Bioorganic & Medicinal Chemistry 17 (2009) 7775–7782

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Structure–activity relationship studies of manzamine A: Amidation of positions 6 and 8 of the β-carboline moiety

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ARTICLE INFO

Article history:
Received 29 July 2009
Revised 14 September 2009
Accepted 16 September 2009
Available online 19 September 2009

Keywords:
Manzamine alkaloids
Amidation
Antimalaria

ABSTRACT

Twenty manzamine amides were synthesized and evaluated for in vitro antimalarial and antimicrobial activities. The amides of manzamine A (**1**) showed significantly reduced cytotoxicity against Vero cells, although were less active than **1**. The structure–activity analysis showed that linear, short alkyl groups adjacent to the amide carbonyl at position 8 are favored for antimalarial activity, while bulky and cyclic groups at position 6 provided the most active amides. Most of the amides showed potent activity against *Mycobacterium intracellulare*. The antimicrobial activity profile for position 8 series was similar to that for antimalarial activity profile, in which linear, slightly short alkyl groups adjacent to the amide carbonyl showed improved activity. Two amides **14** and **21**, which showed potent antimalarial activity in vitro against *Plasmodium falciparum* were further evaluated in vivo in *Plasmodium berghei* infected mice. Oral administration of **14** and **21** at the dose of 30 mg/kg (once daily for three days) caused parasitemia suppression of 24% and 62%, respectively, with no apparent toxicity.

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1. Introduction

When the resistance to chloroquine has spread around the world, the era of inexpensive and available antimalarial drugs had ended.[1] This, in addition to the fact that artemisinins are the only first-line antimalarial drugs that are still effective against all chloroquine-resistance malaria parasites has driven the scientific community and funding agencies to invest additional time and resources for the development of new antimalarial drugs. Malaria causes more than one million deaths every year.[2] The process of drug discovery includes three major stages: (1) target identification and high throughput screening of small molecules (2) lead identification and optimization, and (3) preclinical and clinical studies.[2] Nature has served as the mine for structurally diverse small molecules utilized for target identification for human diseases. Over 63% of the bioactive small molecules reported between 1981 and 2006 are either natural, natural product derived or inspired from natural compounds.[3] The manzamine alkaloids (Fig. 1) represent a unique class of natural products that have shown a diverse range of bioactivities, including antimicrobial,[4–7] antiparasitic,[8] cytotoxicity,[9,10] anti-inflammatory,[11] pesticidial,[12] and were shown to possess activity against HIV-1 and AIDS opportunistic infections.[13] They are particularly attractive candidates for optimization for the control of infectious diseases.[14] The first representative of this family is manzamine A (**1**) isolated in 1986 by Higa.[10] This family has the unique structural feature of having complex polycyclic ring systems coupled with a β-carboline moiety. Manzamine A (**1**) and its 8-hydroxy derivative (**2**), showed the most promising antimalarial activity within this class of compounds. Both showed improved potency against the malaria parasite in vitro and in vivo over the clinically used drugs chloroquine and artemisinin.[15] Oral treatment of **1** (2×100 μmol/kg) and **2** (2×100 μmol/kg) showed 90% reduction in parasitemia. Mice treated with a single dose (50 or 100 μmol/kg) of **1** or **2** also showed significant improvements in survival times over mice treated with chloroquine or artemisinin.[16] This data revealed significant promise for the development of this new class of antimalarial drugs. However, the major drawback of this class of compounds is the toxicity associated with higher dosing schedules. The mechanism of action of **1** as an antimalarial agent is not clear and requires intensive structure–activity relationship (SAR) study for a better understanding of the importance of each moiety of this complex molecule for the antimalarial activity. The first intensive SAR study on manzamine alkaloids was completed by our group and focused on exploring the different functional groups around the molecule.[17] This included reduction of the double bonds in the complex polycyclic ring systems, N-oxidation, 9N-alkylation, 8O-alkylation in **2** and reduction of the carbonyl group in manzamine F (**3**). In addition, ircinal (**4**) was coupled with substituted tryptamines through Pictet–Spengler cyclization. Several analogues were synthesized in this study with good antimalarial

