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Application of multicomponent reactions to antimalarial drug discovery. Part 2: New antiplasmodial and antitrypanosomal 4-aminoquinoline γ- and δ-lactams via a 'catch and release' protocol

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Abstract—A parallel synthesis of a new series of 4-aminoquinoline γ- and δ-lactams synthesized via the Ugi 3-component 4-centre multicomponent reaction is described. The basicity of the quinoline nitrogen was exploited in the purification of compounds via a 'catch and release' protocol. Yields ranging from 60% to 77% and purities as high as 96% were obtained. Compound **29**, the most active against a chloroquine-resistant W2 strain of *Plasmodium falciparum* with an IC_{50} of 0.096 μM, also inhibited recombinant falcipain-2 in vitro (IC_{50} = 17.6 μM). Compound **17** inhibited the growth of *Trypanosoma brucei* with an ED_{50} of 1.44 μM whilst exhibiting a favourable therapeutic index of 409 against a human KB cell line.

1. Introduction

Modern drug discovery is faced with the challenge of designing sequential chemical reactions that are highly efficient in providing a maximum of the elements of structural complexity and diversity with minimal synthetic steps for the assemblage of compounds with interesting properties.[1] Combinatorial chemistry has in the recent past emerged as a powerful tool for the rapid identification, generation and optimization of lead compounds in the drug discovery process.[2,3] Thus, the major driving force behind the increased interest in this field has been the need to discover and develop new chemical entities with desirable properties in a more efficient and cost-effective manner, and most importantly within a short period of time. Presently most drugs on the market are small organic compounds that contain heterocyclic rings.[4,5] However, easy access and availability of suitably functionalized heterocyclic building blocks for the synthesis of diverse libraries is rather limited. As a consequence the development of new, efficient and clean synthetic reactions remains a vital challenge to organic and medicinal chemists.[6]

Multicomponent reactions (MCRs),[7,8] reactions involving at least three starting materials in a one-pot reaction, 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.[9] Compared to conventional multistep organic syntheses MCRs are advantageous owing to their greater atom efficiency, accessibility to large numbers of compounds and complex molecules, wide structural diversity and simplicity of their one-pot procedures making them amenable to combinatorial synthesis. Among the known multicomponent reactions to date, the most valuable reactions are those based on isocyanides. One such reaction is the Ugi 4-component condensation (4CC) reaction[8] combining an amine, aldehyde (or ketone),

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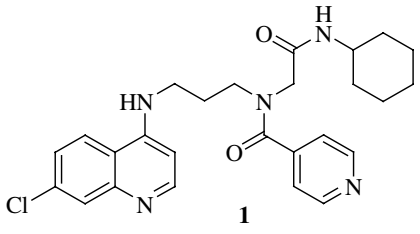
carboxylic acid and isocyanide in a single-stage reaction to afford α-acylamino amides.

As part of our continued drug discovery programme in search of new agents for the treatment of malaria and sleeping sickness, we became interested in the synthesis of heterocyclic compounds via multicomponent reactions built around the 7-chloro-4-aminoquinoline unit, a known antimalarial pharmacophore. Recently, we reported on the synthesis and biological evaluation of new aminoquinoline α-acylamino amides, exemplified by **1** (Fig. 1) by way of the Ugi 4CC reaction.[10] However, despite its synthetic potential, the classical Ugi reaction is limited by giving rise to products that are flexible and peptidic in nature, often being described as being 'non-drug-like' and therefore making them less attractive targets in the search for new drugs. Importantly though, several novel intramolecular variations and secondary transformations of the Ugi 4CC that produce more constrained, biologically relevant heterocyclic compounds including tetrazoles,[11] diketopiperazines,[12] benzodiazepines[13] and morpholines[14] have been developed. In one variant of the Ugi 4CC, termed the Ugi 3-component 4-centre (3C/4C) reaction, two of the functional groups that take part in the reaction are tethered onto one starting material, leading to heterocyclic compounds. This strategy has been used to synthesize β-lactams[15] and δ- and γ-lactams.[16] Combinatorial syntheses in which multicomponent reactions have been employed have largely been dominated by solid-phase methods.[17] To the best of our knowledge the use of polymer-supported reagents as purification and/or isolation tools specifically in the Ugi reaction has not been previously demonstrated.

We targeted synthesis of cyclic compounds via MCRs as these are known to impart molecular rigidity and improve the bioavailability of drugs and drug candidates. Indeed, studies have revealed that the number of rotatable bonds in a drug candidate is a major contributor to good oral bioavailability.[18] As a sequel to our previous efforts we targeted cyclic variants of the Ugi reaction known as γ- and δ-lactams (Scheme 2) with a view to lowering the levels of flexibility often associated with acyclic amides of type **1**.



1

Figure 1. Structure of a first-generation aminoquinoline α-acylamino amide.[10]

2. Chemistry

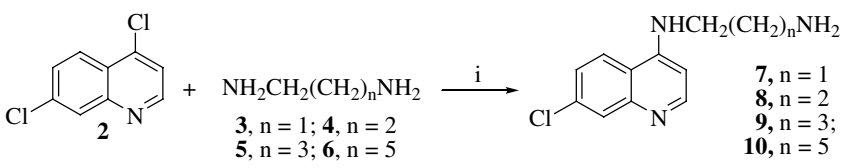
The requisite 1,*x*-diamines **7**–**10** (where *x* represents the position of the N atom in the alkyl chains) needed as amine inputs for the multicomponent synthesis of our exploratory library of γ- and δ-lactams were prepared by way of Scheme 1.[19] Thus, neat mixtures of commercially available 4,7-dichloroquinoline **2** and the alkyl diamines **3**–**6** were condensed at 80 °C for 1 h and then at 135 °C for 3 h. All the diamines were recrystallised from ethylacetate to afford **7**–**10** in excellent yields (Table 1).

We designed δ- and γ-lactams as synthetic targets and planned the synthetic route as shown in Scheme 2. The first step involved the Ugi 3C/4C reaction of diamines **7**–**10**, levulinic (**11a**, $m = 1$) or 4-acetylbutyric acid (**11b**, $m = 2$) and *tert*-butylisocyanide **12a** or cyclohexylisocyanide **12b** in MeOH to give the compounds **14**–**29** in solution in parallel array format.

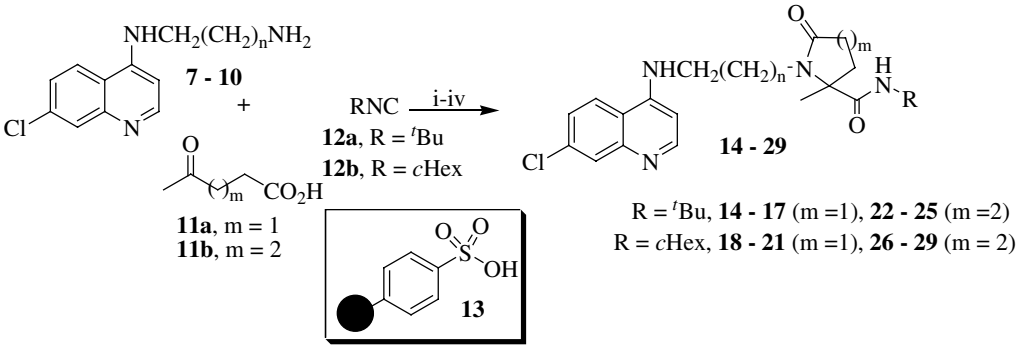
We were then faced with the challenge of having to purify our library by means of conventional chromatography. Concerned about the laborious efforts involved, we sought a protocol wherein purification and isolation would be achieved by simple filtration and washing. Such an approach should greatly accelerate the generation of larger compound libraries with minimal inputs in terms of time and effort, and yield compounds of high purity that are ready for high-throughput screening. Two strategies were thus available to us, viz., solid-phase organic synthesis and use of polymer-assisted solution-phase synthesis. Although the use of solid-phase synthesis of γ- and δ-lactams has been achieved on solid support[16] and is an attractive approach towards yielding high purity compounds, immobilization of the reagent onto and cleavage from solid support introduces two extra steps in the reaction sequence. As an alternative, the use of polymer-bound reagents renders itself as

Table 1. Recrystallised yields of 1-(7′-chloro-4′-amino)-*x*-diamines

Diamine	*n*	Yield (%)
7	1	91
8	2	86
9	3	83
10	5	90



Scheme 1. Reagents and conditions: (i) **3**–**6**, 1-*x*-diamine, 80 °C, 1 h, then 135 °C, 3 h.



