Antiplasmodial, β-haematin inhibition, antitrypanosomal and cytotoxic activity *in vitro* of novel 4-aminoquinoline 2-imidazolines†

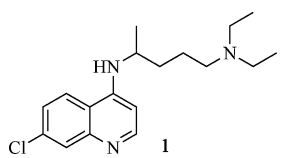
Chitalu C. Musonda,[a] Vanessa Yardley,[b] Renata C. Carvalho de Souza,[c] Kanyile Ncokazi,[a] Timothy J. Egan[a] and Kelly Chibale*[a,d]

Received 28th July 2008, Accepted 19th September 2008
First published as an Advance Article on the web 24th October 2008
DOI: 10.1039/b813007h

A novel series of 4-aminoquinoline-containing 2-imidazolines were synthesized *via* a one-pot 3-component condensation reaction of amine, aldehyde and isocyanoacetate. The products were obtained in high yield as well as purity and were evaluated directly against two strains of *Plasmodium falciparum* and *Trypanosoma brucei.* Compound **21** was the most active across all parasites with ED_{50} = 3.3 nM against a chloroquine (CQ)-sensitive 3D7 strain, ED_{50} = 33 nM against a CQ-resistant K1 strain and ED_{50} = 70 nM against *T. brucei.* Several compounds were able to inhibit formation of β-haematin *in vitro*, suggesting haemozoin formation in the malaria parasite as a possible target. On the other hand, evaluation against a human KB cell line revealed that the compounds were generally non-cytotoxic to the host cells.

Introduction

Sub-Saharan Africa remains the most affected global region in terms of the major parasitic diseases afflicting humanity. For instance, malaria, a parasitic disease transmitted by the female species of the *Anopheles* mosquito, afflicts an estimated 300–500 million people on an annual basis. Approximately 1–2 million of these cases result in death.[1] *Plasmodium falciparum* is the causal agent of malaria, and of the four species that are responsible for transmission of human malaria, *P. falciparum* is the most virulent, accounting for 95% of malaria-related deaths. The problem of endemic malaria has been exacerbated by the emergence of widespread resistance to the once first line treatment drugs such as chloroquine, (CQ) **1**, Fig. 1 and others.



1

Fig. 1

This new dimension to the malaria problem created by the emergence of multiple drug resistance has prompted the need to discover new chemotherapeutic agents against the parasite. Thus, the global significance of malaria cannot be overemphasized. On the other hand, African trypanosomiasis (sleeping sickness) affects both humans as human African trypanosomiasis (HAT) and livestock as nagana in cattle. The causal agent is a parasite within the *Trypanosoma brucei* complex[2] that is spread to humans through the bite of a tsetse fly and is believed to infect 300 000–500 000 people in Africa, mostly in the sub-Saharan regions. If it remains untreated the disease can be fatal. Unfortunately, the chemotherapies employed for the treatment of HAT are usually administered in large doses and are associated with high levels of toxicity or are expensive (*e.g.* DL-α-difluoromethylornithine, DFMO).

Contemporary drug discovery is faced with the challenge of having to design chemical reactions that are highly efficient. To this end, reactions that can provide maximum structural complexity and diversity with minimal synthetic steps to assemble molecules with therapeutic properties would be attractive.[3] One of the most versatile tools that has emerged as a means to rapidly identify, generate and optimize lead compounds in the drug discovery process is combinatorial chemistry.[4,5] A survey of the literature reveals that most drugs on the market are small organic compounds that contain heterocyclic rings.[6,7] However, easy access and availability of suitably functionalized heterocyclic building blocks for the synthesis of diverse libraries is rather limited. Consequently the development of new, efficient and clean synthetic reactions remains a vital challenge to organic and medicinal chemists.[8]

The multicomponent reaction (MCR)[9,10] strategy has sparked widespread interest in the recent past as a means to discovering biologically interesting entities.[11] Multicomponent reactions (MCRs) involve at least three starting materials in a one-pot reaction and by far remain the most efficient method of rapidly introducing molecular diversity. As such they have found widespread use in organic and diversity-oriented synthesis by their ability to access highly functionalized molecules in simple and straightforward one-step transformations.[12] Of the many

