In vitro antimalarial activity, β-haematin inhibition and structure-activity relationships in a series of quinoline triazoles

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Original article

In vitro antimalarial activity, β-haematin inhibition and structure–activity relationships in a series of quinoline triazoles

Mukesh C. Joshi [a], Kathryn J. Wicht [a], Dale Taylor [b], Roger Hunter [a], Peter J. Smith [b], Timothy J. Egan [a, *]

[a] Department of Chemistry, University of Cape Town, Private Bag, Rondebosch 7701, South Africa
[b] Division of Pharmacology, Department of Medicine, University of Cape Town, Observatory 7925, South Africa

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ABSTRACT

A novel series of quinoline triazole amide analogues (**38**–**51**) has been synthesized. Analogues **38**–**44** had a Cl substituent at the 7-position of the quinoline ring, while **45**–**51** had a CN substituent at this position. Compounds **40**, **45** and **49** were found to be the most active in the series against the *Plasmodium falciparum* chloroquine-sensitive D10 strain, with IC_{50} values in the range of 349–1247 nM, with **40** and **45**, but not **49** also exhibiting similar activity against the chloroquine-resistant K1 strain of parasite. Quinoline triazoles **40** and **44** were the most active β-haematin inhibitors, with 50% inhibitory concentrations of 14.7 and 8.9 μM respectively. *In vitro* antimalarial activity of the 7-Cl bearing analogues **38**–**44** exhibited a strong linear dependence of $\log(1/IC_{50})$ on log *P*. Thus, the more lipophilic, the more active it was found be. The 7-CN series **45**–**51** showed no such dependence. The resistance index (IC_{50} K1/IC_{50} D10) also exhibited a linear dependence on log *P*, with a substantially steeper slope in the case of the 7-Cl series. The findings demonstrate the feasibility of producing hydrophilic analogues with strong activity and low cross-resistance with chloroquine.

1. Introduction

Quinolines have formed a historically important class of antimalarial drugs. Although compromised by resistance, they remain of interest (e.g. Ref. [1]). The 4-aminoquinolines, such as chloroquine are known to inhibit the crystallisation of Fe(III)haem to β-haematin, the synthetic counterpart of haemozoin [2], an insoluble product formed in the parasite digestive vacuole following digestion of human haemoglobin [3]. Inhibition of haemozoin formation leads to intraparasitic accumulation of free Fe(III)haem [4], which is highly toxic to the parasite. Several studies have shown that side chain modification of 4-aminoquinolines can lead to increased potency of these compounds against drug-resistant *Plasmodium falciparum* strains [5,6]. Recently, hybrid 4-aminoquinolines with suitable side chains have been designed to act as chloroquine chemosensitizers with strong activity against resistant strains [5–8]. However, many of these compounds were very lipophilic, an undesirable trait from the point of view of adverse drug effects such as hERG inhibition and cytochrome P450 inhibition [9]. Based on the recent observation that 4-amino-7-cyanoquinolines retain good β-haematin inhibition activity and probably also retain antimalarial activity we have proposed that less lipophilic analogues of the 4-amino-7-chloroquinoline antimalarials can be made [10] and might show improved toxicity profiles. Lately, 4-aminoquinolines covalently linked to triazine groups were also synthesized in the expectation of improving activity against drug resistant parasites [11–14] owing to the altered side-chain, suggesting that other nitrogen heterocycles may also be advantageous. Triazoles are an important class of compounds known to exhibit a variety of biological activities including antifungal, antimicrobial [15–17] and antitubercular activity [18] that might also be expected to show improved antimalarial activity. Herein we report the synthesis, antimalarial activity and β-haematin inhibition activity of a series of 7-chloro- and 7-cyano-4-aminoquinolines with side chains containing a triazole as a linker, readily prepared using click chemistry [19–23]. We further report on their structure–activity relationships.

2. Chemistry

The synthesis sequence for producing the triazolyl-amines **29**–**35** for coupling is shown in Scheme 1. According to an established method [24–28] *N*-substituted amines (**1**–**7**, groups R_1 and R_2 with

* Corresponding author. Tel.: +27 21 650 2528; fax: +27 21 650 5195.
E-mail addresses: Timothy.Egan@uct.ac.za, timothy.john.egan@gmail.com (T.J. Egan).

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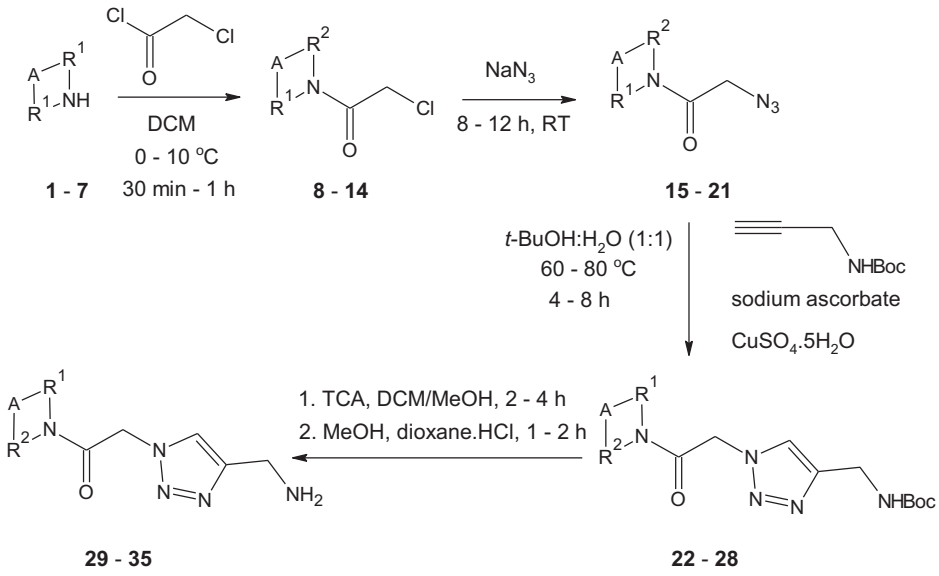
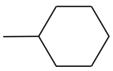
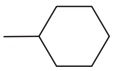
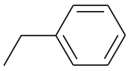


Scheme 1. Three-step sequence for synthesis of the amine coupling partners **29**–**35**.

or without a bridging group A, as specified in Table 1) were reacted with chloroacetyl chloride (1.1 equiv.) in dichloromethane at 0–10 °C for 30 min to 1 h to form amides **8**–**14**. Compounds **8**–**14** were further reacted [25,27,29] with NaN_3 (3.0 equiv.) in dimethylformamide at room temperature for 8–12 h to give azides **15**–**21**, which were then reacted at 60–80 °C for 4–8 h in a click reaction with *N*-Boc-propargylamine (1.6 equiv.) in *t*-BuOH and H_2O (1:1) in the presence of sodium ascorbate (0.4 equiv.) and $CuSO_4 \cdot 5H_2O$ (0.22 equiv.) as catalyst. This furnished compounds **22**–**28**, which were then deprotected by trifluoroacetic acid (5.0 equiv.) in dichloromethane/methanol (2:1). Isolation involved converting the residue to its hydrochloride salt using dioxane HCl to yield triazolyl amines **29**–**35** as hydrochloride salts in a purity of >90% as judged by ^{1}H NMR spectroscopy. The only chromatographic purification utilized in the three-step sequence was for the *N*-Boc derivatives **22**–**28** (Scheme 1).

The final quinoline-triazole analogues **38**–**51** were synthesized by S_NAr reaction of triazolyl-amines **29**–**35** with 4,7-dichloroquinoline (**36**) or 4-chloro-7-cyanoquinoline (**37**, 1.0–2.5 equiv.) using triethylamine (3.0–5.0 equiv.) as base in ethanol at 130–135 °C for 8 h under a nitrogen atmosphere in a sealed tube (Scheme 2). 4-Chloro-7-cyanoquinoline needed to be synthesized by a literature procedure [30], while 4,7-dichloroquinoline was used as received from a commercial supplier. The adducts were quite difficult to isolate as single spots on TLC in view of their high polarity, and it was found that column chromatographic purification using ethyl acetate/methanol/triethylamine as eluent needed to be carried out twice to obtain material of better than 85% purity by HPLC. Final purification to obtain material pure enough for biological testing was achieved using preparative-plate chromatography, which afforded the quinoline triazole analogues (**38**–**51**) in >95% purity by HPLC, except **39** and **43** which were more than 90% pure. A full analysis using ^{1}H and ^{13}C NMR spectroscopy, as well as high resolution mass spectrometry, confirmed the correct structures for the library.