Scheme 2. Reagents and conditions: (i) MeOH, rt, 12–18 h; (ii) **13**, 1 h; (iii) filter, wash; (iv) 3% NH_3/MeOH, filter, wash.

an excellent approach towards synthesis of combinatorial libraries. Several concepts are used in polymer-assisted solution-phase synthesis,[20] among which the 'catch and release' principle clearly stands out. In this concept, a target compound is selectively bound from solution onto the resin by means of a covalent or ionic bond. Excess of starting materials are then removed at the end of the reaction by means of filtration and simple washings and finally, the products are released from the resin support. More recently, a one-pot combinatorial synthesis of functionalized thiazine libraries using an in situ polymer-assisted synthesis/catch and release protocol has been disclosed.[21]

We chose to employ the 'catch and release' concept of polymer-supported reagents as a means of purifying our compound library. We reasoned that the basicity (albeit weak) of the quinoline nitrogen could be exploited for the capture of the products followed by purification and subsequent release of pure products. The quinoline nitrogen is rendered basic due to the resonance or mesomeric release mediated by the 4-amino group, Figure 2.

The reactions were performed in parallel using excess of ketoacids (**11a** and **11b**) and isocyanides (**12a** and **12b**), while the diamines **7**–**10** were used as the limiting reagents (Scheme 2). The ratio of reactants was carefully determined in such a way as to minimize the quantity of amines used in the reactions. Since the presence of the tertiary nitrogen atom in the aminoquinoline ring renders the lactam products basic and, since the 'catch and release' concept relies on the formation of an ionic pair between a basic and an acidic compound, use of excess of the diamines would ultimately be a source of impurities in the final products. The predictable inability of **13** to discriminate between starting amines **7**–**10** and products **14**–**29** in solution thus dictated the use of the former as the limiting reagents.

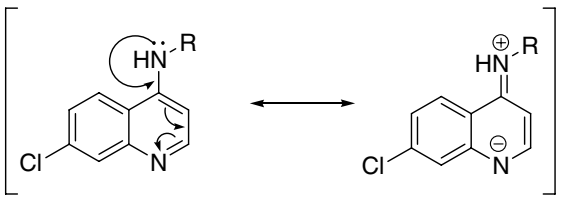


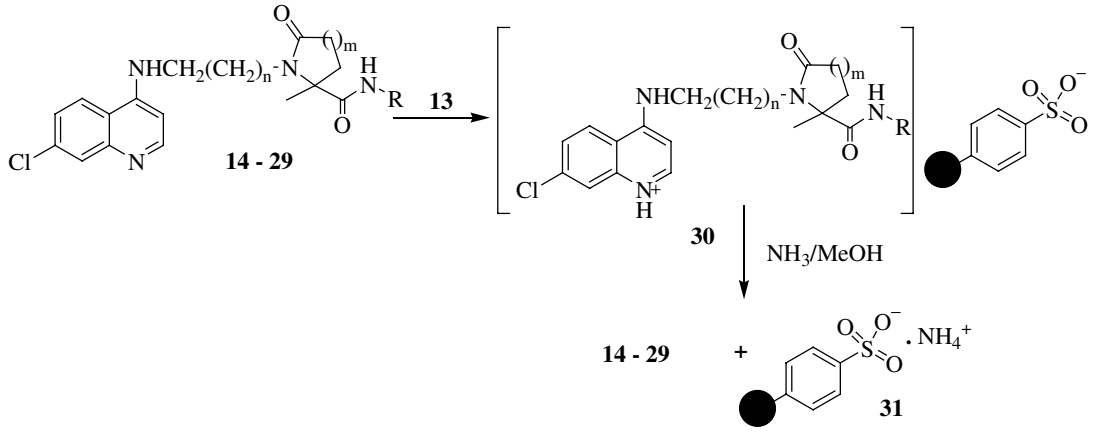
Figure 2. Increased basicity of quinoline nitrogen due to resonance effects.

We found the acidic resin-bound macroporous *para*-toluene sulfonic acid **13** (MP-TsOH)[22] to be an effective tool for achieving the dual purpose of purifying and isolating our target compounds. Since the starting amines from which the lactams derive their basicity are completely consumed in the reactions, we reasoned that the addition of **13** at the end of reaction should achieve selective capture of the target compounds as the strong ion pairs **30** from solution, leaving behind excess of ketoacid and isocyanide in solution, which can then be removed by simple filtration and resin washing with the reaction solvent (Scheme 3).

Therefore, after the reactions were deemed complete as was indicative of TLC, MP-TsOH was added to the reaction mixtures to scavenge the products from solution. After 1 h following addition of MP-TsOH **13**, all reactions revealed complete scavenging of the products from solution (TLC) and were thus filtered and washed repeatedly to remove excess of ketoacids (**11a** and **11b**) and isocyanides (**12a** and **12b**). Release of the products was achieved by agitating the resins in anhydrous methanolic ammonia for 30 min. On addition of methanolic ammonia a base interchange should occur between the ionic pairs **30** and NH_3 to give the free lactams **14**–**29** and the resin-bound ammonium salt **31**. Yields were generally good to excellent and were comparable with those from traditional solution-phase synthesis. More interestingly the purities of compounds **14**–**29** were high enough to permit their use in biological assays (Table 2).

The approach discussed here offers the obvious advantage of providing access to high purity 4-aminoquinoline-based γ- and δ-lactams via multicomponent Ugi 3C/4C reaction in a parallel array format, whilst avoiding the tedious work involved in chromatography; it is also economical in the sense that only a single and readily available commercial reagent is required for the purification of large numbers of compounds.

In summary, we have extended the concept of 'catch and release' as a purification and isolation protocol to the Ugi 3C/4C multicomponent synthesis of conformationally constrained -aminoquinoline-based γ- and δ-lactams using a readily available polymer-supported reagent.



Scheme 3. Formation of ionic pair **30** between lactams **14**–**29** and MP-TsOH **13**.

Table 2. Isolated yields and purities of lactams **14**–**29**

Entry	Compound	*m*	*n*	R	Yield[a] (%)	Yield[b] (%)	Purity[c] (%)
1	**14**	1	1	^{t}Bu	57	60	91
2	**15**	1	2	^{t}Bu	84	79	92
3	**16**	1	3	^{t}Bu	77	73	90
4	**17**	1	5	^{t}Bu	75	69	94
5	**18**	1	1	^{c}Hex[d]	84	75	88
6	**19**	1	2	^{c}Hex[d]	67	70	91
7	**20**	1	3	^{c}Hex[d]	70	68	95
8	**21**	1	5	^{c}Hex[d]	84	77	94
9	**22**	2	1	^{t}Bu	65	75	91
10	**23**	2	2	^{t}Bu	76	71	96
11	**24**	2	3	^{t}Bu	75	69	87
12	**25**	2	5	^{t}Bu	70	74	93
13	**26**	2	1	^{c}Hex[d]	76	66	95
14	**27**	2	2	^{c}Hex[d]	80	72	94
15	**28**	2	3	^{c}Hex[d]	78	74	91
16	**29**	2	5	^{c}Hex[d]	79	72	93

[a] Yields from solution-phase synthesis.
[b] Yields from polymer-assisted solution-phase synthesis.
[c] RP-HPLC purity was determined as area under a curve (λ = 220 and 254 nm).
[d] ^{c}Hex, cyclohexyl.

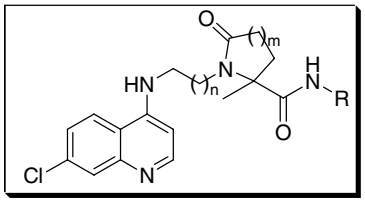
The methodology is general and works because of the presence of the basic quinoline nitrogen site in the molecule. The method has allowed us to quickly isolate and purify biologically interesting compounds in our continued search for new antiprotozoal agents. The disclosure of our simple and economic method for rapidly accessing libraries of 4-aminoquinolines is timely given the recent interest in libraries of 4-aminoquinolines, which have so far been purified by high-throughput HPLC techniques.[23] For biological assay purposes, compounds **14**–**29** were resynthesized and purified by means of preparative layer chromatography.