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0968-0896/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.bmc.2009.09.026

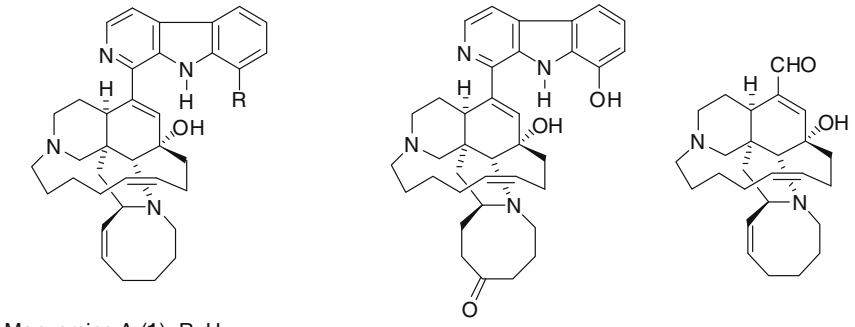


Figure 1. Manzamine alkaloids.

activity but without improvement in regard to cytotoxicity. Experimental evidence that manzamines arrest the cell cycle in the S phase[17] suggests that this toxicity may be due to DNA intercalation by the planer β-carboline moiety. However, no significant modifications of the β-carboline were completed until this study.

In this study, we focused our modification on the β-carboline moiety of **1** as an extension to the previous study for lead optimization as an antimalarial agent. Twenty amides of **1** which differ at positions 6 and 8 in the β-carboline moiety have been synthesized. These amides were evaluated for in vitro and in vivo antimalarial activity in addition to in vitro antimicrobial activity.

2. Results and discussion

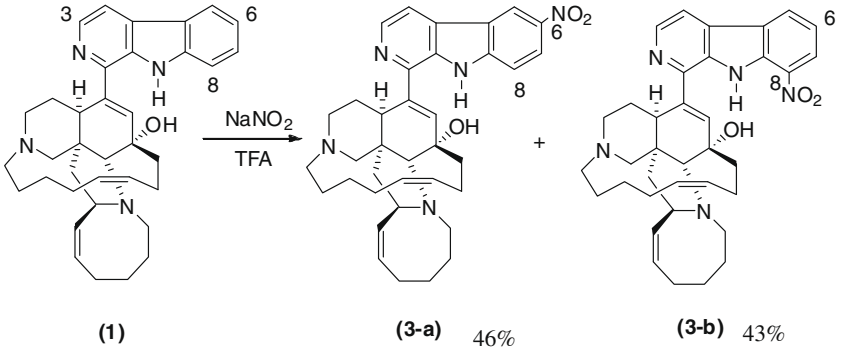
2.1. Chemistry

Manzamine A (**1**) in addition to **2**, **3**, and **4** were purified from the Indonesian sponge *Acanthostrongylophora* sp. through an optimized isolation procedure.[13] Positions 6 and 8 of the β-carboline moiety in **1** could be chemically modified via electrophilic aromatic substitution reactions, due to the activation by the secondary amine functionality in the indole part of the β-carboline moiety. We began our modifications by nitrating the benzene ring of the β-carboline moiety with $NaNO_2$ in the presence of trifluoroacetic acid (TFA) (Scheme 1). This yielded two nitro products, 6-nitromanzamine A (**3a**), and 8-nitromanzamine A (**3b**). The stability of these two nitromanzamine A products, and the feasibility of converting the nitro group to a different functional groups were the main reasons for selecting nitromanzamines as key intermediates for synthesizing additional analogues at positions 6 and 8. Large-scale nitration of **1** and the purification of both nitro products **3a**,**b** were carried out to provide starting material for additional analogues. Both nitro products were reduced to the corresponding 6 and 8-aminomanzamine A (**4a**,**b**) in almost complete conversion (by LC–MS). However, low yield of the amines were recovered after workup step due to their instability, even as the hydrochloric salts. In addition, aminomanzamines were unstable in solution, especially in the presence of chloroform or dichloromethane. This lack of stability created challenges in regard to the yields of the amidation reaction. Adding to the challenge is the conjugation with the remaining two nitrogens of the β-carboline which appear to produce a highly basic amine after reduction of the nitro group.