Table 1
Side chains of amines **1**–**7** in Scheme 1.

	$-R_1$	$-R_2$	A
1	$-CH_2CH_3$	$-CH_2CH_3$	–
2	$-CH(CH_3)_2$	$-CH(CH_3)_2$	–
3			–
4		–H	–
5		$-CH_3$	–
6	CH_2	CH_2	CH_2OCH_2
7	CH_2	CH_2	$CH_2CH_2CH_2$

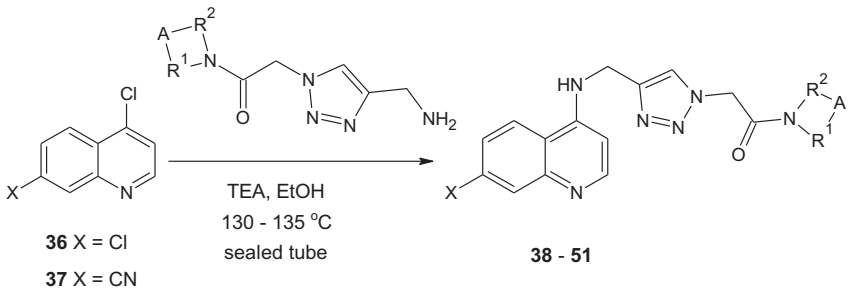
3. Biological and physico-chemical testing

3.1. In vitro antimalarial activity

Quinolone-triazole amides (QTA) **38**–**51** were tested for their *in vitro* antimalarial activity against the *P. falciparum* chloroquine-sensitive (CQS) D10 and chloroquine-resistant K1 strains (Table 2), and compared to chloroquine (CQ) as a standard drug. Compounds **40**, **45** and **49** were the most active analogues in the series against the *P. falciparum* D10 strain. With the exception of **45** and **49**, compounds having a 7-CN group in the quinoline core were less effective than those with a 7-Cl group. Resistance indices varied from low values in **40** and **47** (1.5 and 2.5 respectively) to high in **41** and **49** (10.8 and 12.1 respectively). Compounds **45** and **49** were also tested for cytotoxicity in mammalian (Chinese Hamster Ovarian, CHO) cells. Both compounds were cytotoxic at much higher concentrations than their antimalarial activity (Table 3), with selectivity indices versus the D10 strain of *P. falciparum* (IC_{50}(CHO)/IC_{50}(D10)) of 31 and 125 respectively and versus the K1 strain of 20 and 31 respectively.

3.2. Inhibition of β-haematin formation

Synthesised QTA compounds **38**–**51** were tested for β-haematin inhibiting properties using an NP-40 detergent mediated assay, the details of which have been reported elsewhere [31–33]. Standard



Scheme 2. S_NAr coupling to the final adducts **38**–**51**.

drugs such as CQ and amodiaquine were assayed prior to testing and were found to have IC_{50} values of 18.2 μM and 6.8 μM respectively. The assay results are reported in Table 2. The most active QTA compounds in the group with the 7-Cl substituent are **40** and **44**, which contain dicyclohexyl and piperidinyl substituents on the amides respectively. These results differ from the 7-CN substituent case where the most active compounds (**48** and **49**) contained cyclopentyl and benzyl substituents. There is no statistically significant difference in average IC_{50} values for β-haematin inhibition between the compounds with a 7-Cl substituent and those with a 7-CN substituent on the quinoline ring ($P = 0.25$). Furthermore, differences are generally relatively small between the various compounds, with the most active only about five-fold more active than the least active.

4. Discussion

Most highly active 4-aminoquinoline antimalarials contain side chains with a basic amino group thought to play a crucial role in pH trapping [34,35]. The QTA series contains an amide group which is unable to assist in pH trapping. It is therefore notable that compounds **40** and **45** have parasite IC_{50} values below 1 μM in the CQS D10 strain of parasite. The relatively strong activity of **45** is also noteworthy given that the 7-Cl group was replaced with a more hydrophilic 7-CN group in this analogue. Activity is not a mere result of cytotoxicity, because both **40** and **45** exhibit selective activity against malaria parasites.

Previous studies on 4-amino-7-chloroquinolines have shown that biological activity is often correlated with β-haematin inhibition, albeit only after taking into account varying levels of vacuolar accumulation via pH trapping [5,35]. In the case of the analogues studied here, there is a relatively narrow range of β-haematin inhibition IC_{50} values and the compounds possess only one at best moderately basic nitrogen atom, that in the 4-aminoquinoline group (with a conjugate acid pKa estimated at 7.16 for the 7-Cl series and 5.71 for the 7-CN series, while the triazole is only very weakly basic, with a conjugate acid pKa below 2) [36]. As a result, pH trapping is expected to occur to a much smaller extent for the Cl series and is probably negligible for the 7-CN series, possibly accounting for the generally weaker activity of the latter series relative to the former. On the other hand, the biological activity (expressed as log $(1/IC_{50})$ or $-\log IC_{50}$) was found to be strongly linearly correlated with calculated log of the partition coefficient (log *P*) in the case of the compounds with a 7-Cl group (**38**–**44**) as shown in Fig. 1a. Thus, in the case of the 7-Cl series, *in vitro* antimalarial activity simply increases with lipophilicity, probably reflecting the ease with which the compound reaches the digestive vacuole of the parasite. Of interest in this respect is compound **40**. This compound has two cyclohexyl groups attached to the amide N, resembling the reversed chloroquine motifs reported by Andrews et al. [6]. Indeed, identical terminal amide side chains with ethyl or piperazinyl linkers were described by these authors, with the former showing good activity. By contrast with the 7-Cl series, no correlation of *in vitro* antimalarial activity with lipophilicity was found for compounds **45**–**51** with a 7-CN group (Fig. 1b).

The resistance index was found to decrease with increasing log *P* in both the 7-Cl and 7-CN series. The gradient is much larger in the former series (Fig. 1c). As a result, the most hydrophobic analogues show almost no cross-resistance with chloroquine, while the least hydrophobic compounds show strong cross-resistance. Thus compound **40** is the most active against both the CQS D10 and CQR K1 parasite strains, and exhibits little evidence of cross-resistance with CQ. Interestingly, the linear dependence of resistance index on log *P* suggests that the lack of cross-resistance in this analogue may be related to its physico-chemical properties, rather than its resemblance to a CQ chemosensitizing pharmacophore.

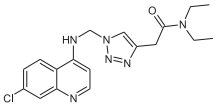
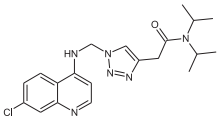
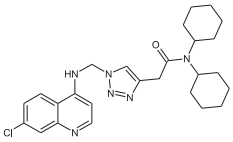
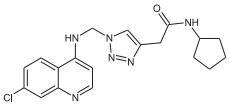
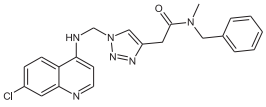
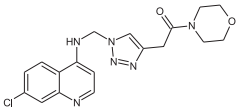
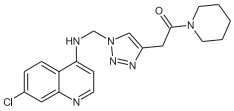
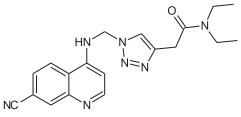
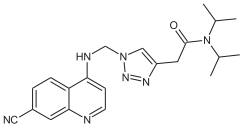
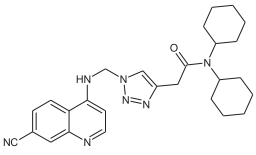
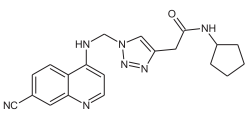
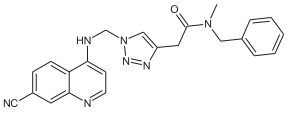
The dependence of resistance index on log *P* is much less steep in the case of the 7-CN series (Fig. 1d). This means that these compounds have comparable resistance index values to the 7-Cl compounds of similar polarity at the hydrophobic end of the scale, but lower resistance index values at the hydrophilic end.

