 μM, 10 μM, 1 μM, 500 nM, and 100 nM) were introduced in eppendorf tubes. The eppendorf tubes were then incubated at 37 °C for 72 h. After incubation, the different solutions were centrifuged at 14000 rpm and the supernatant was discarded. The pellet was washed with a 2% solution of sodium dodecyl sulfate (SDS) and also a 1 M solution of Na_2CO_3 (pH 9). The pellet was then dissolved in a 1 M solution of NaOH, and the absorbance of this solution was measured at 400 nm. A graph was plotted for each product, and the IC_{50} value was determined by comparison with a control (without drug).

In vivo growth inhibition assay of P. vinckei:[60,61] Groups of three female Swiss mice OF1 (22–26 g, Charles River Laboratories France, Domaine de Oncins–BP 0109–69592 L'Arbresle Cedex) were acclimatized one week before experiments. Each animal was weighted the first day of the experiment in order to adjust dosage to mice weight and identified individually by a ring on their ear. Female Swiss mice were infected at day 0 by intravenous (iv) injection in

the caudal vein of 10^7 infected erythrocytes in 200 μL 0.9% NaCl. These injections lead to an initial parasitemia at day 1 between 0.5% and 1.5%. Mice were treated once a day for four days (days 1–4). The compounds were dissolved in DMSO and administered in 100 μL ip and/or po. On day 5, parasitemia levels were monitored in Giemsa-stained blood smears and by flow cytometry on blood samples (Yoyo-1 iodide (491/509), Invitrogen). In the absence of treatment, the *P. vinckei* malaria infection was lethal to the mice, and death followed on day 6–7 with parasitemia higher than 85%. The ED_{50} value was determined on day 5 only, when treatment induced a microscopically verified clearance of blood parasitemia. In addition, survival of mice that had cleared their parasitemia was monitored until at least one month after the end of treatment. Absence of parasitemia and lethality within the 30 days shows a complete cure without recrudescence. All protocols used for in vivo experiments were approved by the local ethics committee at CNRS-Université de Montpellier II, 34095 Montpellier (France)

Cytotoxicity:[62] HL-60 cells were cultured in RPMI-1640 media supplemented with 25 mM HEPES, 300 mg L^{-1} L-glutamine, 10% fetal bovine serum (FBS; Biowest, Nuaillé, France), 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin. Cells were cultured at 37 °C in a 5% CO_2 humidified atmosphere. For drug treatments, triazoles were dissolved in DMSO, with the concentration of the solvent in the media controlled to 0.5%. Cells were seeded in 96-well plates at 4×10^4 cells per well and exposed to the indicated drug for the indicated time. Cell viability was quantified by measuring the total cellular concentration of adenosine triphosphate (ATP), using the CellTiter-Glo luminescent assay (Promega, Southampton, UK) in accordance with the manufacturer's instructions. The concentrations of each compound that induced a 50% decrease in cellular ATP level (IC_{50}) were calculated using GraFit (Erithacus Software, Horley, UK).

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