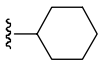
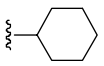
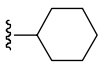
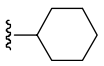
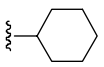
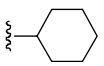
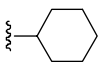
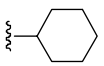
3. Biological results and discussion

Initially all lactams **14**–**29** were tested for their activities against parasite cultures of the chloroquine-resistant W2 strain of *P. falciparum* (IC_{50} chloroquine = 0.24 μM). It was found that the activities of the compounds were dependent upon the size of the lactam ring for compounds containing the same *N*-alkyl substituent. Thus, compounds containing the six-membered lactam ring were in general more active than those with the five-membered ring. From the modest library, compound **29** with an IC_{50} of 0.096 μM exhibited the best activity, being 2.5 times as potent in this strain as chloroquine (Table 3).

Compounds **17** (IC_{50} = 0.18 μM), **21** (IC_{50} = 0.27 μM) and **22** (IC_{50} = 0.21 μM) exhibited comparable activities to chloroquine, while **18** (IC_{50} = 0.35 μM) and **24** (IC_{50} = 0.37 μM) exhibited slightly reduced activities. The remaining compounds on the other hand were less efficacious than the control drug. In terms of methylene spacer length, compounds with the 6-carbon spacer were generally more efficacious, while in the case of the five-membered γ-lactams, those containing the cyclohexyl ring turned out to be more potent than those with the *tert*-butyl group. On the contrary, the *tert*-butyl-containing δ-lactams were more efficacious than the cyclohexyl counterparts.

Table 3. In vitro antiplasmodial activities of γ- and δ-lactams **14–29**



Compound	*m*	*n*	R	IC_{50} (μM)	
				W2 *P. falciparum*	Rec. F-2[a]
Chloroquine	—	—	—	0.24	nd
14	1	1		0.67	>10
15	1	2		0.98	>10
16	1	3		1.11	>10
17	1	5		0.18	>10
18	1	1		0.35	>10
19	1	2		0.63	>10
20	1	3		0.52	>10
21	1	5		0.27	>10
22	2	1		0.21	>10
23	2	2		0.31	>10
24	2	3		0.37	>10
25	2	5		0.46	>10
26	2	1		0.87	>10
27	2	2		0.83	>10
28	2	3		0.87	>10
29	2	5		0.096	17.62

nd, not determined.
[a] Rec. F-2, recombinant falcipain-2.

The generally modest activities of these compounds may be due to their overall reduced basicities. Whereas chloroquine accumulation in the acidic food vacuole of the parasite is dependent upon presence of two protonatable nitrogen atoms, the absence of a second protonatable nitrogen is likely to have implications for accumulation and hence antiplasmodial activity. Consequently, these lactams may be accumulating to much lower concentrations than those required to significantly inhibit plasmodial growth. Moreover, studies have revealed that chloroquine adopts a bioactive conformation in which the inter-nitrogen separation (N-(quinoline)–N-(diethyl)) of 0.83 Å[24] is equivalent to that of the central Fe atom and the two oxygens in the carboxylate groups of haem,[24] strongly suggesting that ferriprotoporphyrin (haem) is the 'receptor' target. Such similarity is unlikely to be present in these series of compounds in the absence of a second basic nitrogen in the side chain. Therefore, it may be possible that the modest antiplasmodial activities displayed by these compounds may result from their inability to interact with haem or from not being delivered to the requisite site of action within the acidic food vacuole. However, the reduced basicities of the compounds relative to chloroquine do not fully explain the observed antiplasmodial activities. Some compounds (such as **17**, **21** and **22**) have comparable activities to chloroquine and yet they lack the terminal basic nitrogen, which is more critical for the accumulation in the acidic food vacuole. The varying degrees of activity of the compounds in W2, a chloroquine-resistant strain, support the assertion that *P. falciparum* resistance is compound specific. Indeed, modifications to the side chain of chloroquine have led to derivatives which are able to circumvent chloroquine resistance.[25] The most notable result from this limited series of compounds is that for **29** (W2 IC_{50} = 0.096 μM, falcipain-2 IC_{50} = 17.7 μM). To our knowledge, this is the first report of an aminoquinoline lactam (albeit weak) inhibitor of falcipain-2. The antiplasmodial activity of **29** may in part be due to the inhibition of falcipain-2, which may be attacking a carbonyl group of one of the amide bonds of this compound (**29**), forming a tetrahedral intermediate.[26]

For comparative purposes, pentamidine, a drug used for the treatment of African sleeping sickness, was employed as the control drug in the determination of the ED_{50}s of compounds **14–29**. On the other hand, podophyllotoxin was used as the standard in the cytotoxicity assays against a human KB cell.

The data in Table 4 reveal that pentamidine exhibited superior antitrypanosomal activity (ED_{50} = 0.0002 μM), with the majority of compounds only being active in the low micromolar range. The most active compound in the series, **17**, was several hundred-fold less efficacious than the standard drug pentamidine. Despite these low antitrypanosomal activities, however, compounds **15** and **18–23** were active at or below 20 μM and were all several orders of magnitude less toxic to the mammalian cells employed in the assay as is revealed by the cytotoxicity ED_{50}s of podophyllotoxin versus **14–29**. Since *Trypanosoma brucei* exists in the bloodstream as

Table 4. In vitro antitrypanosomal activities of compounds **14–29**

Compound	ED_{50} μg/ml	ED_{50} (μM)	Cytotoxicity μg/ml	Cytotoxicity ED_{50} (μM)	Therapeutic index
Pentamidine	0.00007	0.0002	—	—	—
Podophyllotoxin	—	—	0.00024	0.41	—
14	nd[a]	nd	nd	nd	nd
15	3.32	8	48.6	117	15
16	18.5	43	>300	697	16
17	0.66	1.44	269.8	589	409
18	5.28	12.3	29.6	69	6
19	9.04	20.4	194.5	440	22
20	7.57	16.6	>300	658	54
21	5.56	11.5	44.7	92	8
22	7.74	18.6	44.7	107	6
23	6.1	14.2	63.1	147	10
24	>30	>67.5	41.4	93	—
25	>30	>63.5	65.2	138	—
26	13.25	30	186.5	420	14
27	12.32	27	241.8	528	20
28	>30	>64	166.8	353	—
29	nd	nd	nd	nd	nd

[a] nd, not determined.

extracellular trypomastigotes, the problems that are usually associated with drug penetration of the macrophage are absent and the compounds are likely to reach their intended sites of action.

In summary, we have disclosed a simple, convenient and general protocol for the parallel synthesis of 4-aminoquinolines. Although only one compound (**29**) was identified as being more potent than chloroquine in the *P. falciparum* chloroquine-resistant strain (W2), overall, the lactams described herein represent a new class of 4-aminoquinolines possessing dual antiplasmodial and antitrypanosomal activities.

4. Experimental

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at ambient temperature using a Varian Mercury (300 MHz) or a Varian Unity Spectrometer (400 MHz) and TMS was used as an internal standard. Chemical shift values (δ) are given in ppm relative to TMS ($\delta = 0.00$). Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded at 75 MHz or 100 MHz with the same instruments and internal standard. Deuterated methanol (CD_3OD) and chloroform ($CDCl_3$) were used in the determination of spectra for the amines and lactams, respectively. Mass spectra were recorded by means of a VG micromass 16 F spectrometer at 70 eV with accelerating voltage 4 kV. Accurate masses were determined using a VG-70E spectrometer and VG (Micromass) 70-SE magnetic sector mass spectrometer. Infrared spectra were measured in solution form using chloroform on a satellite FT-IR spectrophotometer. Melting points were determined on a Reichert-Jung Thermovar (temperature range 0–350 °C) on coverslips and are uncorrected. Preparative layer chromatography (PLC) was performed on silica 60 F_{254} coated glass plates. Reactions were monitored by thin-layer chromatography (TLC) using silica gel coated plates and visualized under an ultra-violet lamp. HPLC was performed at ambient temperature on a Thermo Separations Products® instrument, fitted with an Xtera® C_{18} 5 μm, 4.6 × 150 mm column and operating on a UV detector. HPLC-grade acetonitrile and ultrafiltered water were used as the eluting solvents at a concentration of 30% CH_3CN/70% H_2O in the presence of a 25 mM phosphate buffer.

4.1. General procedure for the synthesis of diamines 7–10

A mixture of 4,7-dichloroquinoline (0.98 g, 4.94 mmol) and the 1,*x*-diamine (24.7 mmol, 5 equiv) was heated at 80 °C for 1 h without stirring and then at 135 °C for 3 h with stirring. The reaction mixture was cooled to room temperature, basified with 10% NaOH (30 ml), extracted with hot EtOAc (3× 50 ml) and dried (Na_4SO_4). The solvent was removed in vacuo and the residue recrystallised from EtOAc to afford the diamine in high yield.