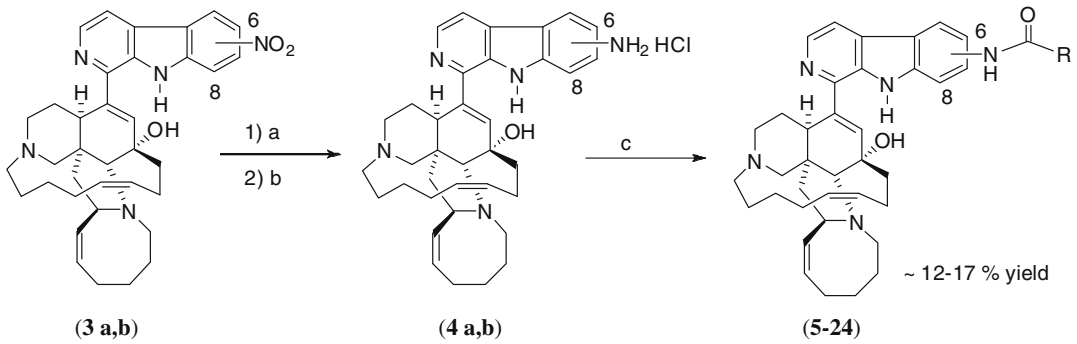
Amidation of aminomanzamines at positions 6 and 8 were carried out by reacting the amines as a hydrochloric acid salts with a slight excess of different acyl chlorides in the presence of the catalyst DMAP, and the base triethylamine at room temperature in dry THF under nitrogen atmosphere (Scheme 2). Twenty amides were synthesized in both positions with yields ranging between ~12% and 17%. The structures of the amidated manzamine A series were confirmed by 1D and 2D NMR spectroscopy (Table 1).

2.2. In vitro antimalarial activity

All amides were evaluated in vitro for antimalarial activity against chloroquine sensitive (D6, Sierra Leone) and resistant (W2, IndoChina) clones of *Plasmodium falciparum*. In addition, they were also tested for toxicity on normal African green monkey kidney fibroblast cells (Vero) (Table 2). Manzamine A (**1**) showed the highest activity with an IC_{50} of 8.0 nM (D6 clone) and 11 nM (W2 clone). This activity is more potent than the standard antimalarials chloroquine and artemisinin which show an IC_{50} values of 50 nM and 46 nM, respectively, against the D6 clone. Manzamine A (**1**) has a TC_{50} of 365 nM against Vero cells, providing therapeutic indexes of 44 (D6 clone) and 25 (W2 clone).[18] Like manzamine A almost all the amides showed similar antimalarial activity against



Scheme 1. Nitration of manzamine A.



(**3 a,b**) (**4 a,b**) (**5-24**)

Scheme 2. General procedure for amidation of manzamine. Reagents and conditions: (a) Zn, AcOH/MeOH (5%), rt, 10 min; (b) 1 equiv concd HCl; (c) 1.2 equiv RCOCl, 1 equiv Et_3N, cat. DMAP, THF, rt, 1 h.