5. Conclusion

The successful synthesis of a series of quinoline–triazole amides using click chemistry has produced several compounds with good *in vitro* antimalarial activity. More importantly, these compounds have proved to be valuable probes of activity and resistance in the 4-aminoquinolines. Several observations are particularly noteworthy. Firstly, replacement of the 7-Cl group with a 7-CN group, which results in significantly more polar compounds, does not render the compound inactive. Furthermore, the CN group decouples the tight correlation between activity and lipophilicity seen in the 7-Cl series. This is important, because reduced lipophilicity is often accompanied by decreased adverse drug effects and is also beneficial for oral bioavailability [9]. Attempting to reduce lipophilicity in 7-Cl analogues simply decreases activity, whereas some of the hydrophilic CN compounds retain strong activity. Secondly, the observation of much lower levels of cross-resistance in the 7-CN series suggests that the 7-chloroquinoline ring itself is involved in resistance. Many studies have pointed to the importance of the side-chain in determining resistance, while the role of the 4-amino-7-chloroquinoline core has been less clear. It seems from this work that this group is itself part of the pharmacophore involved in the resistance mechanism. This highlights the need to move away from this motif. Thirdly, the 4-amino-7-cyanoquinoline series exhibits much lower levels of resistance in hydrophilic analogues than the 7-Cl series. This suggests that strongly active hydrophilic analogues with relatively little cross resistance with CQ can be prepared.

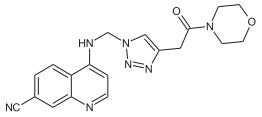
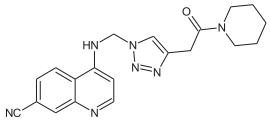
It may be possible to improve the activities of these compounds by reducing the amide groups to amines, increasing pH trapping to produce compounds with activities similar to chloroquine, but not

Table 2
IC_{50} data for *in vitro* antimalarial activity against the CQS D10 and CQR K1 strains of *P. falciparum* and β-haematin (β-H) inhibition of compounds **38**–**51**, together with resistance index, IC_{50}(K1)/IC_{50}(D10), calculated log *P* values. Values for CQ are given for comparison.

Compound	Structure	Parasite IC_{50} (nM)		RI	β-H IC_{50} (μM)	log *P*
		D10	K1			
38		1440	10,498	7.3	20.3	2.45
39		1592	9518	6.0	32.7	3.27
40		348.8	518.6	1.5	14.7	4.93
41		2473	26,681	10.8	30.5	2.70
42		1910	9077	4.8	21.7	3.54
43		8387	69,624	8.3	28.7	1.42
44		2063	11,287	5.5	8.9	2.48
45		584.5	2399	4.1	46.3	1.74
46		3969	14,440	3.6	23.7	2.57
47		2599	6563	2.5	26.5	4.23
48		12,131	37,297	3.1	16.1	2.00
49		1247	15,057	12.1	15.9	2.84

(*continued on next page*)

Table 2 (*continued*)

Compound	Structure	Parasite IC_{50} (nM)		RI	β-H IC_{50} (μM)	log *P*
		D10	K1			
50		27,518	130,625	4.7	44.6	0.72
51		13,619	43,439	3.2	32.7	1.78
CQ		17.4	354.7	20.3	18.2	3.81

exhibiting cross-resistance with chloroquine. However there is a caveat. The finding that secondary amides with either 7-Cl or 7-CN groups show high-level resistance may indicate that *N*-dealkylation of the tertiary amines would result in a secondary amine metabolite with similar high-level resistance. This will need careful investigation.

6. Experimental

6.1. Chemistry

Solvents, acids, and common salts were obtained from Saarchem, South Africa, while 4,7-dichloroquinoline **36** and other chemicals were obtained from Sigma–Aldrich. 4-Chloro-7-cyanoquinoline **37** was synthesized according to a literature procedure [30]. Pre-coated silica-gel plates as well as silica-gel for column chromatography were obtained from Merck. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury spectrometer at 300 MHz and a Bruker spectrometer at 400 MHz. All spectra were recorded in either d-chloroform (references = 7.26 ppm for ^{1}H and 77.16 ppm for ^{13}C NMR), d_4-methanol (references = 3.31 ppm for ^{1}H and 49.00 ppm for ^{13}C NMR), or d_6-dimethylsulphoxide (references = 2.50 for ^{1}H and 39.52 ppm for ^{13}C NMR), which were obtained from Sigma–Aldrich. Mass spectra were recorded on a VG Micromass 16F spectrometer operating at 70 eV with an accelerating voltage of 4 kV and a variable temperature source. Accurate mass determinations were performed on a Kratos Limited MS9/50 spectrometer. All mass spectra were obtained using electron-impact techniques. The elemental analysis (HRMS-ES$^+$) of final compounds were recorded using a Waters Synapt G2 instrument. All the final compounds were subjected to HPLC to evaluate purity using an Agilent Technologies 1220 Infinity LC Gradient system with an Eclipse Plus C18 column (5 μm, 4.6 × 150 mm). Purity exceeded 95% except where otherwise stated. Melting points for all final products were measured using a BioCote SMP-10 instrument.

6.2. Synthesis

6.2.1. General method for the synthesis of ***8*–*14***

To a series of substituted amines (**1**–**7**) in dichloromethane (0.05 g/mL) was added chloroacetyl chloride (1.1 equiv.) followed by the addition of triethylamine (2.5 equiv.) at 0–10 °C. The reaction was continued at the same temperature for 30 min to 1 h. The progress of reaction was monitored by TLC. After completion of the reaction, the reaction mixture was washed with saturated $NaHCO_3$ solution and washed with brine solution The excess organic solvent was removed under reduced pressure and the crude compound used for further reaction without purification.

*6.2.1.1. 2-Chloro-N,N-diethylacetamide (**8**) [24].* Light-yellow liquid; yield (78%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.13 (t, *J* = 7.2 Hz, 3H), 1.22 (t, *J* = 7.2 Hz, 3H), 3.36 (q, *J* = 7.2 Hz, 2H), 3.38 (q, *J* = 7.2 Hz, 2H), 4.04 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 12.7, 14.5, 40.7, 41.3, 42.6, 165.8.

*6.2.1.2. 2-Chloro-N,N-diisopropylacetamide (**9**).* Dark-brown liquid; yield (96%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24 (d, *J* = 7.6 Hz, 6H), 1.39 (d, *J* = 7.6 Hz, 6H), 3.44 (m, 1H), 3.96 (m, 1H), 4.01 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 20.4, 21.0, 43.3, 46.5, 50.0, 165.2.

*6.2.1.3. 2-Chloro-N,N-dicyclohexylacetamide (**10**).* Brown-solid powder; yield (82%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21–1.81 (m, 20H), 3.02 (m, 1H), 3.43 (m, 1H), 4.01 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 25.4 (2), 26.0, 26.6, 29.7, 31.4, 43.6, 56.6, 59.1, 165.6.

*6.2.1.4. 2-Chloro-N-cyclopentylacetamide (**11**) [25].* Brown, crystalline solid; yield (92%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38–1.47 (m, 2H), 1.59–1.74 (m, 4H), 1.97–2.05 (m, 2H), 4.01 (s, 2H), 4.25 (m, 1H), 6.47 (br. s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 23.8, 33.1, 42.8, 51.7, 165.5.

*6.2.1.5. N-Benzyl-2-chloro-N-methylacetamide (**12**) [26].* Dark-yellow liquid; yield (~100%); ^{1}H NMR (400 MHz, $CDCl_3$): δ (major isomer) 2.98 (s, 3H), 4.12 (s, 2H), 4.58 (s, 2H), 7.17–7.35 (m, 5H); (minor isomer) 2.95 (s, 3H), 4.08 (s, 2H), 4.58 (s, 2H), 7.17–7.35 (m, 5H); ^{13}C NMR (400 MHz, $CDCl_3$): δ (major isomer) 35.2, 41.5, 51.5, 126.6, 128.1, 128.9, 136.6, 166.8; (minor isomer) 34.5, 41.2, 53.8, 126.6, 127.8, 129.2, 135.9, 167.0.