[a]*Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa. E-mail: Kelly.Chibale@uct.ac.za; Fax: +27 21 689 7499; Tel: +27 21 650 2553*
[b]*Department of Infectious & Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, UK*
[c]*Centro de Pesquisas René Rachou, FIOCRUZ, 30190-002 Belo Horizonte, Brazil*
[d]*Institute of Infectious Disease and Molecular Medicine, University of Cape Town Medical School, Observatory, 7925, South Africa*

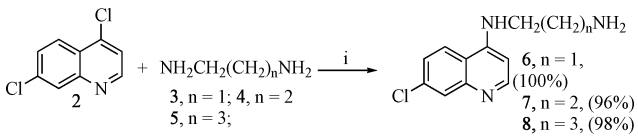
† Electronic supplementary information (ESI) available: Experimental details for the synthesis of all compounds and biological evaluation. See DOI: 10.1039/b813007h

MCRs known to date, the most valuable ones are those based on isocyanides, also known as isocyanide-based MCRs (IMCRs).[11] One such very early reaction is the Ugi 4-component condensation (4CC) reaction[10] that combines an amine, aldehyde (or ketone), carboxylic acid and isocyanide in a single-stage reaction to afford α-acylamino amides. Various expressions of the Ugi MCR have been documented,[11] with those delivering heterocyclic scaffolds such as 2-imidazolines being particularly attractive.

Our interest in the 2-imidazoline scaffold stemmed from the array of biological properties exhibited by compounds containing this sub-structure. Indeed, imidazoline derivatives exhibit a wide variety of biological activities including potent antidiabetic[13] and antihypertensive[14] properties. Despite the great potential of 2-imidazolines in chemotherapy, however to our knowledge, they have not been previously investigated as antimalarial compounds, or indeed as antiparasitic agents in general. We were thus interested in exploring the potential of the 2-imidazoline scaffold as an antiplasmodial as well as an antitrypanosomal entity. In our[15] continued efforts to discover new antiplasmodial and antitrypanosomal agents by way of established MCRs and known antiprotozoal pharmacophores, we elected to generate novel 4-aminoquinoline-containing 2-imidazolines *via* MCR chemistry. To this end we set out to explore the potential of hybridizing the 2-imidazoline sub-structure and the 4,7-diaminoquinoline sub-structure (a well known antimalarial pharmacophore) *via* the recently disclosed 3-component condensation of amine, aldehyde and isocyanoacetate.[16]

Chemistry

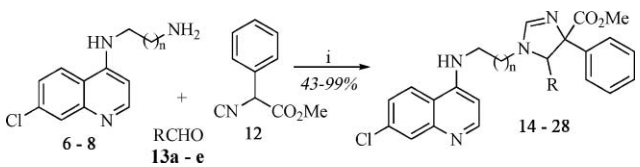
The design of the target 4-aminoquinoline-containing 2-imidazolines was such that the 4-aminoquinoline substructure was incorporated in the amine input of the MCR while using commercially available aldehydes. Thus, the synthesis of the requisite 4-aminoquinoline amines followed the approach reported previously (Scheme 1)[17] in which commercially available 4,7-dichloroquinoline **2** was treated with a 5-fold excess of terminal alkyldiamines **3–5** in the absence of solvent, initially at 80 °C for 1 h followed by temperature elevation to 135 °C for a further 3 h. In all three cases, the yields were excellent.



Scheme 1 *Reagents and conditions* i) **3–5**, 80 °C, 1 h then 135 °C, 3 h.

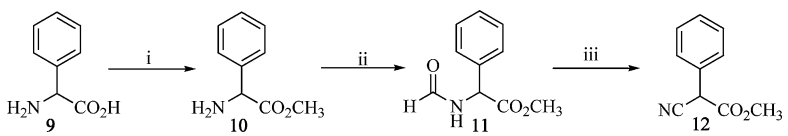
Next, commercially available racemic phenylglycine **9** was esterified *via* the acid chloride using $SOCl_2$/MeOH at ambient temperature (Scheme 2).[18]