Table 1
The synthesized manzamine A amides

Compound	R	Entry	Yield (%)
6-Acetamidomanzamine A	CH_3	**5**	14
8-Acetamidomanzamine A		**15**	13
6-*n*-Propamidomanzamine A		**6**	14
8-*n*-Propamidomanzamine A		**16**	14
6-*n*-Butamidomanzamine A		**7**	14
8-*n*-Butamidomanzamine A		**17**	14
6-Isobutamidomanzamine A		**8**	12
8-Isobutamidomanzamine A		**18**	14
6-*n*-Pentamidomanzamine A		**9**	13
8-*n*-Pentamidomanzamine A		**19**	15
6-Pivalamidomanzamine A		**10**	12
8-Pivalamidomanzamine A		**20**	12
6-*n*-Hexamidomanzamine A		**11**	14
8-*n*-Hexamidomanzamine A		**21**	14
6-*n*-Octamidomanzamine A		**12**	12
8-*n*-Octamidomanzmaine A		**22**	15
6-(*t*-Butyl)-acetamidomanzamine A		**13**	15
8-(*t*-Butyl)-acetamidomanzamine A		**23**	14
6-Cyclohexamidomanzamine A		**14**	17
8-Cyclohexamidomanzamine A		**24**	16

D6 and W2 clones of *P. falciparum*. Introduction of an acetamido functionality at position 8 (**15**) reduced the antimalarial activity to an IC_{50} of 182 nM against D6 clone. Increasing the length of the alkyl chain adjacent to the amide carbonyl (2, 3, 4, 5 and 7 carbons) (**16**, **17**, **19**, **21** and **22**) in position 8 series showed improvement in in vitro antimalarial activity with IC_{50} values of 123, 35, 53, 32, and 55 nM, respectively, against D6 clone. Branched alkyl groups adjacent to the amide carbonyl were not favored at position 8. For example, 8-isobutamidomanzamine A (**18**) showed reduced activity with IC_{50} values of 158 and 205 nM, against D6 and W2 clones, respectively. This is relative to 8-*n*-butamidomanzamine A (**17**, 35 nM against D6), which has the same number of carbon atoms as a linear chain. Similar results were obtained when adding bulkier groups such as *t*-butyl either directly attached to the amide carbonyl (**20**, IC_{50} = 170 and 232 nM against D6 and W2 clones, respectively) or separated by one carbon as in 8-*t*-butylacetamidomanzamine A (**23**, IC_{50} = 139 and 127 nM against D6 and W2 clones, respectively). Furthermore, adding a cyclohexyl group as in 8-cyclohexamidomanzamine A (**24**) markedly diminished the antimalarial activity (IC_{50} = 950 and 995 nM against D6 and W2 clones, respectively). This data suggests that relatively short linear alkyl groups (2–7 carbons) attached to the amide carbonyl at position 8 are preferred over branched and bulky groups for antimalarial activity.

The antimalarial activity profile for position 6 amides was completely opposite to that of the amides at position 8. The acetamido group at position 6 drastically eliminated the antimalarial activity (**5**, IC_{50} = 1288 and 1982 nM against D6 and W2 clones, respectively). Increasing the number of carbons attached to the amide carbonyl (2, 3, 4, 5 and 7 carbons) **6**, **7**, **9**, **11**, and **12** also resulted in similar reduction in the antimalarial activity with IC_{50} values of 1162, 1152, 1235, 680 and 696 nM against D6, respectively. The amide with an isopropyl group attached to the amide carbonyl **8** also showed reduced antimalarial activity (IC_{50}s of 1105 and 1547 nM against D6 and W2 clones, respectively). Addition of a *t*-butyl group at position 6 slightly improved the activity as in 6-pivalamidomanzamine A (**10**) and 6-*t*-butylacetamidomanzamine A (**13**) with IC_{50} values of 231 and 211 nM against D6, compared to the linear alkyl groups. 6-Cyclohexamidomanzamine A (**14**) showed the best antimalarial activity among the 6-amide series with an IC_{50} = 34 nM against D6 clone. This data suggests that bulky groups at position 6 are preferred for antimalarial activity. It was interesting to note that all the amides in both series (6 and 8) did not show cytotoxicity to Vero cells.