4.2. *N*-(2-Aminoethyl)-7-chloroquinolin-4-amine (3)

Yellow crystals (1.0 g, 91%); mp 115–117 °C; R_f (NH_3/MeOH 1:49) 0.20; δ_H (300 MHz, CD_3OD) 8.35 (d, 1H, $J = 5.4$, H-2), 8.1 (d, 1H, $J = 9.0$, H-5), 7.77 (d, 1H, $J = 2.1$, H-8), 7.38 (dd, 1H, $J = 2.1$, 9.0, H-6), 6.6 (d, 1H, $J = 5.4$, H-3), 3.43 (t, 2H, $J = 6.0$, ArNCH_2), 2.97 (t, 2H, $J = 6.0$, NCH_2); δ_C (75 MHz, $CDCl_3$); δ_C (75 MHz, CD_3OD) 152.9, 150.5, 134, 128.9, 126.1, 125.2, 122, 117, 98, 53.6, 46.1.

4.3. *N*-(3-Aminopropyl)-7-chloroquinolin-4-amine (4)

Yellow crystals (1.0 g, 86%), mp 124–127 °C; R_f (NH_3/MeOH 1:49) 0.21; δ_H (300 MHz, CD_3OD) 8.33 (d, 1H, $J = 5.4$, H-2), 8.12 (d, 1H, $J = 9.0$, H-5), 7.75 (d, 1H, $J = 2.1$, H-8), 7.42 (dd, 1H, $J = 2.1$, 9.0, H-6), 6.62 (d, 1H, $J = 5.4$, H-3), 3.44 (t, 2H, $J = 6.0$, ArNCH_2), 2.98 (t, 2H, $J = 6.0$, NCH_2), 1.89 (m, 2H, CH_2); δ_C (75 MHz, $CDCl_3$); δ_C (75 MHz, CD_3OD) 152.9, 150.5,

134, 128.9, 126.4, 125.1, 122.2, 117, 98.8, 43.6, 41.4, 30.1.

4.4. *N*-(4-Aminobutyl)-7-chloroquinolin-4-amine (5)

Yellow crystals (1.02 g, 83%), mp 45–47 °C; R_f (NH_3/MeOH 1:49) 0.22; δ_H (300 MHz, CD_3OD) 8.48 (d, 1H, J = 5.4, H-2), 7.94 (d, 1H, J = 9.0, H-5), 7.69 (d, 1H, J = 2.1, H-8), 7.27 (dd, 1H, J = 2.1, 9.0, H-6), 6.33 (d, 1H, J = 5.4, H-3), 3.39 (t, 2H, J = 6.0, ArNCH_2), 3.04 (t, 2H, J = 6.0, NCH_2), 1.89 (m, 2H, CH_2), 1.62 (m, 2H, NCH$_2$CH_2); δ_C (75 MHz, CD_3OD) 152.9, 150.5, 134, 128.9, 126, 124.9, 122, 117, 98.8, 43.6, 41.4, 30.1, 28.9.

4.5. *N*-(6-Aminohexyl)-7-chloroquinolin-4-amine (6)

Yellow needles (1.23 g, 90%), mp 136–138 °C; R_f (NH_3/MeOH 1:49) 0.22; δ_H (300 MHz, CD_3OD) 8.39 (d, 1H, J = 5.4, H-2), 8.18 (d, 1H, J = 9.0, H-5), 7.79 (d, 1H, J = 2.1, H-8), 7.44 (dd, 1H, J = 2.1, 9.0, H-6), 6.68 (d, 1H, J = 5.4, H-3), 3.36 (t, 2H, J = 6.0, ArNCH_2), 2.67 (t, 2H, J = 6.0, NCH_2) 1.77 (m, 2H, CH_2), 1.51 (m, 6H, 3× CH_2); δ_C (75 MHz, $CDCl_3$); δ_C (75 MHz, CD_3OD) 152.9, 150.5, 134, 128.9, 126.3, 124.9, 122, 117, 98.6, 42.6, 41.1, 31.8, 28.2, 26.7, 26.5.

4.6. General procedure for the synthesis of lactams 14–29

Amines **7**–**10** (0.23 mmol, 1.0 equiv) were dispensed in 16 separate vials and dissolved in MeOH (5 ml). A solution of levulinic acid or 4-acetylbutyric acid (0.27 mmol, 1.2 equiv) was added via micro syringe and the reaction mixtures stirred at room temperature for 30 min. At this point isocyanide was added (0.27 mmol, 1.2 equiv) and stirring continued for an additional 18 h. After the reactions were deemed complete (TLC), MP-TSOH with a loading capacity of 1.46 mmol/g (0.46 g, 0.67 mmol) was added to each of the vials which were transferred to an orbital shaker. After 1 h, the resins were filtered and washed with MeOH and suspended in a solution of 3% NH_3 in MeOH and filtered after 30 min of shaking at room temperature. The solvent was removed in vacuo and concentrated to afford the lactams.

4.7. *N*-*tert*-Butyl-1-(2-(7-chloroquinolin-4-ylamino)ethyl)-2-methyl-5-oxopyrrolidine-2-carboxamide (14)

White amorphous powder (54 mg, 60%), mp 112–114 °C; R_f (MeOH/DCM 1:9) 0.20; IR ν_{max} ($CHCl_3$)/cm^{-1} 3304 (NH), 3031 (ArCH), 2934 (CH), 1712 (C=O), 1627 (C=C), 1580 (C=N); δ_H (400 MHz, $CDCl_3$) 8.51 (d, 1H, J = 5.6, H-2), 7.94 (d, 1H, J = 2.0, H-8), 7.87 (d, 1H, J = 9.0, H-5), 7.40 (dd, 1H, J = 2.0, 9.0, H-6), 6.29 (d, 1H, J = 5.6, H-3), 3.68 (t, 2H, J = 6.0, ArNCH_2CH$_2$N), 3.43 (t, 2H, J = 6.0, ArNCH$_2$CH_2N), 2.55–2.49 (m, 2H, CH_2-4′), 2.32 (m, 1H, H-3′α), 1.99 (m, 1H, H-3′β), 1.56 (s, 3H, CCH_3), 1.24 (s, 9H, C(CH_3)$_3$) δ_C (100 MHz, $CDCl_3$) 178.4, 173.0, 151, 150.6, 134.0, 129.0, 126.0, 125.2, 122.0, 117.0, 98.2, 69.3, 52.4, 44.2, 40.3, 33.2, 29.3, 28.5 (3C), 22.6; HRMS (FAB) m/z 402.17884, (M^+ $C_{21}H_{27}ClN_4O_2$ requires 402.17874); HPLC purity: 91%, t'_R = 1.49 min.

4.8. *N*-*tert*-Butyl-1-(3-(7-chloroquinolin-4-ylamino)propyl)-2-methyl-5-oxopyrrolidine-2-carboxamide (15)

Yellow oil (74 mg, 79%); R_f (MeOH/DCM 1:9) 0.21; IR ν_{max} ($CHCl_3$)/cm^{-1} 33344 (NH), 3019 (ArCH), 2984 (CH), 1719 (C=O), 1648 (C=C), 1532 (C=N); δ_H (400 MHz, $CDCl_3$) 8.50 (d, 1H, J = 5.6, H-2), 7.92 (d, 1H, J = 2.0, H-8), 7.85 (d, 1H, J = 9.0, H-5), 7.44 (dd, 1H, J = 2.0, 9.0, H-6), 6.30 (d, 1H, J = 5.6, H-3), 3.66 (t, 2H, J = 6.0, ArNCH_2 (CH$_2$)$_2$N), 3.41 (t, 2H, J = 6.0, ArN(CH$_2$)$_2$CH_2N), 2.54–2.50 (m, 2H, CH_2-4′), 2.33–2.21 (m, 1H, H-3′α), 1.97–1.88 (m, 1H, H-3′β), 1.84–1.80 (m, 2H, ArCH$_2$CH_2CH$_2$N), 1.48 (s, 3H, CCH_3), 1.33 (s, 9H, (CH_3)$_3$); δ_C (100 MHz, $CDCl_3$) 178.6, 173.3, 151.0, 150.7, 134.0, 129.0, 126.2, 125.4, 122.2, 117.3, 98.6, 69.7, 52.3, 44.2, 40.4, 33.2, 30.2, 29.3, 28.4 (3C), 22.3; HRMS (FAB) m/z 416.19790, (M^+ $C_{22}H_{29}ClN_4O_2$ requires 416.19837); HPLC purity: 92%, t'_R = 2.04 min.