The free base was then formylated by the method described by Orru *et al.*,[16] whereby a suspension of the amino ester **10** was refluxed in DCM in the presence of the preformed mixed anhydride of Ac_2O and HCOOH. The resultant formamide **11** was isolated by repeated dilution of the mixture with water and continued evaporation without the need to perform any extractive processes. Using this method of isolation, **11** was obtained in quantitative yield and with high purity. Finally, dehydration of the formamide **11** to the isocyanide **12** was achieved by treatment of the former with $POCl_3$ in anhydrous DCM in the presence of 5 equivalents of Et_3N. The low product yield (20%) of **12** was attributed to the decomposition of the product during column chromatography of the crude material on the (SiO_2) gel column. Unfortunately, neither use of Et_3N-deactivated SiO_2 gel nor basic alumina could improve the yield of **12**. Having synthesized **12**, we attempted the multicomponent reaction to generate a modest exploratory library of the 4-aminoquinoline-containing 2-imidazolines. Deliberately, we restricted ourselves to the use of amines **3–5** and randomly chose five aldehydes (**13a–e**, Scheme 3) and carried out the multicomponent reactions as described, Scheme 3. The multicomponent reaction to 2-imidazolines was previously optimized as reported by Orru *et al.*[16] and accordingly, MeOH was found to be one of the better solvents for the reaction. Thus, our synthesis of the 2-imidazolines was conducted in MeOH. Orru and co-workers conducted their synthesis at room temperature in the presence of a drying agent (Na_2SO_4) to remove the water produced during the condensation of amine and aldehyde. Perhaps the low temperature at which the reactions were performed was responsible for the prolonged reaction times that were seen with these reactions (up to 18 h). In our laboratories we found that performing the reactions at elevated temperatures greatly accelerated the forward reactions towards formation of the 2-imidazoline heterocycle.



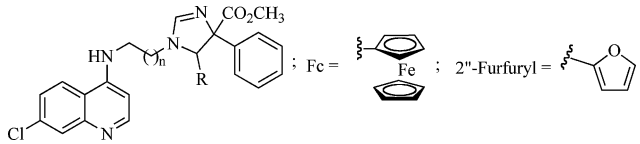
Scheme 3 *Reagents and conditions* i) MeOH, 45 °C, 2 h.

Typically, all reactions were allowed to proceed for 20 min prior to addition of isocyanoacetate **12** and were deemed complete within a space of 2 h (TLC) after addition of **12**. A temperature of 45 °C was established to be optimal, although higher temperatures were tolerated. The preclusion of a drying agent from the reaction mixtures was found to have no effect on the isolated yields of the products. The average diastereoselectivity reported by Orru *et al.* was 1 : 3 *syn vs. anti* diastereomer, whereas in our cases the diastereoselectivites were comparable and in some cases as high as 1 : 5. Thus on this basis our chosen high temperature of



Scheme 2 *Reagents and conditions* i) (a) $SOCl_2$, MeOH, rt, 2 h, (b) K_2CO_3, MeOH, 15 min, rt, 100%; ii) HCOOH, Ac_2O, DCM, reflux, 2 h, 100%; iii) $POCl_3$, DCM, −25 °C, 3 h, 20%.

Table 1 Isolated yields and structures of **14–28**



General structure of 4-aminoquinoline-containing 2-imidazolines **14–28**

Compound	n	R	% Yield	*dr*
14	1	H	84	—
15	1	CH_3	82	1 : 3
16	1	Fc	76	1 : 3
17	1	^{i}Bu	86	1 : 3
18	1	2″-Furfuryl	95	1 : 4
19	2	H	78	—
20	2	CH_3	55	1 : 4
21	2	Fc	77	1 : 4
22	2	^{i}Bu	56	1 : 5
23	2	2″-Furfuryl	96	1 : 5
24	3	H	67	—
25	3	CH_3	43	1 : 3
26	3	Fc	74	1 : 3
27	3	^{i}Bu	52	1 : 3
28	3	2″-Furfuryl	99	1 : 4

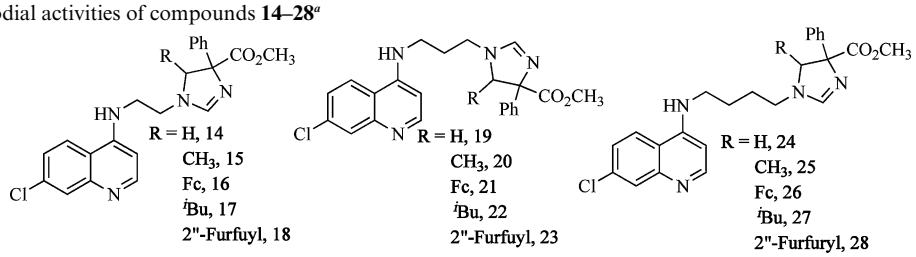
45 °C did not appear to compromise the diastereoselectivity of the reaction. Table 1 shows the isolated yields and diastereomeric ratios of compounds **14–28**.