2.3. In vivo antimalarial activity

With the objective of finding the analogues with reduced toxicity and equivalent or better antimalarial activity as compared to manzamine A, the two most potent amides, 6-cyclohexamidomanzamine A (**14**, IC_{50} = 34 nM) and 8-*n*-hexamidomanzamine A (**21**, IC_{50} = 32 nM) were evaluated in vivo in a *P. berghei* mouse malaria model. The treatment with compounds **14** and **21** through oral administration caused only moderate suppression in parasitemia of 24% and 62%, respectively, at three doses (once daily for three days) of 30 mg/kg with no apparent toxicity. These results indicate that the amides **14** and **21** were less toxic in vivo; however, their antimalarial potency in vivo was also compromised, as compared to manzamine A.

2.4. In vitro antimicrobial activity

In vitro antimicrobial activities of the manzamine amides were investigated against *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Cryptococcus neoformans*, *Mycobacterium intracellulare* and *Aspergillus fumigatus* (Table 3).

2.4.1. Bioactivity against *M. intracellulare*

Manzamine A (**1**) showed potent activity against *M. intracellulare* with an IC_{50} value of 0.640 μM which is slightly more potent

Table 2
In vitro antimalarial activity of manzamine amides against chloroquine sensitive (D6, Sierra Leone) and resistant (W2, IndoChina) strains of *Plasmodium falciparum*

Compound	Entry	*P. falciparum* (D6 Clone) IC_{50} (nM)	*P. falciparum* (W2 Clone) IC_{50} (nM)	Cytotoxicity (Vero) IC_{50} (nM)
6-Acetamidomanzamine A	**5**	1288	1982	NC
8-Acetamidomanzamine A	**15**	182	231	NC
6-*n*-Propamidomanzamine A	**6**	1162	1937	NC
8-*n*-Propamidomanzamine A	**16**	123	87	NC
6-*n*-Butamidomanzamine A	**7**	1152	1894	NC
8-*n*-Butamidomanzamine A	**17**	35	121	NC
6-Isobutamidomanzamine A	**8**	1105	1547	NC
8-Isobutamidomanzamine A	**18**	158	205	NC
6-*n*-Pentamidomanzamine A	**9**	1235	1482	NC
8-*n*-Pentamidomanzamine A	**19**	53	49	NC
6-Pivalamidoanzamine A	**10**	231	417	NC
8-Pivalamidomanzamine A	**20**	170	232	NC
6-*n*-Hexamidomanzamine A	**11**	680	786	NC
8-*n*-Hexamidomanzamine A	**21**	32	65	NC
6-*n*-Octamidomanzamine A	**12**	696	754	NC
8-*n*-Octamidomanzamine A	**22**	55	43	NC
6-(*t*-Butyl)-acetamidomanzamine A	**13**	211	302	NC
8-(*t*-Butyl)-acetamidomanzamine A	**23**	139	127	NC
6-Cyclohexamidomanzamine A	**14**	34	53	NC
8-Cyclohexamidomanzamine A	**24**	950	995	NC
Manzamine A	**1**	8.0	11	365
8-Hydroxymanzamine A	**2**	11	14	
Chloroquine		50	484	—
Artemisinin		46	28	—

NC, no cytotoxicity up to 470 nM.

Table 3
In vitro antimicrobial data of manzamine amides (all values in μM)