4.9. *N*-*tert*-Butyl-1-(4-(7-chloroquinolin-4-ylamino)butyl)-2-methyl-5-oxopyrrolidine-2-carboxamide (16)

White amorphous powder (71 mg, 73%), mp 203–205 °C; R_f (MeOH/DCM 1:9) 0.21; IR ν_{max} ($CHCl_3$)/cm^{-1} 3323 (NH), 3011 (ArCH), 2965 (CH), 1726 (C=O), 1651 (C=C), 1545 (C=N); δ_H (400 MHz, $CDCl_3$) 8.46 (d, 1H, J = 5.6, H-2), 7.91 (d, 1H, J = 2.0, H-8), 7.83 (d, 1H, J = 9.0, H-5), 7.43 (dd, 1H, J = 2.0, 9.0, H-6), 6.24 (d, 1H, J = 5.6, H-3), 3.38 (t, 2H, J = 6.0, ArNCH_2 (CH$_2$)$_2$CH$_2$N), 3.00 (t, 2H, J = 6.0, ArNCH$_2$ (CH$_2$)$_2$CH_2N), 2.51–2.38 (m, 2H, CH_2-4′), 2.27–2.18 (m, 1H, H-3′α), 1.91–1.87 (m, 1H, H-3′β), 1.83–1.70 (m, 4H, ArNCH$_2$CH_2CH_2CH$_2$N), 1.48 (s, 3H, CCH_3), 1.39 (s, 9H, C(CH_3)$_3$); δ_C (100 MHz, $CDCl_3$) 178.4, 173.0, 151.0, 150.6, 134.0, 129.0, 126.0, 125.2, 122.0, 117.0, 98.8, 69.3, 52.4, 44.2, 40.3, 33.2, 30.3, 29.3, 29.0, 28.5 (3C), 22.6; HRMS (FAB) m/z 430.21355, (M^+ $C_{23}H_{31}ClN_4O_2$ requires 430.21150); HPLC purity: 90%, t'_R = 2.19 min.

4.10. *N*-*tert*-Butyl-1-(6-(7-chloroquinolin-4-ylamino)hexyl)-2-methyl-5-oxopyrrolidine-2-carboxamide (17)

White amorphous powder (71 mg, 73%), mp 203–205 °C; R_f (MeOH/DCM 1:9) 0.21; IR ν_{max} ($CHCl_3$)/cm^{-1} 3323 (NH), 3011 (ArCH), 2965 (CH), 1726 (C=O), 1651 (C=C), 1545 (C=N); δ_H (400 MHz, $CDCl_3$) 8.46 (d, 1H, J = 5.6, H-2), 7.91 (d, 1H, J = 2.0, H-8), 7.83 (d, 1H, J = 9.0, H-5), 7.43 (dd, 1H, J = 2.0, 9.0, H-6), 6.24 (d, 1H, J = 5.6, H-3), 3.38 (t, 2H, J = 6.0, ArNCH_2 (CH$_2$)$_5$N), 3.00 (t, 2H, J = 6.0, ArN(CH$_2$)$_5$CH_2N), 2.51–2.38 (m, 2H, CH_2-4′), 2.27–2.18 (m, 1H, H-3′α), 1.91–1.87 (m, 1H, H-3′β), 1.83–1.70 (m, 4H, ArNCH$_2$CH_2 (CH$_2$)$_2$CH_2 (CH$_2$)$_2$N), 1.48 (s, 3H, CCH_3), 1.39 (s, 9H, C(CH_3)$_3$), 1.23–1.01 (m, 4H, ArN(CH$_2$)$_2$CH_2CH_2 (CH$_2$)$_2$N); δ_C (100 MHz, $CDCl_3$) 178.5, 172.8, 151.2, 150.7, 134.2, 129.1, 126.2, 125.4, 122.0, 117.0, 99.0, 69.5, 52.5, 44.4, 40.2, 33.4, 30.2, 29.4, 29.0, 28.4 (3C), 22.8; HRMS (FAB) m/z 430.24355, (M^+ $C_{25}H_{35}ClN_4O_2$ requires 458.24485); HPLC purity: 90%, t'_R = 2.19 min.

4.11. 1-(2-(7-Chloroquinolin-4-ylamino)ethyl)-*N*-cyclohexyl-2-methyl-5-oxopyrrolidine-2-carboxamide (18)

White amorphous powder (72 mg, 75%), mp 210–210 °C; R_f (MeOH/DCM 1:9) 0.20; IR ν_{max} ($CHCl_3$)/cm^{-1} 3334 (NH), 3013 (ArCH), 2988 (CH), 1725 (C=O), 1650(C=C), 1555 (C=N); δ_H (300 MHz, $CDCl_3$) 8.46 (d, 1H, J = 5.4, H-2), 7.91 (d, 1H, J = 2.1, H-8), 7.86 (d, 1H, J = 9.0, H-5), 7.41 (dd, 1H, J = 2.1, 9.0, H-6), 6.33 (d, 1H, J = 5.4, H-3), 3.68 (m, 3H, ArNCH_2CH$_2$N, H-1″), 3.48 (t, 2H, J = 6.0, ArNCH$_2$CH_2N), 2.52–2.50 (m, 2H, CH_2-4′), 2.40–2.36 (m, 1H, H-3′α), 2.10–1.99 (m, 1H, H-3′β), 1.78 (br t, 2H, J = 12.0, CH-2″α, CH-6″α), 1.59 (m, 5H, CH-2″β, CH-6″β, CCH_3), 1.26–0.99 (m, 6H, CH_2-3″, CH_2-4″, CH_2-5″); δ_C (75 MHz, $CDCl_3$) 178.7, 172.0, 151.1, 150.0, 146.8, 135.0, 126.7, 126.0, 122.5, 117.0, 98.5, 67.8, 48.9, 44.3, 40.2, 33.4, 32.7 (2C), 29.4, 25.2, 24.7 (2C), 22.6; HRMS (FAB) m/z 428.19404, (M^+ $C_{23}H_{29}ClN_4O_2$ requires 428.19790); HPLC purity: 88%, t'_R2.02 = min.

4.12. 1-(3-(7-Chloroquinolin-4-ylamino)propyl)-*N*-cyclohexyl-2-methyl-5-oxopyrrolidine-2-carboxamide (19)

Yellow oil (69 mg, 70%); R_f (MeOH/DCM 1:9) 0.20; IR ν_{max} ($CHCl_3$)/cm^{-1} 3309 (NH), 3010 (ArCH), 2973 (CH), 1718 (C=O), 1649 (C=C), 1556 (C=N); δ_H (300 MHz, $CDCl_3$) 8.48 (d, 1H, J = 5.4, H-2), 7.89 (d, 1H, J = 2.0, H-8), 7.81 (d, 1H, J = 9.0, H-5), 7.44 (dd, 1H, J = 2.1, 9.0, H-6), 6.31 (d, 1H, J = 5.4, H-3), 3.58 (m, 3H, ArNCH_2 (CH$_2$)$_2$N, H-1″), 3.28 (t, 2H, J = 6.0, ArN(CH$_2$)$_2$CH_2N), 2.53–2.48 (m, 2H, CH_2-2′), 2.39–2.26 (m, 1H, H-3′α), 2.16–1.90 (m, 3H, H-3′β, ArNCH$_2$CH_2CH$_2$N), 1.78 (br t, 2H, J = 12.0, CH-2″α, CH-6″α), 1.60–1.40 (m, 5H, CH-2″β, CH-6″β, CCH_3), 1.38–0.98 (m, 6H, CH_2-3″, CH_2-4″, CH_2-5″); δ_C (75 MHz, $CDCl_3$) 178.5, 172.1, 151.3, 150.0, 146.5, 135.2, 126.5, 125.2, 122.7, 117.0, 99.0, 67.6, 49, 44.2, 40.4, 33.5, 32.8 (2C), 30.3, 29.6, 25.1, 24.5 (2C), 22.8, HRMS (FAB) m/z 442.21325, (M^+ $C_{24}H_{31}ClN_4O_2$ requires 442.21355); HPLC purity: 91%, t'_R = 2.35 min.