*6.2.1.6. 2-Chloro-1-(morpholin-4-yl)ethanone (**13**) [27].* Light-reddish solid; yield (92%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.52 (m, 2H), 3.61 (m, 2H), 3.66–3.74 (m, 4H), 4.05 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 40.7, 42.6, 46.9, 66.6, 66.7, 165.4.

*6.2.1.7. 2-Chloro-1-(piperidin-1-yl)ethanone (**14**) [28].* Dark-yellow liquid; yield (92%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.55–1.60 (m, 2H), 1.61–1.68 (m, 4H), 3.45 (t, *J* = 5.7 Hz, 2H), 3.57 (t,

Table 3
Cytotoxicity and selectivity index values of compounds **40** and **45**. The cytotoxicity of emetine is given for comparison.

Compound	D10 IC_{50} (μM)	K1 IC_{50} (μM)	CHO IC_{50} (μM)	SI (D10)	SI (K1)
40	0.349	0.519	10.7	30.7	20.6
45	0.585	2.40	73.2	125	30.5
Emetine	–	–	0.041	–	–

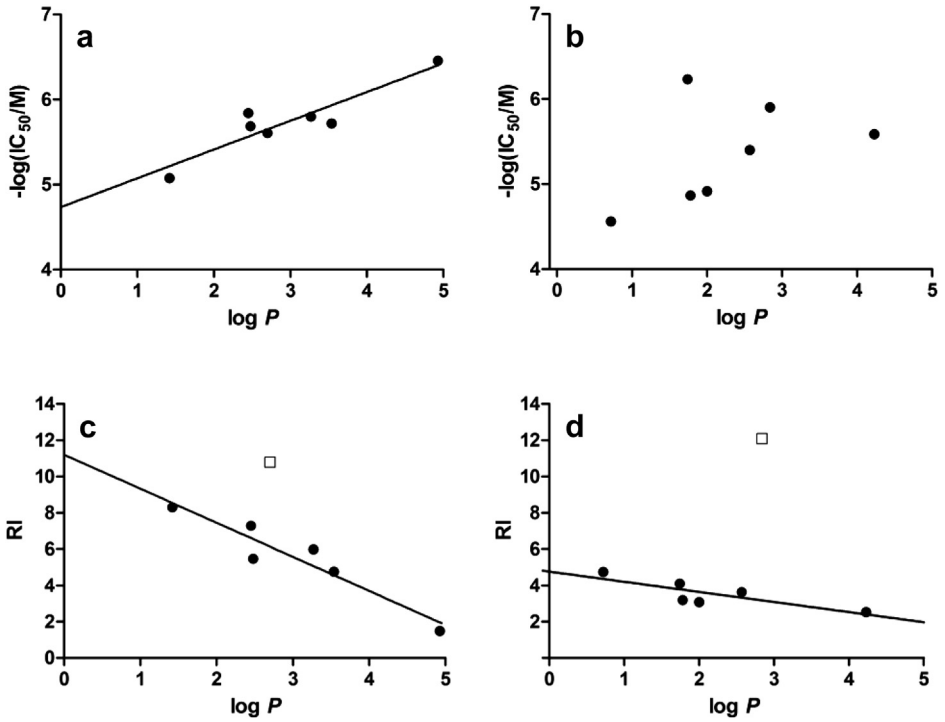


Fig. 1. Quantitative structure–activity relationships were observed in the QTA series. (a) The 7-Cl series (D10 strain) with $-\log IC_{50} = 0.34(7)\log P + 4.7(2)$, $r^2 = 0.84$, $P = 0.0039$. (b) In the 7-CN series there is no relationship between biological activity and lipophilicity ($r^2 = 0.23$, $P = 0.28$). (c) In the 7-Cl series $RI = -1.9(3)\log P + 11(1)$, $r^2 = 0.90$, $P = 0.0039$. (d) In the 7-CN series $RI = -0.6(2)\log P + 4.8(4)$, $r^2 = 0.70$, $P = 0.038$. The open squares in (c) and (d) represent secondary amide compounds that show much higher levels of cross-resistance with chloroquine and were not included in correlation analyses.

$J = 5.7$ Hz, 2H), 4.06 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 24.4, 25.5, 26.4, 41.3, 43.4, 47.5, 165.0.

6.2.2. General method for the synthesis of ***15–21***

To a mixture of **8–14** in dimethylformamide (0.067 g/mL) was added sodium azide (3.0 equiv.) at room temperature and the reaction allowed to proceed for 8–12 h. The progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was subjected to an extraction with ethyl acetate with saturated $NaHCO_3$ solution, and the organic extracts washed with brine solution and finally water. The organic solvent was removed under reduced pressure and the crude compounds were used for further reaction without any purification, based on a high-level purity (>95%) by ^{1}H NMR spectroscopy.

*6.2.2.1. 2-Azido-N,N-diethylacetamide (**15**).* Dark-yellow liquid; yield (99%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.14 (t, $J = 7.2$ Hz, 3H), 1.18 (t, $J = 7.2$ Hz, 3H), 3.22 (q, $J = 7.2$ Hz, 2H), 3.40 (q, $J = 7.2$ Hz, 2H), 3.88 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 13.0, 14.4, 40.8, 41.7, 50.5, 166.5; EI-MS (m/z) %: 156 (M^+, 12), 100 (100), 72 (80).

*6.2.2.2. 2-Azido-N,N-diisopropylacetamide (**16**).* Dark-brown liquid; yield (92%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22 (d, 6H), 1.39 (d, 6H), 3.53 (m, 1H), 3.73 (m, 1H), 3.85 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 20.6, 21.1, 46.5, 48.4, 52.3, 165.8; (EI-MS (m/z) %:185 ($M + H)^+$, 9), 86 (100).

*6.2.2.3. 2-Azido-N,N-dicyclohexylacetamide (**17**).* Brown solid; yield (82%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.10–1.90 (m, 20H), 3.05 (m, 1H), 3.18 (m, 1H), 3.86 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 25.3 (2), 26.1, 26.6, 30.1, 31.5, 52.3, 56.5, 57.7, 166.1; EI-MS (m/z) %: 264 (M^+, 5), 83 (100).

*6.2.2.4. 2-Azido-N-cyclopentylacetamide (**18**) [25].* Brown solid; yield (88%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.36–1.46 (m, 2H), 1.58–1.74 (m, 4H), 1.95–2.04 (m, 2H), 3.92 (s, 2H), 4.21 (m, 1H), 6.20 (br. s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 23.8, 33.1, 51.3, 52.9, 166.1; EI-MS (m/z) %: 168 (M^+, 18), 69 (100).

*6.2.2.5. 2-Azido-N-benzyl-N-methylacetamide (**19**).* Brown liquid; yield (97%); ^{1}H NMR (300 MHz, $CDCl_3$): δ (major isomer) 2.87 (s, 3H), 3.97 (s, 2H), 4.61 (2H, s), 7.15 (m, 1H), 7.23–7.42 (m, 4H); (minor isomer) 3.01 (s, 3H), 3.95 (s, 2H), 4.46 (s, 2H), 7.15 (m, 1H), 7.23–7.42 (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$): δ (major isomer) 34.2, 50.7, 51.4, 126.3, 128.7, 129.2, 136.5, 167.2; (minor isomer) 34.5, 50.8, 53.0, 126.3, 128.2, 129.7, 135.6, 167.6; EI-MS (m/z) %: 204 (17), 91 (100).

*6.2.2.6. 2-Azido-1-(morpholin-4-yl)ethanone (**20**) [27].* Yellow powder; yield (50%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.38 (m, 2H), 3.64 (m, 2H), 3.68 (m, 4H), 3.92 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 42.4, 45.7, 50.7, 66.5, 66.8, 165.9; EI-MS (m/z) %: 170 (M^+, 17), 113 (100).

*6.2.2.7. 2-Azido-1-(piperidin-1-yl)ethanone (**21**) [29].* Brown liquid; yield (97%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.53–1.70 (m, 6H), 3.30 (m, 2H), 3.57 (m, 2H), 3.91 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 24.5, 25.6, 26.5, 43.3, 46.3, 50.9, 165.2; EI-MS (m/z) %: 168 (M^+, 33), 112 (100).