Biological evaluation

***In vitro* antiplasmodial activity 14–28.** The *in vitro* antiplasmodial activity of compounds **14–28** were determined on two different strains of the malaria parasite, namely CQ-sensitive 3D7 and CQ-resistant K1, while the determination of compound cytotoxicity was done on a human KB cell line. The results of these screens are tabulated in Table 2.

From the results shown in Table 2, it is apparent that most compounds indeed show great promise as antiplasmodial agents as most have activities comparable to chloroquine or even better. In two separate independent experiments, the chloroquine ED_{50} was determined to be 0.02 μM and 1.03 μM, whereas in a third experiment (compounds marked '^{b}'), the ED_{50} of chloroquine was 0.16 μM. When the compounds were tested against 3D7, it was found that 7 out of 15 compounds possessed greater potencies in comparison to chloroquine (ED_{50} chloroquine = 0.02 μM). With an ED_{50} of 0.34 μM, **14** was the least active, exhibiting 17 times less efficacy than chloroquine and was the only compound whose activity did not approach that of the standard drug. As can also be seen from the table, the next least active compound, **19** (ED_{50} = 0.095 μM) was 5 times less active than chloroquine. On the other hand, the most potent compound in this assay, **26** (ED_{50} = 0.00048 μM), showed a 42-fold improvement in activity over chloroquine in the (CQS) 3D7 strain. In terms of SAR, compounds with the shortest ethylene spacer **14**, **15**, **16**, **17**, and **18** were generally less efficacious than those with the longer carbon spacers (in the chloroquine-sensitive strain); in fact the former were all less active than chloroquine (including **19** and **28**). Furthermore, compounds possessing a substituent at the 5′-position were generally more active than unsubstituted compounds except for **28**; notably, compounds with the ferrocenyl (**16**, **21** and **26**) and isobutyl groups (**17**, **22** and **27**) were the most active among their respective groups. Another general trend that was seen was that compounds with

Table 2 *In vitro* antiplasmodial activities of compounds **14–28**[a]



Compound	3D7 ED_{50} (μM)	K1 ED_{50} (μM)	Cytotoxicity (μM)	Therapeutic index a	Therapeutic index b
Chloroquine	*0.02*	*1.03*	nd	—	—
14	0.34	0.49[b]	587.8	1722	1200
15	0.047	0.54	99.0	2106	183
16	0.051	0.29	41.2	808	142
17	0.043	0.15	81.2	1889	541
18	0.084	0.38[b]	111.4	1326	293
19	0.095	0.97[b]	112.3	1182	116
20	0.023	0.69[b]	52.5	2283	76
21	0.0033	0.033	8.4	2550	255
22	0.0063	0.084	46.8	7435	557
23	0.0041	0.38[b]	17.6	4297	46
24	0.0023	0.46[b]	111.0	48246	241
25	0.022	nd	14.0	636	—
26	0.00048	0.016	2.6	5375	163
27	0.0020	0.020	6.1	3047	305
28	0.04	0.44	24.9	622	57

[a] All compounds were screened as diastereomers. [b] Chloroquine (diphosphate salt of) ED_{50} was determined as 0.16 μM; nd = not determined; therapeutic index a = cytotoxicity/3D7 ED_{50}; therapeutic index b = cytotoxicity/K1 ED_{50}.

the longer 4-carbon spacer **24**, **25**, **26** and **27** were the more active within their respective groups. As can be seen from the table, all compounds show favourable therapeutic indices, with the most active compound **26** being 5375 times more cytotoxic to the parasite (3D7) than the mammalian KB cells used in the cytotoxicity assays. Even more notable was compound **24** whose cytotoxicity towards the parasite over the mammalian cells was over 48 000.

Against the chloroquine-resistant K1 strain, the results show that most compounds had activities comparable to those of chloroquine except **14**, **16**, **18**, **19**, **20** and **28**. Ferrocenic compound **26** (ED_{50} = 0.016 μM) and isobutyl compound **27** (ED_{50} = 0.02 μM) were again the most potent in the resistant strain within the modest library.