4.13. 1-(4-(7-Chloroquinolin-4-ylamino)butyl)-*N*-cyclohexyl-2-methyl-5-oxopyrrolidine-2-carboxamide (20)

White amorphous solid (70 mg, 68%), mp 182–184 °C; R_f (MeOH/DCM 1:9) 0.21; IR ν_{max} ($CHCl_3$)/cm^{-1} 3331 (NH), 3014 (ArCH), 2984 (CH), 1711 (C=O), 1647 (C=C), 1553 (C=C); δ_H (300 MHz, $CDCl_3$) 8.46 (d, 1H, J = 5.4, H-2), 7.91 (d, 1H, J = 2.0, H-8), 7.86 (d, 1H, J = 9.0, H-5), 7.41 (dd, 1H, J = 2.1, 9.0, H-6), 6.33 (d, 1H, J = 5.4, H-3), 3.68 (m, 3H, ArNCH_2(CH$_2$)$_3$N, H-1″), 3.27 (t, 2H, J = 6.0, ArN(CH$_2$)$_3$CH_2N), 2.51–2.48 (m, 2H, CH_2-4′), 2.40–2.38 (m, 1H, H-3′α), 2.00–1.98 (m, 3H, H-3′β, ArCH$_2$CH_2 (CH$_2$)CH$_2$N), 1.78 (m, 4H, CH-2″α CH-6″α, ArCH$_2$ (CH$_2$)$_2$CH_2CH$_2$N), 1.61–1.48, (m, 5H, CH-2″β, CH-6″β, CCH_3), 1.38–0.98 (m, 6H, CH_2-3″, CH_2-4″, CH_2-5″); δ_C (75 MHz, $CDCl_3$) 178.8, 172.3, 151.3, 150.2, 146.5, 135.2, 126.8, 125.7, 122.4, 117.2, 98.7, 67.7, 48.6, 44.4, 40.6, 33.5, 32.4 (2C), 30.1, 29.3, 29, 25.3, 24.5 (2C), 22.3; HRMS (FAB) m/z 456.23033, (M^+ $C_{25}H_{33}ClN_4O_2$ requires 456.22920); HPLC purity: 95%, t'_R = 2.90 min.

4.14. 1-(6-(7-Chloroquinolin-4-ylamino)hexyl)-*N*-cyclohexyl-2-methyl-5-oxopyrrolidine-2-carboxamide (21)

Yellow foam (84 mg, 77%); R_f (MeOH/DCM 1:9) 0.23; IR ν_{max} ($CHCl_3$)/cm^{-1} 3323 (NH), 3026 (ArCH), 2954 (CH), 1716 (C=O), 1652 (C=C), 1583 (C=N); δ_H (300 MHz, $CDCl_3$) 8.46 (d, 1H, J = 5.4, H-2), 7.92 (d, 1H, J = 2.0, H-8), 7.81 (d, 1H, J = 9.0, H-5), 7.41 (dd, 1H, J = 2.1, 9.0, H-6), 6.33 (d, 1H, J = 5.4, H-3), 3.43 (m, 3H, ArNCH_2 (CH$_2$)$_5$N, NCH), 3.22 (t, 2H, J = 6.0, ArN(CH$_2$)$_5$CH_2N), 2.50–2.46 (m, 2H, CH_2-4′), 2.41–2.39 (m, 1H, H-3′α), 2.00–1.98 (m, 3H, H-3′β, ArCH$_2$CH_2 (CH$_2$)$_4$N), 1.70–1.62 (m, 4H, CH-2″α, CH-6″α, ArCH$_2$ (CH$_2$)$_4$CH_2CH$_2$N), 1.59 (m, 5H, CH-2″β, CH-6″β, CCH_3), 1.25–0.98 (m, 10H, CH_2-3″, CH_2-4″, CH_2-5″, ArN(CH$_2$)$_2$CH_2CH_2 (CH$_2$)$_2$N); δ_C (75 MHz, $CDCl_3$) 178.6, 172.0, 151.4, 150.0, 146.7, 135.0, 126.3, 125.4, 122.5, 117.0, 98.9, 67.8, 48.7, 44.3, 40.2, 33.4, 32.3 (2C), 31.5, 29.5, 28.2, 26.7, 26.5, 25.2, 24.7 (2C), 22.8; HRMS (FAB) m/z 484.26050, (M^+ $C_{27}H_{37}ClN_4O_2$ requires 484.26024); HPLC purity: 94%, t'_R = 2.46 min.

4.15. *N-tert*-Butyl-1-(2-(7-chloroquinolin-4-ylamino)ethyl)-2-methyl-6-oxopiperidine-2-carboxamide (22)

White gum (70 mg, 75%); R_f (MeOH/DCM 1:9) 0.20; IR ν_{max} ($CHCl_3$)/cm^{-1} 3317 (NH), 3030 (ArCH), 2974 (CH), 1715 (C=O), 1646 (C=C), 1578 (C=N); δ_H (300 MHz, $CDCl_3$) 8.44 (d, 1H, J = 5.7, H-2), 8.08 (d, 1H, J = 9.0, H-5), 7.9 (d, 1H, J = 2.1, H-8), 7.40 (dd, 1H, J = 2.1, 9.0, H-6), 6.26 (d, 1H, J = 5.4, H-3), 3.25 (t, 2H, J = 6.0, ArNCH_2CH$_2$N), 3.0 (t, 2H, J = 6.0, ArNCH$_2$CH_2N), 2.56–2.40 (m, 2H, CH_2-5′), 2.20–2.14 (m, 2H, CH_2-3′), 1.80–1.74 (m, 2H, CH_2-4′), 1.55 (s, 3H, CCH_3), 1.32 (s, 9H, C(CH_3)$_3$); δ_C (75 MHz, $CDCl_3$) 178.7, 172.4, 151.6, 150.4, 147.9, 134.8, 126.3, 125.7, 122.3, 117.4, 98.2, 66.5, 51.4, 42.7, 41.0, 36.1, 34.0, 29.0, 28.6 (3C), 17.4; HRMS (EI) 416.19785 (M^+ $C_{22}H_{29}ClN_4O_2$ requires 416.19790); HPLC purity: 91%, t'_R = 2.12 min.

4.16. *N-tert*-Butyl-1-(3-(7-chloroquinolin-4-ylamino)propyl)-2-methyl-6-oxopiperidine-2-carboxamide (23)

Cream white paste (69 mg, 71%); R_f (MeOH/DCM 1:9) 0.20; IR ν_{max} ($CHCl_3$)/cm^{-1} 3322 (NH), 3035 (ArCH), 2956 (CH), 1721 (C=O), 1647 (C=C), 1583 (C=N); δ_H (300 MHz, $CDCl_3$) 8.38 (d, 1H, J = 5.7, H-2), 8.10 (d, 1H, J = 9.0, H-5), 7.88 (d, 1H, J = 2.1, H-8), 7.42 (dd, 1H, J = 2.1, 9.0, H-6), 6.34 (d, 1H, J = 5.7, H-3), 3.26 (t, 2H, J = 6.0, ArNCH_2 (CH$_2$)$_2$N), 3.10 (t, 2H, J = 6.0, ArN(CH$_2$)$_2$CH_2N), 2.57–2.40 (m, 2H, CH_2-5′), 2.20–2.12 (m, 2H, CH_2-3′), 1.94–1.89 (m, 2H, CH_2-4′), 1.83–1.72 (m, 2H, ArNCH$_2$CHCH$_2$N), 1.49 (s, 3H, CH_3), 1.38 (s, 9H, C(CH_3)$_3$); δ_C (75 MHz, $CDCl_3$) 178.8, 172.2, 151.5, 150.2, 147.6, 134.7, 126.4, 125.8, 122.6, 117.2, 98.6, 66.5, 51.7, 42.5, 42.1, 36.3, 34.2, 30.4, 29.2, 28.8 (3C), 17.7; HRMS (EI) 430. 21319 (M^+ $C_{23}H_{31}ClN_4O_2$ requires 430.21355); HPLC purity: 96%, t'_R = 1.81 min.