6.2.3. General method for the synthesis of ***22–28***

To each of the azides **15–21** in *t*-BuOH (0.067 g substrate/mL) was added *N*-Boc-propargylamine (1.6 equiv.) and the mixture vigorously stirred at room temperature in a round-bottomed flask. In a second round-bottomed flask a yellow suspension was prepared by vigorously stirring a solution of sodium ascorbate (0.40 equiv.) and $CuSO_4 \cdot 5H_2O$ (0.22 equiv.) in distilled water. This yellow suspension was then poured into the first round-bottomed flask with vigorous stirring such that the ratio of *t*-BuOH to water

was 1:1. The reaction temperature was then increased from room temperature to 60–80 °C and the reaction vigorously stirred for 4–8 h. The progress of the reaction was monitored by TLC. Upon reaction completion, excess solvent was removed from the reaction mixture and the crude mixture dissolved in ethyl acetate, which was washed with 80% brine solution. The organic solvent was dried over anhydrous $MgSO_4$ and excess organic solvent was removed under reduced pressure. The compounds were purified by flash column chromatography using ethyl acetate/methanol/triethylamine as eluent.

*6.2.3.1. tert-Butyl ({1-[2-(diethylamino)-2-oxoethyl]-1H-1,2,3-triazol-4-yl}methyl)carbamate (**22**).* Dark-brown oil; yield (93%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.13 (t, $J = 7.2$ Hz, 3H), 1.25 (t, $J = 7.2$ Hz, 3H), 1.42 (s, 9H), 3.41 (m, 4H), 4.42 (s, 2H), 5.28 (s, 2H), 5.37 (br. s, 1H), 7.77 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 12.9, 14.5, 28.5, 36.2, 41.1, 42.1, 51.2, 79.7, 124.1, 146.3, 155.9, 164.0; EI-MS (*m*/*z*) %: 311 (M^+, 10), 255 (98), 100.02 (100).

*6.2.3.2. tert-Butyl ({1-[2-(diisopropylamino)-2-oxoethyl]-1H-1,2,3-triazol-4-yl}methyl)carbamate (**23**).* Light-yellow powder; yield (84%); ^{1}H NMR (400 MHz $CDCl_3$): δ 1.18–1.40 (m, 12H), 1.44 (s, 9H), 3.57 (m, 1H), 3.97 (m, 1H), 4.41 (d, $J = 5.6$ Hz, 2H), 5.07 (br. s, 1H), 5.14 (s, 2H), 7.67 (s, 1H); ^{13}C NMR (400 MHz $CDCl_3$): δ 20.6, 20.8, 28.6, 36.7, 46.8, 48.8, 52.7, 79.8, 123.6, 145.8, 155.9, 163.5; EI-MS (*m*/*z*) %: 339 (M^+, 10), 86 (100).

*6.2.3.3. tert-Butyl ({1-[2-(dicyclohexylamino)-2-oxoethyl]-1H-1,2,3-triazol-4-yl}methyl)carbamate (**24**).* Brown powder; yield (~100%); ^{1}H NMR (300 MHz, $DMSOd_6$): δ 1.39 (s, 9H), 1.25–1.80 (m, 20H), 3.06 (m, 1H), 3.53 (m, 1H), 4.18 (d, $J = 6.0$ Hz, 2H), 5.34 (s, 2H), 7.19 (br. s, 1H), 7.71 (s, 1H); ^{13}C NMR (400 MHz, $DMSOd_6$): δ 24.6, 25.1 (2), 25.7, 28.2, 29.4, 30.3, 35.6, 51.8, 54.9, 56.1, 77.8, 124.1, 145.1, 155.5, 164.1; EI-MS (*m*/*z*) %: 419 (M^+, 2), 69 (100).

*6.2.3.4. tert-Butyl ({1-[2-(cyclopentylamino)-2-oxoethyl]-1H-1,2,3-triazol-4-yl}methyl)carbamate (**25**).* Light-yellow powder; yield (93%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.34 (s, 2H), 1.44 (s, 9H), 1.61 (m, 4H), 1.94 (m, 2H), 4.17 (m, 1H), 4.42 (s, 2H), 4.98 (s, 2H), 5.18 (br. s, 1H), 6.05 (br. s, 1H), 7.67 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 23.8, 28.5, 32.9, 36.3, 51.8, 53.3, 79.9, 124.0, 146.7, 156.0, 164.5; EI-MS (*m*/*z*) %: 323 (M^+, 8), 113 (100).

*6.2.3.5. tert-Butyl ({1-[2-(N-benzyl-N-methylamino)-2-oxomethyl]-1H-1,2,3-triazol-4-yl}methyl)carbamate (**26**).* Yellow powder; yield (76%); ^{1}H NMR (300 MHz, $DMSOd_6$): δ (major isomer) 1.40 (s, 9H), 3.02 (s, 3H), 4.20 (d, $J = 5.7$ Hz, 2H), 4.54 (s, 2H), 5.49 (s, 2H), 7.20–7.43 (m, 5H), 7.79 (s, 1H); (minor isomer) 1.40 (s, 9H), 2.83 (s, 3H), 4.20 (d, $J = 5.7$ Hz, 2H), 4.67 (s, 2H), 5.44 (s, 2H), 7.20–7.43 (m, 5H), 7.81 (s, 1H); ^{13}C NMR (400 MHz, $DMSOd_6$): (major isomer only) δ 28.0, 33.7, 35.6, 50.3, 51.6, 77.6, 123.9, 126.8, 127.3, 128.5, 136.9, 145.2, 155.5, 165.7; EI-MS (*m*/*z*) %: 359 (M^+, 10), 91(100).

*6.2.3.6. tert-Butyl ({1-[2-(morpholin-1-yl)-2-oxoethyl]-1H-1,2,3-triazol-4-yl}methyl)carbamate (**27**).* Yellow powder; yield (92%); ^{1}H NMR (300 MHz $CDCl_3$): δ 1.44 (s, 9H), 3.56 (m, 2H), 3.64 (m, 2H), 3.70 (m, 4H), 4.42 (d, $J = 5.9$ Hz, 2H), 5.13 (br. s, 1H), 5.21 (s, 2H), 7.68 (s, 1H); ^{13}C NMR (400 MHz $CDCl_3$): δ 28.5, 36.4, 42.7, 45.9, 51.0, 66.5, 66.7, 79.8, 123.6, 146.0, 155.9, 163.7; EI-MS (*m*/*z*) %: 325 (M^+, 10), 269 (100).

*6.2.3.7. tert-Butyl ({1-[2-oxo-2-(piperidin-1-yl)ethyl]-1H-1,2,3-triazol-4-yl}methyl)carbamate (**28**).* Yellow powder; yield (44%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (s, 9H), 1.54–1.75 (m, 6H), 3.46 (m, 2H), 3.56 (m, 2H), 2H, 4.41 (d, $J = 7.8$ Hz, 2H), 5.13 (br. s, 1H), 5.20 (s, 2H), 7.67 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 24.3, 25.4, 26.4, 28.5, 36.4, 43.6, 46.6, 51.2, 79.7, 123.7, 145.8, 155.9, 163.1; EI-MS (*m*/*z*) %: 323 (M^+, 14), 125 (100).

*6.2.4. General method for the synthesis of **29**–**35***

To each of **22**–**28** in 2:1 dichloromethane/methanol (0.1 g compound/mL) was added trifluoroacetic acid (5.0 equiv.) at 0 °C and the reaction left at room temperature for 2–4 h. The progress of the reaction was monitored by TLC. After completion of the reaction, excess solvent was removed, the crude mixture dissolved in anhydrous methanol (0.1 g substrate/mL), and dioxane–HCl solution added at 0 °C to give a final concentration of 0.067 g substrate/mL. After 0.5 h, the excess solvent was removed under high vacuum and the crude solid washed several times with 80% ethyl acetate/petroleum ether to remove organic by-products. The residues **29**–**35** were each obtained as a hydrochloride salt, which were pure enough (>90%) by ^{1}H NMR spectroscopy for the final S_NAr reaction.