A similar trend in activity in K1 as seen in 3D7 with regard to the size of the carbon spacer between the side chain nitrogens was only observed for compounds **16** *vs.* **21** *vs.* **26** and **17** *vs.* **22** *vs.* **27**. Among these compounds, **26** and **27** showed the best activities. These two compounds were >100 and >80 times more active in the chloroquine-resistant strain than the standard drug chloroquine respectively. Despite their lower antiplasmodial activities, compounds **15**, **16** and **17** all exhibited several hundred-fold greater efficacy than chloroquine (IC_{50} = 1.03 μM). The therapeutic indices reveal that the compounds were generally nontoxic to the mammalian KB cell line used in the assay. The low toxicities associated with the compounds are manifest in the high therapeutic indices obtained, from which it can be seen that the majority of compounds are over 100 times more cytotoxic to the parasite than the human cell line. Considering the more active ferrocenyl and isobutyl-substituted compounds, it is noted that the former (**16**, **21** and **26**) are generally more cytotoxic than the latter (**17**, **22** and **27**).

In vitro inhibition of β-haematin formation

The inhibition of β-haematin formation was determined using the recently disclosed method called the pyridine hemichrome inhibition of β-haematin (Phiβ) assay.[19] This high throughput method was chosen over other assays given its low cost, accuracy and short assay time. Using the method, compounds are screened for inhibitory activity below a cut-off point of 5 equivalents prior to determination of IC_{50} values. Compounds with values higher than this cut-off point are not examined further for IC_{50} determination.

Results for the screen are given in Table 3 and, for comparative purposes, the Phiβ IC_{50} of CQ (the standard) is also given in the table. It was determined that six of these compounds (**22–28**, except **26**) were stronger inhibitors of β-haematin formation *in vitro* than CQ (IC_{50} = 1.91 ± 0.3). Among the compounds, **27** was the strongest inhibitor with an IC_{50} of 1.26 equivalents. It was also established that compounds bearing the ferrocenyl subunit (**16**, **21** and **26**) did not inhibit the formation of β-haematin at or below the desirable cut-off point, suggesting that the compounds are inhibiting plasmodial growth by other means other than inhibition of haemozoin formation. Considering the more active inhibitors, the data suggest that generally there is a correlation between the antiplasmodial activity and inhibition of β-haematin formation. This is most clearly evident with compounds **22** and **27** against both strains. Whilst the same generalization does not hold for compounds **23** and **24** against the CQ-resistant K1 strain where the compounds are weaker inhibitors than CQ, it does hold true in the CQ-sensitive 3D7 strain. However, it must be noted that biological activity is likely to depend on both β-haematin inhibition and the degree of accumulation in the food vacuole as a result of pH trapping. The pH trapping effect is determined by the pK_a of the compounds, which are unknown. Thus a quantitative correlation is not expected. Overall the strong β-haematin inhibition activity of these compounds indicates that this is their mode of action.

Table 3 *In vitro* inhibition of β-haematin formation by **14–28**

Compound	Phiβ equiv IC_{50}
CQ	1.91 ± 0.3
14	5.60 ± 0.1
15	4.50 ± 0.2
16	2.68 ± 0.08
17	3.45 ± 0.3
18	> 5
19	4.98 ± 0.07
20	2.42 ± 0.2
21	> 5
22	1.53 ± 0.2
23	1.41 ± 0.08
24	3.09 ± 0.4
25	1.32 ± 0.2
26	> 5
27	1.26 ± 0.2
28	1.35 ± 0.1

In vitro antitrypanosomal activity

Determination of the ED_{50}s identified the ferrocenyl compound **21** as the most potent (ED_{50} = 0.070 μM, Table 4), which besides this encouraging activity remains far inferior to the efficacy of the control drug pentamidine. Encouragingly though, **21** exhibits good selectivity of parasite cytotoxicity over the mammalian KB cell line used in this assay as is revealed by its high therapeutic index of 120. Since *T. brucei* exists in the bloodstream as extracellular trypomastigotes, the problems that are usually associated with drug penetration of the macrophage are absent and the compound is more likely to reach its site of action. In this regard **21** is a potential valid lead compound.