4.17. *N-tert*-Butyl-1-(4-(7-chloroquinolin-4-ylamino)butyl)-2-methyl-6-oxopiperidine-2-carboxamide (24)

Cream white paste (69 mg, 69%); R_f (MeOH/DCM 1:9) 0.21 IR ν_{max} ($CHCl_3$)/cm^{-1} 3332 (NH), 3027 (ArCH), 2966 (CH), 1715 (C=O), 1636 (C=C), 1582 (C=C); δ_H (300 MHz, $CDCl_3$) 8.37 (d, 1H, J = 5.7, H-2), 8.06 (d, 1H, J = 9.0, H-5), 7.81 (d, 1H, J = 2.1, H-8), 7.38 (dd, 1H, J = 2.1, 9.0, H-6), 6.24 (d, 1H, J = 5.7, H-3), 3.22 (t, 2H, J = 6.0, ArNCH_2 $(CH_2)_3$N), 3.11 (t, 2H, J = 6.0, ArN$(CH_2)_3CH_2$N), 2.53–2.41 (m, 2H, CH_2-5′), 2.22–2.13 (m, 2H, CH_2-3′), 1.97–1.95 (m, 2H, CH_2-4′), 1.85–1.74 (m, 4H, ArNCH_2CH_2CH_2N), 1.52 (s, 3H, CH_3), 1.34 (s, 9H, C(CH_3)$_3$); δ_C (75 MHz, $CDCl_3$) 178.5, 172.6, 151.3, 150.5, 147.7, 134.2, 126.4, 125.5, 122.4, 117.2, 98.3, 66.3, 51.5, 42.8, 41.2, 36.3, 34.2, 30.5, 29.1, 28.7, 28.8 (3C), 17.9; HRMS (EI) 444.23007 (M^+ $C_{24}H_{33}ClN_4O_2$ requires 444.22920); HPLC purity: 87%, t'_R = 1.95 min.

4.18. *N-tert*-Butyl-1-(6-(7-chloroquinolin-4-ylamino)hexyl)-2-methyl-6-oxopiperidine-2-carboxamide (25)

White paste (79 mg, 74%); R_f (MeOH/DCM 1:9) 0.20; IR ν_{max} ($CHCl_3$)/cm^{-1} 3319 (NH), 3031 (ArCH), 2987 (CH), 1707 (C=O), 1653 (C=C), 1567 (C=N); δ_H (300 MHz, $CDCl_3$) 8.35 (d, 1H, J = 5.7, H-2), 8.0 (d, 1H, J = 9.0, H-5), 7.88 (d, 1H, J = 2.1, H-8), 7.41 (dd, 1H, J = 2.1, 9.0, H-6), 6.28 (d, 1H, J = 5.7, H-3), 3.30 (t, 2H, J = 6.0, ArNCH_2 $(CH_2)_5$N), 3.00 (t, 2H, J = 6.0, ArN$(CH_2)_5CH_2$N), 2.56–2.40 (m, 2H, CH_2-5′), 2.20–2.14 (m, 2H, CH_2-3′), 2.05–2.01 (m, 2H, CH_2-4′), 1.90–1.80 (m, 2H, ArNCH_2C$H_2$$(CH_2)_4$N), 1.82–1.70 (m, 4H, ArN$(CH_2)_2CH_2CH_2$ $(CH_2)_2$N), 1.62–1.53 (m, 5H, ArN$(CH_2)_2CH_2$ $(CH_2)_2$N, CCH_3), 1.30 (s, 9H, C(CH_3)$_3$); δ_C (75 MHz, $CDCl_3$) 178.8, 172.6, 151.7, 150.5, 147.8, 134.7, 126.5, 125.8, 122.4, 117.6, 99.0, 66.6, 51.3, 42.8, 41.3, 36.5, 34.3, 31.8, 29.3, 28.8 (3C), 28.1, 26.5, 26.6, 17.7; HRMS (EI) 472.26001 (M^+ $C_{26}H_{37}ClN_4O_2$ requires 472.26050); HPLC purity: 92%, t'_R = 1.93 min.

4.19. 1-(2-(7-Chloroquinolin-4-ylamino)ethyl)-*N*-cyclohexyl-2-methyl-6-oxopiperidine-2-carboxamide (26)

Off-white crystals (65 mg, 66%); R_f (MeOH/DCM 1:9) 0.20; IR ν_{max} ($CHCl_3$)/cm^{-1} 3331 (NH), 3015 (ArCH), 2976 (CH), 1722 (C=O), 1617 (C=C), 1559 (C=N); δ_H (300 MHz, $CDCl_3$) 8.46 (d, 1H, J = 5.4, H-2), 7.91 (d, 1H, J = 2.0, H-8), 7.81 (d, 1H, J = 9.0, H-5), 7.41 (dd, 1H, J = 2.1, 9.0, H-6), 6.33 (d, 1H, J = 5.4, H-3), 3.68 (m, 1H, H-1″), 3.32 (t, 2H, J = 6.0, ArNCH_2CH_2N), 3.10 (t, 2H, J = 6.0, ArNCH_2CH_2N), 2.57–2.42 (m, 2H, CH_2-5′), 2.20–2.12 (m, 2H, CH_2-3′), 1.81–1.70 (m, 4H, CH-2″α, CH-6″α, CH_2-4′), 1.64–1.60 (m, 5H, CH-2″β CH-6″β, CCH_3), 1.25–1.22 (m, 3H, CH-3″α, CH-4″α, CH-5″α), 1.22–1.01 (m, 3H, CH-3″β, CH-4″β, CH-5″β); δ_C (75 MHz, $CDCl_3$) 178.4, 172.0, 151.3, 150.2, 146.9, 135.2, 126.6, 125.5, 122.7, 117.3, 98.8, 66.4, 49.8, 44.6, 43.9, 36.0, 33 (2C), 32.6, 30.0, 25, 24.8 (2C), 17.8; HRMS (EI) 442.21343 (M^+ $C_{24}H_{31}ClN_4O_2$ requires 442.21355); HPLC purity: 95%, t'_R = 2.14 min.

4.20. 1-(3-(7-Chloroquinolin-4-ylamino)propyl)-*N*-cyclohexyl-2-methyl-6-oxopiperidine-2-carboxamide (27)

Cream white paste (74 mg, 72%); R_f (MeOH/DCM 1:9) 0.20; IR ν_{max} ($CHCl_3$)/cm^{-1} 3327 (NH), 3027 (ArCH), 2988 (CH), 1718 (C=O), 1630 (C=C), 1580 (C=N); δ_H (300 MHz, $CDCl_3$) 8.43 (d, 1H, J = 5.4, H-2), 7.85 (d, 1H, J = 2.0, H-8), 7.78 (d, 1H, J = 9.0, H-5), 7.40 (dd, 1H, J = 2.1, 9.0, H-6), 6.30 (d, 1H, J = 5.4, H-3), 3.66 (m, 1H, H-1″), 3.30 (t, 2H, J = 6.0, ArNCH_2 $(CH_2)_2$N), 3.11 (t, 2H, J = 6.0, ArN$(CH_2)_2CH_2$N), 2.56–2.43 (m, 2H, CH_2-5′), 2.20–2.12 (m, 2H, CH_2-3′), 1.99–1.97 (m, 2H, CH_2-4′), 1.84–1.71 (m, 4H, CH-2″α, CH-6″α, CH_2-4′), 1.67–1.65 (m, 5H, CH-2″β, CH-6″β, CCH_3), 1.26–1.24 (m, 3H, CH-3″α, CH-4″α, CH-5″α), 1.10–1.00 (m, 3H, CH-3″β, CH-4″β, CH-5″β); δ_C (75 MHz, $CDCl_3$) 178.3, 172.2, 151.4, 150.3, 146.8, 135.1, 126.5, 125.6, 122.8, 117.5, 98.4, 66.7, 49.4, 44.8, 43.3, 36.2, 33.0 (2C), 32.4, 30.3, 26.5, 25.2, 24.7 (2C), 18.0; HRMS (EI) 456.22954 (M^+ $C_{25}H_{33}ClN_4O_2$ requires 456.22920); HPLC purity: 94%, t'_R = 2.51 min.