*6.2.5. General method for the synthesis of 38–51 with 4,7-dichloroquinoline (**36**) or 4-chloro-7-cyanoquinoline (**37**)*

To a stirred solution of each of **29**–**35** in anhydrous EtOH (5.0 mL) under a N_2 atmosphere was added triethylamine (5.0 equiv.) and the reaction mixture was stirred for 15–20 min at 0 °C. 4,7-Dichloroquinoline (2.5 equiv) (**36**) or 4-chloro-7-cyanoquinoline (**37**) was added and the mixture allowed to reach room temperature before being heated under pressure in a sealed cycloaddition tube at 130–135 °C for 8 h. The progress of the reaction was monitored by TLC. On completion of the reaction the mixture was decanted into a round-bottomed flask and excess solvent removed under reduced pressure. The crude reaction mixture was then washed with 25% ethyl acetate/petroleum ether to remove excess **36**. The remaining crude mixture was then purified twice by column chromatography using mixtures of ethyl acetate/methanol/triethylamine (100% ethyl acetate to 80:15:5) as an eluent. Owing to the highly polar nature of all of these compounds, impurities were always included in the product. Thus, for analytical and biological purposes they were further purified by prep-TLC using ethyl acetate/methanol/triethylamine (92:5:3) as mobile phase (double development of plate) to give **38**–**51**.

*6.2.5.1. 2-(4-{[(7-Chloroquinolin-4-yl)amino]methyl}-1H-1,2,3-triazol-1-yl)-N,N-diethylacetamide (**38**).* White powder; yield (22%); Mp: 213–216 °C; ^{1}H NMR (300 MHz, CD_3OD): δ 1.12 (t, $J = 7.2$ Hz, 3H), 1.27 (t, $J = 7.2$ Hz, 3H), 3.40 (q, $J = 7.2$ Hz, 2H), 3.47 (q, $J = 7.2$ Hz, 2H), 4.71 (s, 2H), 5.40 (s, 2H), 6.65 (d, $J = 5.4$ Hz, 1H), 7.43 (dd, $J = 2.1$ Hz, $J = 9.0$ Hz, 1H), 7.80 (d, $J = 2.1$ Hz, 1H), 7.93 (s, 1H), 8.12 (d, $J = 9.0$ Hz, 1H), 8.37 (br. s, 1H); ^{13}C NMR (400 MHz, $DMSO-d_6$): δ 12.8, 13.9, 37.8, 39.8, 40.7, 50.4, 99.3, 117.4, 124.1, 124.4, 124.6, 126.9, 133.7, 143.8, 148.3, 150.1, 151.2, 164.5; HRMS-ES$^+$: Observed: 373.1551; Calculated: 373.1544 $[M + H]^+$ for $C_{18}H_{22}ClN_6O$; HPLC: 97.6%.

*6.2.5.2. 2-(4-{[(7-Chloroquinolin-4-yl)amino]methyl}-1H-1,2,3-triazol-1-yl)-N,N-diisopropylacetamide (**39**).* Off-white powder; yield (13%); Mp: 135–141 °C; ^{1}H NMR (300 MHz, CD_3OD): δ 1.30–1.36 (m, 6H), 3.61 (m, 1H), 4.08 (m, 1H), 4.88 (s, 2H), 5.42 (s, 2H), 7.00 (d, $J = 5.6$ Hz, 1H), 7.68 (dd, $J = 1.5$ Hz, $J = 6.6$ Hz, 1H), 7.88 (d, $J = 1.5$ Hz, 1H), 8.01 (s, 1H), 8.34 (d, $J = 6.6$ Hz, 1H), 8.42 (d, $J = 5.6$ Hz, 1H); ^{13}C NMR (400 MHz, CD_3OD): δ 20.8 (4× Me), 39.8, 47.7, 50.0, 53.3, 100.5, 121.8, 125.6, 126.7, 128.4, 138.0, 140.3, 141.9, 145.7, 149.3, 166.0; HRMS-ES$^+$: Observed: 401.1862; Calculated: 401.1857 $[M + H]^+$ for $C_{20}H_{26}ClN_6O$; HPLC: 90.1%.

*6.2.5.3. 2-(4-{[(7-Chloroquinolin-4-yl)amino]methyl}-1H-1,2,3-triazol-1-yl)-N,N-dicyclohexylacetamide (**40**).* Light-yellow powder;

Mp: 165–171 °C; yield (20%); ^{1}H NMR (300 MHz, CD_3OD): δ 1.12–1.84 (m, 20H), 3.12 (m, 1H), 3.57 (m, 1H), 4.75 (s, 2H), 5.40 (s, 2H), 6.74 (d, J = 4.5 Hz, 1H), 7.50 (dd, J = 1.5 Hz, 6.8 Hz, 1H), 7.82 (d, J = 1.5 Hz, 1H), 7.92 (s, 1H), 8.17 (d, J = 6.8 Hz, 1H), 8.38 (d, J = 4.5 Hz, 1H); ^{13}C NMR (400 MHz, CD_3OD): δ 26.2, 26.5, 26.8, 27.3, 30.9, 32.1, 39.5, 53.6, 57.8, 59.1, 100.5, 124.6, 126.1 (2), 126.9, 137.5, 145.4, 147.6, 150.6, 153.5, 166.3; HRMS-ES$^+$: Observed 481.2470; Calculated 481.2483 $[M + H]^+$ for $C_{26}H_{34}ClN_6O$; HPLC: 95.9%.

*6.2.5.4. 2-(4-{[(7-Chloroquinolin-4-yl)amino]methyl}-1H-1,2,3-triazol-1-yl)-N-cyclopentylacetamide (**41**).* Off-white powder; Mp: 215–218 °C (decomp.); yield (10%); ^{1}H NMR (300 MHz, CD_3OD): δ 1.46–1.75 (m, 6H), 1.90–1.97 (m, 2H), 4.11 (m, 1H), 4.74 (s, 2H), 5.09 (s, 2H), 6.71 (d, J = 3.9 Hz, 1H), 7.49 (dd, J = 1.5 Hz, 6.5 Hz, 1H), 7.82 (d, J = 1.5 Hz, 1H), 7.97 (s, 1H), 8.17 (d, J = 6.5 Hz, 1H), 8.39 (br. s, 1H); ^{13}C NMR (400 MHz, CD_3OD): δ 24.7, 33.4, 39.5, 52.8, 53.2, 100.4, 124.8 (2), 125.9. 127.3, 133.9, 139.5, 145.0, 148.9, 149.0, 166.9; HRMS-ES$^+$: Observed 385.1544; Calculated: 385.1544 $[M + H]^+$ for $C_{19}H_{22}ClN_6O$; HPLC: 95.4%.

*6.2.5.5. N-Benzyl-2-(4-{[(7-chloroquinolin-4-yl)amino]methyl}-1H-1,2,3-triazol-1-yl)-N-methylacetamide (**42**).* Off-white powder; Mp: 152–156 °C; yield (24%); ^{1}H NMR (300 MHz, CD_3OD): δ (major isomer) 3.08 (s, 3H), 4.60 (s, 2H), 4.82 (s, 2H), 5.53 (s, 2H), 6.84 (d, J = 5.7 Hz, 1H), 7.24–7.33 (m, 5H), 7.56 (dd, J = 1.8, 9.0 Hz, 1H), 7.83 (d, J = 1.8 Hz, 1H), 8.00 (s, 1H), 8.25 (d, J = 9.0 Hz, 1H), 8.38 (br. s, 1H); ^{13}C NMR (300 MHz,DMSO-d_6): δ (major isomer) 33.9, 38.0, 50.4, 50.7, 99.2, 116.6, 124.7, 124.9, 125.5, 127.0, 127.1, 127.5, 128.4, 135.6, 137.0, 143.0, 144.1, 147.6, 152.4, 165.9; HRMS-ES$^+$: Observed 421.1538; Calculated: 421.1544 $[M + H]^+$ for $C_{22}H_{22}ClN_6O$; HPLC: 97.65%.