Discussion

The improved activities of the novel 4-aminoquinoline-2-imidazolines may be speculated to be a result of several factors. It is known that the 4-amino-7-chloroquinoline subunit is an antimalarial pharmacophore that inhibits haem dimerization and crystallization (biomineralization) into nontoxic haemozoin.[20] Therefore, the primary antiplasmodial activities associated with these compounds may be arising from inhibition of haem biomineralization causing a build up of toxic haem, which may result in parasite death. It is also possible that the increase in basicity caused by the two nitrogens of the imidazoline ring may be improving the accumulation of the compounds in the acidic food vacuole of the parasite *via* pH trapping.[21] The presence of the heterocycle in the lateral chain may also be resulting in secondary interactions with other targets apart from haem, resulting in the observed antiplasmodial activities. Considering the ferrocenyl compounds **16**, **21** and **26**, whose antiplasmodial activities seem to

Table 4 *In vitro* activity against *T. brucei*

Compound	ED_{50} (μg ml^{-1})	ED_{50} (μM)	Cytotoxicity ED_{50} (μg ml^{-1})	Cytotoxicity ED_{50} (μM)	Therapeutic index
Pentamidine	> 0.0001	> 0.0003	—	—	—
Podophyllotoxin	0.002	—	0.00024	—	—
14	13.94	34.2	239.9	587.8	17
15	1.83	4.3	41.8	99.0	23
16	1.19	2.0	24.4	41.2	21
17	1.12	2.7	37.7	81.2	30
18	2.25	4.7	52.8	111.4	24
19	4.88	11.6	47.7	112.3	10
20	1.40	3.2	22.9	52.5	16
21	0.43	0.07	5.1	8.4	120
22	0.61	1.3	22.4	46.8	36
23	1.81	3.7	8.6	17.6	5
24	3.93	9.0	48.4	111.0	12
25	1.06	2.4	6.3	14.0	6
26	0.43	0.7	1.6	2.6	4
27	0.38	0.8	3.0	6.1	8
28	2.48	4.9	12.5	24.9	5

be superior to those of their counterparts, but did not necessarily show the strongest inhibition of β-haematin formation, this result may be explained on the basis of the influence of the ferrocenyl moiety on the pharmacodynamic behaviour of these compounds. Previous studies have shown that the ferrocenyl moiety alters the shape, volume, lipophilicity, basicity and electronic profile of the parent molecule, and by implication its pharmacodynamic behaviour.[22,23] Ferroquine, the ferrocenyl analogue of chloroquine, which has been partially studied suggests haemozoin formation to be the target.[24] The aforementioned studies also pointed to the importance of physico-chemical properties of ferroquine as being important in the significant antiplasmodial activity displayed by ferroquine. It may also be possible that the increased lipophilicities of ferrocenic 4-aminoquinoline 2-imidazolines compared to chloroquine and the other 4-aminoquinoline 2-imidazolines is favouring their transport across the parasitic cell membrane, causing much increased concentrations within the parasite. The lipophilicity may also be important in circumventing chloroquine resistance for a number of the described 2-imidazolines found to be more potent than chloroquine against resistant strains. It is known that the chloroquine resistance mechanism is much less effective for certain classes of aminoquinolines as already mentioned. Within the context of lipophilicity, the chloroquine resistance mechanism does not seem to recognize lipophilic compounds.[25,26] For example, quinolines such as mefloquine are more lipophilic than 4-aminoquinolines such as chloroquine. It may be suggested that mutations in the *pfcrt* gene, the gene implicated in chloroquine resistance, may not favour or recognize more lipophilic chloroquine-based 4-aminoquinolines such as the 4-aminoquiniline 2-imidazolines described herein.

Conclusion

The multicomponent reaction strategy has been exploited to synthesize novel 4-aminoquinoline-containing 2-imidazolines in which diversity is introduced in a single step. The compounds have shown great promise both as antiplasmodial and antitrypanosomal agents and form the basis of extensive SAR studies in search of new drugs based on the already known 4-aminoquinoline pharmacophore. Within the context of malaria, the mechanism-of-action studies suggest haemozoin formation to be the target. Finally, while we acknowledge the inherently high lipophilicities of the compounds described herein, it must be stressed that the outlined 3CC strategy can be employed to discover compounds with improved physicochemical properties. Work to generate other longer chain-linkers between the aminoquinoline ring and the imidazoline subunits, as well as new functional groups on the latter is currently under way in our laboratories and will be reported in due course elsewhere.