4.21. 1-(4-(7-Chloroquinolin-4-ylamino)butyl)-*N*-cyclohexyl-2-methyl-6-oxopiperidine-2-carboxamide (28)

Off-white gum (78 mg, 74%); R_f (MeOH/DCM 1:9) 0.21; IR ν_{max} ($CHCl_3$)/cm^{-1} 3322 (NH), 3302 (ArCH), 2974 (CH), 1721 (C=O), 1627 (C=C), 1580 (C=N); δ_H (300 MHz, $CDCl_3$) 8.44 (d, 1H, J = 5.4, H-2), 7.90 (d, 1H, J = 2.0, H-8), 7.86 (d, 1H, J = 9.0, H-5), 7.39 (dd, 1H, J = 2.1, 9.0, H-6), 6.28 (d, 1H, J = 5.4, H-3), 3.68 (m, 1H, H-1″), 3.33 (t, 2H, J = 6.0, ArNCH_2 $(CH_2)_3$N), 3.11 (t, 2H, J = 6.0, ArN$(CH_2)_3CH_2$N), 2.53–2.44 (m, 2H, CH_2-5′), 2.3–2.13 (m, 2H, CH_2-3′), 1.80–1.72 (m, 6H, CH-2″α, CH-6″α, CH_2-4′, ArNCH_2CH_2 $(CH_2)_2$N), 1.66–1.63 (m, 7H, CH-2″β, CH-6″β, ArN$(CH_2)_2CH_2CH_2$N, CCH_3), 1.24–1.22 (m, 3H, CH-3″α, CH-4″α, CH-5″α), 1.0–0.98 (m, 3H, CH-3″β, CH-4″β, CH-5″β); δ_C (75 MHz, $CDCl_3$) 178.2, 172.1, 151.2, 150.3, 146.7, 135.3, 126.4, 125.5, 122.9, 117.5, 99.0, 66.3, 49.5, 44.4, 41.3, 36.3, 33.4 (2C), 32.2, 30.3, 27.3, 25.6, 25.2, 24.7 (2C), 18.3; HRMS (EI) 470.24466 (M^+ $C_{26}H_{35}ClN_4O_2$ requires 470.24485); HPLC purity: 91%, t'_R = 2.08 min.

4.22. 1-(6-(7-Chloroquinolin-4-ylamino)hexyl)-*N*-cyclohexyl-2-methyl-6-oxopiperidine-2-carboxamide (29)

Off-white hygroscopic solid (81 mg, 72%); R_f (MeOH/DCM 1:9) 0.22; IR ν_{max} ($CHCl_3$)/cm^{-1} 3319 (NH), 3011 (ArCH), 2984 (CH), 1734 (C=O), 1622 (C=C), 1558 (C=N); δ_H (300 MHz, $CDCl_3$) 8.44 (d, 1H, J = 5.4, H-2), 7.93 (d, 1H, J = 2.0, H-8), 7.80 (d, 1H, J = 9.0, H-5), 7.38 (dd, 1H, J = 2.1, 9.0, H-6), 6.30 (d, 1H, J = 5.4, H-3), 3.60 (m, 1H, H-1″), 3.34 (t, 2H, J = 6.0, ArNCH_2 $(CH_2)_5$N), 3.12 (t, 2H, J = 6.0, ArN$(CH_2)_5CH_2$N), 2.57–2.42 (m, 2H, CH_2-5′), 2.20–2.12 (m, 2H, CH_2-3′), 1.80–1.70 (m, 6H, CH-2″α, CH-6″α, CH_2-4′, ArNCH_2CH_2 $(CH_2)_4$N), 1.65–1.63 (m, 5H, CH-2″β, CH-6″β, CCH_3), 1.40–1.36 (m, 6H, ArNCH_2CH_2 $(CH_2)_3CH_2$N), 1.23–1.18 (m, 3H, CH-3″α, CH-4″α, CH-5″α), 1.10–0.99 (m, 3H, CH-3″β, CH-4″β, CH-5″β); δ_C (75 MHz, $CDCl_3$) 178.1, 172.2,

151.1, 150.4, 146.7, 135.3, 126.5, 125.4, 122.6, 117.1, 98.6, 66.5, 49.6, 44.5, 43.7, 36.2, 33.1 (2C), 32.3, 30.6, 30.3, 27.7, 27.4, 26.4, 25.0, 24.5 (2C), 18.1; HRMS (EI) 498.27656 (M^+ $C_{28}H_{39}ClN_4O_2$ requires 498.27612); HPLC purity: 93%, $t'_R = 2.36$ min.

4.23. In vitro activities of compounds against falcipain-2

IC_{50} values against the recombinant enzyme (falcipain-2) were determined as described by Greenbaum and coworkers.[27] Thus, an equal amount of recombinant protein (~1 nM) was incubated with different concentrations of inhibitors (added from 100x stock solutions in DMSO) in 100 mM sodium acetate (pH 5.5)-10 mM dithiothreitol for 30 min at room temperature before addition of the substrate benzoxycarbonyl-Leu-Arg-7-amino-4-methyl-coumarin (final concentration = 25 μM). Fluorescence was continuously monitored for 30 min at room temperature in a Labsystems Fluoroscan® Ascent spectrofluorometer. IC_{50} values were determined from plots of activity over inhibitor concentration with GraphPad Prism® software. IC_{50} values for rhodesain were determined similarly at 3 nM.

4.24. In vitro activities of compounds against W2 *P. falciparum*

W2-strain *P. falciparum* parasites (1% parasitaemia, 2% hematocrit) were cultured in 0.5 ml of medium in 48-well culture dishes. Appropriate inhibitors from 10 mM stocks in DMSO were added to cultured parasites to a final concentration of 20 μM. From 48-well plates, 125 μM of culture was transferred to two 96-well plates (duplicates). Serial dilutions (1%) of inhibitors were made to final concentrations of 10 μM, 2 μM, 0.4 μM, 80 nM, 16 nM and 3.2 nM. Cultures were maintained at 37 °C for 2 days after which the parasites were washed and fixed with 1% formaldehyde in PBS. After two days, parasitaemia was measured by flow cytometry using the DNA stain YOYO-1 as a marker for cell survival.[27]

4.25. In vitro activities against *T. b. brucei* S427

The bloodstream form trypomastigotes were maintained in MEM with Earle's salts supplemented with 25 mM HEPES, 1 g/l additional glucose, 10 ml/l MEM non-essential aminoacids (100×), 0.2 mM 2-mercaptoethanol, 2 mM Na-pyruvate, 0.1 mM hypoxanthine, 0.05 mM bathocuprionedisulfonic acid, 0.15 mM L-cysteine and 15% heat-inactivated, foetal calf serum.

All cultures and assays were conducted at 37 °C under an atmosphere of 5% CO_2/95% air mixture.

4.26. Drug sensitivity assays

Stock drug solutions were prepared in 100% DMSO unless otherwise suggested by the supplier at 20 mg/ml, and ball milled or sonicated if necessary. The stocks were kept at 4 °C. For the assays, the compound was further diluted to the appropriate concentration using complete medium.

Assays were performed in sterile 96-well microtitre plates, each well containing 100 μl of parasite culture (1×10^4 bloodstream forms) with or without serial drug dilutions at 37 °C for 72 h in 5% CO_2. The highest concentration for the test compounds was 30 μg/ml. Each drug was tested in triplicate. A 3-fold serial dilution was performed down to a suitable concentration to obtain an IC_{50} value. Initial testing was at 30, 10, 3 and 0.1 μg/ml. The control drug was Pentamidine and was diluted down to 0.0001 μg/ml (12 dilutions). Control wells were without drug, blanks were medium only. After 72 h of incubation, the plates were inspected under an inverted microscope to assure growth of the controls and to determine the minimum inhibitory concentration (MIC): this is the lowest drug concentration at which no trypanosomes with normal morphology and motility as compared to the control wells can be seen.

Twenty microliters of Alamar Blue™ was added to each well and the plates incubated for another 2–4 h. Then the plates were read on a Gemini Plate Reader (Molecular Devices) using an excitation wavelength of 530 nm and an emission wavelength of 580 nm (cut off 550 nm).[28]

4.27. Primary screen

The preliminary screen uses the *T. b. brucei* strain. The compounds were tested at 7 concentrations (drug concentration ranges from 30 μg/ml to 0.1 μg/ml in 3-fold dilutions). In this assay pentamidine had an ED_{50} value of 0.1–0.02 ng/ml.

4.28. Secondary screen

Active compounds (IC_{50} <0.2 μg/ml) were tested again over the appropriate dose range against *T. b. rhodesiense* STIB 900 to confirm IC_{50} and IC_{90} values in comparison to standard pentamidine and internal chemical group standard if available.

4.29. Toxicity tests

4.29.1. Cell cultures. KB cells—a cell line derived from a human carcinoma of the nasopharynx, typically used as an assay for antineoplastic agents. KB cells were maintained as monolayers in RPMI 1640 + 10% HIFCS. All cultures and assays were conducted at 37 °C under an atmosphere of 5% CO_2/95% air mixture.

4.29.2. Drug toxicity assays. Stock drug solutions were prepared in 100% DMSO unless otherwise suggested by the supplier at 20 mg/ml, and ball milled or sonicated if necessary. The stocks were kept at 4 °C. For the assays, the compound was further diluted to the appropriate concentration using complete medium.

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