*6.2.5.6. 2-(4-{[(7-Chloroquinolin-4-yl)amino]methyl}-1H-1,2,3-triazol-1-yl)-1-(morpholine-1-yl)ethanone (**43**).* Light-yellow powder; Mp: 170–175 °C; yield (12%); ^{1}H NMR (300 MHz, CD_3OD): δ 3.55–3.62 (m, 4H), 3.63–3.74 (m, 4H), 4.77 (d, J = 2.5 Hz, 2H), 5.46 (s, 2H), 6.74 (d, J = 6.0 Hz, 1H), 7.50 (dd, J = 2.1, 9.0 Hz, 1H), 7.82 (d, J = 2.1 Hz, 1H), 7.94 (s, 1H), 8.17 (d, J = 9.0 Hz, 1H), 8.38 (br. s, 1H); ^{13}C NMR (400 MHz, CD_3OD): δ 39.5, 43.6, 46.5, 51.6, 67.3, 100.3, 112.2, 118.5, 124.6, 126.0, 127.2, 132.4, 139.5, 141.2, 148.9, 154.2, 166.0; HRMS-ES$^+$: Observed 387.1339; Calculated 387.1336 $[M + H]^+$ for $C_{18}H_{20}ClN_6O$; HPLC: 94.1%.

*6.2.5.7. 2-(4-{[(7-Chloroquinolin-4-yl)amino]methyl}-1H-1,2,3-triazol-1-yl)-1-(piperidin-1-yl)ethanone (**44**).* Off-white powder; Mp: 238–242 °C; yield (20%); ^{1}H NMR (300 MHz, CD_3OD): δ 1.51–1.58 (m, 2H), 1.62–1.73 (m, 4H), 3.52 (m, 4H), 4.72 (s, 2H), 5.42 (s, 2H), 6.66 (d, J = 5.7 Hz, 1H), 7.45 (dd, J = 2.1, 9.0 Hz, 1H), 7.80 (d, J = 2.1 Hz, 1H), 7.91 (s, 1H), 8.13 (d, J = 9.0 Hz, 1H), 8.37 (d, J = 5.7 Hz, 1H); ^{13}C NMR (400 MHz, CD_3OD): δ 25.0, 26.3, 27.1, 39.4, 44.5, 47.2, 52.0, 100.3, 118.6, 124.2, 125.9, 126.5, 126.9, 136.9, 143.0, 145.5, 151.3 (2), 165.5; HRMS-ES$^+$: Observed 385.1546; Calculated 385.1544 $[M + H]^+$ for $C_{19}H_{22}ClN_6O$; HPLC: 95.8%.

*6.2.5.8. 2-(4-{[(7-Cyanoquinolin-4-yl)amino]methyl}-1H-1,2,3-triazol-1-yl)-N,N-diethylacetamide (**45**).* Off-white powder; Mp: 207–210 °C; yield (31%); ^{1}H NMR (300 MHz, CD_3OD): δ 1.12 (t, J = 7.2 Hz, 3H), 1.27 (t, J = 7.2 Hz, 3H), 3.41 (q, J = 7.2 Hz, 2H), 3.47 (q, J = 7.2 Hz, 2H), 4.74 (s, 2H), 5.41 (s, 2H), 6.79 (d, J = 5.7 Hz, 1H), 7.69 (dd, J = 1.4, 8.8 Hz, 1H), 7.95 (s, 1H), 8.19 (d, J = 1.4 Hz, 1H), 8.32 (d, J = 8.8 Hz, 1H), 8.49 (br. s, 1H); ^{13}C NMR (400 MHz, CD_3OD): δ 13.0, 14.3, 39.4, 42.0, 42.8, 51.8, 101.8, 114.3, 118.9, 122.7, 124.3, 126.0, 126.6, 133.5, 145.0, 146.8, 152.0, 152.5, 166.3; HRMS-ES$^+$: Observed 364.1888; Calculated 364.1886 $[M + H]^+$ for $C_{19}H_{22}N_7O$; HPLC: 99.2%.

*6.2.5.9. 2-(4-{[(7-Cyanoquinolin-4-yl)amino]methyl}-1H-1,2,3-triazol-1-yl)-N,N-diisopropylacetamide (**46**).* Off-white powder; Mp: 198–201 °C; yield (25%); ^{1}H NMR (300 MHz, CD_3OD): δ 1.28 (d, J = 6.9 Hz, 6H), 1.36 (d, J = 6.9 Hz, 6H), 3.61 (m, 1H), 4.08 (m, 1H), 4.73 (s, 2H), 5.39 (s, 2H), 6.79 (d, J = 5.7 Hz, 1H), 7.68 (dd, J = 1.5 Hz, 8.7 Hz, 1H), 7.93 (s, 1H), 8.20 (d, J = 1.5 Hz, 1H), 8.31 (d, J = 8.7 Hz, 1H), 8.50 (br. s, 1H); ^{13}C NMR (400 MHz, CD_3OD): δ 20.8 (4), 39.5, 47.7, 50.0, 53.3, 102.0, 114.3, 119.2, 122.7, 124.5, 126.3, 126.6, 134.1, 145.4, 147.6, 152.5, 152.7, 166.0; HRMS-ES$^+$: Observed 392.2192; Calculated 392.2199 $[M + H]^+$ for $C_{21}H_{26}N_7O$; HPLC: 98.8%.

*6.2.5.10. 2-(4-{[(7-Cyanoquinolin-4-yl)amino]methyl}-1H-1,2,3-triazol-1-yl)-N,N-dicyclohexylacetamide (**47**).* Light-yellow powder; Mp: 147–151 °C; yield (10%); ^{1}H NMR (300 MHz, CD_3OD): δ 1.36–1.85 (m, 18H), 2.33 (m, 2H), 3.12 (m, 1H), 3.56 (m, 1H), 4.75 (s, 2H), 5.40 (s, 2H), 6.82 (d, J = 5.9 Hz, 1H), 7.72 (dd, J = 1.5, 8.8 Hz, 1H), 7.93 (s, 1H), 8.20 (d, J = 1.5 Hz, 1H), 8.33 (d, J = 8.8 Hz, 1H), 8.50 (d, J = 5.9 Hz, 1H); ^{13}C NMR (400 MHz, CD_3OD): δ 26.2, 26.4, 26.8, 27.3, 30.9, 32.1, 39.5, 53.6, 57.8, 59.1, 101.9, 114.8, 119.1, 122.6, 124.7, 126.2, 126.9, 133.3, 145.3, 146.8, 152.0, 153.0, 166.3; HRMS-ES$^+$: Observed 472.2833; Calculated 472.2825 $[M + H]^+$ for $C_{27}H_{34}N_7O$; HPLC: 96.2%.

*6.2.5.11. 2-(4-{[(7-Cyanoquinolin-4-yl)amino]methyl}-1H-1,2,3-triazol-1-yl)-N-cyclopentylacetamide (**48**).* Light-yellow powder; Mp: 250–252 °C; yield (25%); ^{1}H NMR (300 MHz, CD_3OD): δ 1.45–1.97 (m, 8H), 4.10 (qu, J = 6.6 Hz, 1H), 4.73 (s, 2H), 5.08 (s, 2H), 6.77 (d, J = 5.7 Hz, 1H), 7.68 (dd, J = 1.5, 8.7 Hz, 1H), 7.97 (s, 1H), 8.19 (d, J = 1.5 Hz, 1H), 8.31 (d, J = 8.7 Hz, 1H), 8.48 (d, J = 5.7 Hz, 1H); ^{13}C NMR (400 MHz, CD_3OD-$CDCl_3$): δ 24.6, 33.4, 39.4, 52.8, 53.2, 101.9, 114.4, 119.1, 122.6, 124.4, 125.7, 126.6, 133.9, 145.4, 147.3, 152.4 (2), 166.9; HRMS-ES$^+$: Observed 376.1891; Calculated 376.1886 $[M + H]^+$ for $C_{20}H_{22}N_7O$; HPLC: 98.0%.