Acknowledgements

This work was supported by the South African National Research Foundation under grant number 2053362 (KC). The investigation also received support from UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) (VY).

References

1 WHO: *The World Health Report*, 2005, http://www.rbm.who.int/wmr2005/html/map1.htm.
2 M. O'Sullivan, *Curr. Med. Chem.: Anti-infective Agents*, 2005, **4**, 355–378.
3 A. Domling, *Curr. Opin. Chem. Biol.*, 2002, **6**, 303–313.
4 M. A. Gallop, R. W. Barret, W. J. Dower, S. P. Fodor and E. M. Gordon, *J. Med. Chem.*, 1994, **37**, 1233–1251.
5 A. Golisade, J. Wiesner, C. Herforth, H. Joma and A. Link, *Bioorg. Med. Chem.*, 2002, **10**, 769–777.
6 S. J. Teague, A. M. Davis, P. D. Leeson and T. Oprea, *Angew. Chem. Int. Ed.*, 1999, **38**, 3743–3748.
7 R. W. Armstrong, A. P. Combs, P. A. Tempest, S. D. Brown and T. A. Keating, *Acc. Chem. Res.*, 1996, **29**, 123–131.
8 R. V. A. Orru and M. de Greef, *Synthesis*, 2003, 1471–1499.
9 C. Hulme and V. Gore, *Curr. Med. Chem*, 2003, **10**, 51–80.
10 I. Ugi, *Angew. Chem. Int. Ed.*, 1962, **1**, 8–21.
11 A. Domling, *Chem. Rev.*, 2006, **106**, 17–89.
12 I. Ugi, *Angew. Chem. Int. Ed.*, 2000, **39**, 3168–3210.
13 H. Y. Li, Drummond, I. DeLucca and G. A. Boswell, *Tetrahedron*, 1996, **52**, 1153–1162.
14 F. Rondu, G. Le Bihan, X. Wang, A. Lamouri, E. Toubaoul, G. Dive, T. Bellahsene, B. Pfeiffer, P. Renard, B. Guardiola-Lemaitre, D. Sanchez, L. Penicaudi, A. Ktora and J. J. Godford, *J. Med. Chem.*, 1997, **40**, 3793–3863.
15 (*a*) C. C. Musonda, D. Taylor, J. Lehman, J. Gut, P. J. Rosenthal and K. Chibale, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 3901–3905; (*b*) C. C.

Musonda, J. Gut, P. J. Rosenthal, V. Yardley, R. C. Carvalho, de Souza and K. Chibale, *Bioorg. Med. Chem.*, 2006, **14**, 5605–5615; (*c*) C. C. Musonda, S. Little, V. Yardley and K. Chibale, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 4733–4736.
16 R. S. Bon, C. Hong, M. J. Bouma, R. F. Schmitz, F. J. J. de Kanter, M. Lutz, A. Spek and R. V. A. Orru, *Org. Lett.*, 2003, **5**, 3759–3762.
17 D. De, L. D. Byers and D. J. Krogstad, *J. Heterocyclic Chem.*, 1997, **34**, 315–320.
18 T. Kihlberg, F. Karimi and B. Lngstroem, *J. Org. Chem.*, 2002, **67**, 3687–3692.
19 K. K. Ncokazi and T. J. Egan, *Anal. Biochem.*, 2005, **338**, 306–319.
20 T. J. Egan, *Drug Des. Rev.*, 2004, **1**, 93–110.
21 A. F. G. Slater, *Pharmacol. Ther.*, 1999, **57**, 203–235.
22 D. Dive and C. Biot, *ChemMedChem*, 2008, **3**, 383–391.
23 C. Biot, *Curr. Med. Chem. Anti-Infect. Agents.*, 2004, **3**, 135–147.
24 C. Biot, D. Taramelli, I. Forfar-Bares, L. A. Maciejewski, M. Boyce, G. Nowogrocki, J. S. Brocard, N. Basilico, P. Olliaro and T. J. Egan, *Mol. Pharm.*, 2005, **2**, 185–193.
25 K. Chibale, *IUBMB Life*, 2002, **53**, 249–252.
26 D. C. Warhurst, *Current Science*, 2007, **92**, 1556–1560.