*6.2.5.12. N-Benzyl-2-(4-{[(7-cyanoquinolin-4-yl)amino]methyl}-1H-1,2,3-triazol-1-yl)-N-methylacetamide (**49**).* Light-yellow powder; Mp: 175–178 °C; yield (25%); ^{1}H NMR (300 MHz, CD_3OD): δ (major isomer) 3.06 (s, 3H), 4.60 (s, 2H), 4.74 (s, 2H), 5.51 (s, 2H), 6.77 (t, J = 5.7 Hz 1H), 7.28 (m, 5H), 7.67 (dd, J = 1.5 Hz, 1H), 7.97 (s, 1H), 8.18 (d, J = 1.5 Hz, 1H), 8.30 (d, J = 8.7 Hz, 1H), 8.48 (d, J = 5.7 Hz, 1H); ^{13}C NMR (400 MHz, CD_3OD): δ (major isomer) 34.6, 39.4, 52.0, 52.4, 101.7, 114.4, 119.0, 122.6, 124.3, 125.9, 126.5, 128.5, 128.8, 129.6, 133.9, 134.0, 145.3, 147.3, 152.4, 152.5, 167.6; HRMS-ES$^+$: Observed 412.1891; Calculated 412.1886 $[M + H]^+$ for $C_{23}H_{22}N_7O$; HPLC: 100.00%.

*6.2.5.13. 4-[({4-[2-(Morpholin-4-yl)-2-oxoethyl]-1H-1,2,3-triazol-1-yl}methyl)amino]quinoline-7-carbonitrile (**50**).* Off-white powder; Mp: 193–196 °C; yield (23%); ^{1}H NMR (300 MHz, CD_3OD): δ 3.58 (m, 4H), 3.65–3.73 (m, 4H), 4.75 (s, 2H), 5.45 (s, 2H), 6.79 (d, J = 6.0 Hz, 1H), 7.69 (dd, J = 1.5, 9.0 Hz, 1H), 7.94 (s, 1H), 8.18 (d, J = 1.5 Hz, 1H), 8.31 (d, J = 9.0 Hz, 1H), 8.48 (d, J = 6.0 Hz, 1H); ^{13}C NMR (300 MHz, CD_3OD-$CDCl_3$): δ 39.4, 43.6, 46.4, 51.7, 67.2, 101.6, 114.5, 118.7, 122.1, 124.4, 125.9, 126.6, 132.7, 146.7, 145.8, 151.1, 152.7, 165.7; HRMS-ES$^+$: Observed 378.1678; Calculated 378.1678 $[M + H]^+$ for $C_{19}H_{20}N_7O_2$; HPLC: 100.00%.

*6.2.5.14. 4-[({4-[2-Oxo-2-(piperidin-1-yl)ethyl]-1H-1,2,3-triazol-1-yl}methyl)amino]quinoline-7-carbonitrile (**51**).* Light-yellow powder; Mp: 220–223 °C; yield (21%); ^{1}H NMR (300 MHz, CD_3OD): δ 1.51–1.71 (m, 6H), 3.52 (m, 4H), 4.72 (s, 2H), 5.42 (s, 2H), 6.76 (d, J = 5.7 Hz, 1H), 7.45 (dd, J = 2.4, 9.0 Hz), 7.80 (d, J = 2.4 Hz, 1H), 7.91 (s, 1H), 8.13 (d, J = 9.0 Hz, 1H), 8.37 (d, J = 5.7 Hz, 1H); ^{13}C NMR (400 MHz, CD_3OD): δ 25.0, 26.3, 27.1, 39.4, 44.5, 47.2, 52.0, 101.8, 114.1, 119.1, 122.7, 124.3, 125.9, 126.4, 134.2, 145.4, 147.7, 152.1, 152.7,

165.5; HRMS-ES$^+$: Observed 376.1883; Calculated 376.1886 $[M + H]^+$ for $C_{20}H_{22}N_7O$; HPLC: 100.00%.

6.3. Biology

Haemin (≥98%; Fluka), chloroquine diphosphate salt, amodiaquine, dimethyl sulfoxide (DMSO), sodium acetate trihydrate, acetic acid, acetone, HEPES and pyridine were purchased from Sigma–Aldrich. NP-40 was obtained from Pierce Biotechnology, Rockford, IL. Flat bottom, 96 well plates (catalogue number 655 180; Cellstar) were purchased from Greiner Bio-One.

6.3.1. Cell culture

6.3.1.1. Parasite survival assay. The samples were tested in triplicate on one occasion against a chloroquine-sensitive strain of *P. falciparum* (D10) and a chloroquine-resistant strain K1. Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen [37]. Quantitative assessment of antimalarial activity *in vitro* was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler [38].

The test samples were prepared as 20 mg/ml stock solutions in 100% DMSO and sonicated to enhance solubility. Samples were tested as a suspension if not completely dissolved. Stock solutions were stored at −20 °C. Further dilutions were prepared on the day of the experiment. Briefly, samples were serially diluted with complete medium to produce solutions of varying concentration across 96-well plates (100 μL). Parasitized red blood cells (100 μL, 2% parasitaemia, 2% haematocrit) were added to each well (test and control). Unparasitized red blood cells (100 μL) were added to blank wells. The plates were incubated for 48 h at 37 °C under 3% O_2, 4% CO_2 and 93% N_2. Thereafter, the contents of each well were resuspended and 15 μL was transferred to other plates containing Malstat reagent in each well (100 μL). Finally, 25 μL of nitroblue tetrazolium (NBT)/phenazine ethosulfate (PES) was added to each well and the plates were allowed to develop in the dark for 5–10 min and the absorbance of the blue formazan product in each well was measured at 620 nm on a microplate reader. Chloroquine (CQ) was used as a reference drug in all experiments. A full dose–response was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC_{50}). Test samples were used at a starting concentration of 1000 ng/ml, which was then serially diluted 2-fold in complete medium to give 10 concentrations, with the lowest concentration being 2 ng/ml. The same dilution technique was used for all samples. CQ was tested at a starting concentration of 100 ng/ml. The highest concentration of solvent to which the parasites were exposed had no measurable effect on the parasite viability (data not shown). The IC_{50} values were obtained using a non-linear least-squares dose–response curve fitting analysis via GraphPad Prism v.4.0 software [39].

6.3.1.2. Cytotoxicity testing. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT)-assay was used as a colorimetric assay for cellular growth and survival [40]. The tetrazolium salt MTT was used to measure all growth and chemosensitivity. The samples were tested in triplicate on one occasion against a mammalian cell-line, Chinese Hamster Ovarian (CHO).

The test samples were prepared to a 20 mg/ml stock solution in 100% DMSO and were tested as a suspension. Test compounds were stored at −20 °C until use. Dilutions were prepared on the day of the experiment. Emetine was used as the reference drug in all experiments. The initial concentration of emetine was 100 μg/ml, which was serially diluted in complete medium with 10-fold dilutions to give 6 concentrations, the lowest being 0.001 μg/ml. The same dilution technique was applied to the all test samples. The highest concentration of solvent to which the cells were exposed had no measurable effect on the cell viability (data not shown). The 50% inhibitory concentration (IC_{50}) values were obtained from full dose–response curves, using a non-linear dose–response curve fitting analysis via GraphPad Prism v.4 software.

6.3.2. Biophysical testing

6.3.2.1. Detergent mediated assay for β-haematin inhibition. The β-haematin formation inhibition assay method described by Carter et al. [31] was modified for manual liquid delivery [41]. Stock solutions of the test compounds were prepared at 10 mM, 2 mM and 0.4 mM by dissolving each sample in DMSO with sonication. Test compounds were delivered to a 96 well plate in triplicate from 0 to 500 μM (final concentration) with a total DMSO volume of 10 μL in each well. Deionized H_2O (70 μL) and NP-40 (20 μL; 30.6 μM) were then added. A 25 mM haematin stock solution was prepared by sonicating haemin in DMSO, for complete dissolution, and then suspending 178 μL of this in a 2 M acetate buffer (pH 4.8). The homogenous suspension (100 μL) was then added to the wells to give final buffer and haematin concentrations of 1 M and 100 μM respectively. The plate was covered and incubated at 37 °C for 5–6 h in a water bath. Analysis was carried out using the pyridine–ferrichrome method developed by Ncokazi and Egan [32]. A solution of 50% (v/v) pyridine, 30% (v/v) H_2O, 20% (v/v) acetone and 0.2 M HEPES buffer (pH 7.4) was prepared and 32 μL added to each well to give a final pyridine concentration of ~5% (v/v). Acetone (60 μL) was then added to assist with haematin dispersion. The UV–vis absorbance of the plate wells was read on a SpectaMax plate reader. Sigmoidal dose–response curves were fitted to the absorbance data using GraphPad Prism v3.02 to obtain a 50% inhibitory concentration (IC_{50}) for each compound.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.08.046.

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