Compound	Entry	IC_{50}/MIC			
		C. albicans	*C. neoformans*	*M. intracellulre*	*A. fumigatus*
6-Acetamidomanzamine A	**5**	—/—	—/—	9.911/16.51	—/—
8-Acetamidomanzamine A	**15**	—/—	33.35/—	1.651/2.064	—/—
6-*n*-Propamidomanzamine A	**6**	—/—	—/—	5.650/8.071	—/—
8-*n*-Propamidomanzamine A	**16**	>32.28/—	NT/NT	1.517/2.018	>32.28/—
6-*n*-Butamidomanzamine A	**7**	—/—	—/—	4.736/7.893	—/—
8-*n*-Butamidomanzamine A	**17**	—/—	23.68/—	0.868/1.973	—/—
6-Isobutamidomanzamine A	**8**	—/—	—/—	3.157/3.946	—/—
8-Isobutamidomanzamine A	**18**	—/—	3.157/—	1.026/1.973	—/—
6-*n*-Pentamidomanzamine A	**9**	—/—	—/—	2.316/3.861	—/—
8-*n*-Pentamidomanzamine A	**19**	—/—	4.633/—	0.695/0.973	—/—
6-Pivalamidomanzamine A	**10**	—/—	—/—	1.544/3.861	—/—
8-Pivalamidomanzamine A	**20**	—/—	—/—	0.54/0.973	—/—
6-*n*-Hexamidomanzamine A	**11**	—/—	—/—	15.12/30.22	—/—
8-*n*-Hexamidomanzamine A	**21**	—/—	3.779/—	0.030/0.468	—/—
6-*n*-Octamidomanzamine A	**12**	—/—	—/—	17.40/29.01	—/—
8-*n*-Octamidomanzamine A	**22**	—/—	1.015/29.01	0.508/1.813	—/—
6-(*t*-Butyl)-acetamidomanzamine A	**13**	—/—	—/—	1.360/1.889	—/—
8-(*t*-Butyl)-acetamidomanzamine A	**23**	—/—	14.36/—	1.511/3.779	—/—
6-Cyclohexamidomanzamine A	**14**	—/—	22.27/—	1.262/1.856	—/—
8-Cyclohexamidomanzamine A	**24**	—/—	—/—	11.14/14.85	—/—
Manzamine A	**1**	3.656	1.848	0.640	
8-Hydroxymanzamine A	**2**	6.205	3.546	0.177	
Amphotericin B		0.487/1.352	0.920/2.705		1.623/2.701
Ciprofloxacin				1.056	

IC_{50}, the concentration that affords 50% inhibition of growth; minimum inhibitory concentration (MIC) is the lowest test concentration that allows no detectable growth; amphotericin B and ciprofloxacin are used as positive antifungal and antibacterial controls, respectively, '— not' active, 'NT' not tested.

than ciprofloxacin, (IC_{50} = 1.056 μM). The amides at position 6 with linear alkyl groups (**5**, **6**, **7**, **9** and **11**) showed significantly reduced activity against *M. intracellulare* with IC_{50} values of 9.911 5.650, 4.736, 2.316 and 15.12 μM, respectively. Similar results were obtained when adding branched acyclic alkyl groups such as isopropyl (**8**, IC_{50} = 3.157 μM). 6-Cyclohexamidomanzamine A (**14**) and 6-pivalamidomanzamine A (**10**) showed improved activities within the amides of the position 6 series with IC_{50} values of 1.262 and 1.544 μM, respectively. These results indicated that amidation at position 6 is not favorable for activity against *M. intracellulare.* Position 8 analogues showed better antimycobacterial potency compared to the position 6 series. The amide with acetamido (**15**) and propamido (**16**) groups at position 8 showed moderate activities with an IC_{50}'s of 1.651 and 1.517 μM, respectively. 8-*n*-Butamidomanzamine A (**17**), 8-isobutamidomanzamine A (**18**) and 8-*n*-pentamidomanzamine A (**19**) showed activities close to manzamine A (**1**) and the control with IC_{50} values of 0.868, 1.026 and 0.695 μM, respectively, while 8-*n*-octamidomanzamine A (**22**) was slightly more potent than manzamine (IC_{50} = 0.508 μM). 8-*n*-Hexamidomanzamine A (**21**) was the most potent amide with an IC_{50} of 0.030 μM, which is one order of magnitude more potent than the control as well as **1**. Marked loss of activity in

8-cyclohexamidomanzamine A (**24**, IC_{50} = 11.14 μM) suggests that linear acyclic alkyl groups as the amide functionality at position 8 are more favorable than bulkier groups, which is similar to the activity profile for antimalarial activity.

2.4.2. Bioactivity against *C. neoformans*

All the amides were screened for anticryptococcal activity against *C. neoformans*. The amides were either not active (**5**–**13** and **24**) or showed lower activity compared to **1** (IC_{50} = 1.848 μM). None of the amides were active against *C. albicans, E. coli and P. aeruginosa.*

3. Conclusion

In conclusion, 20 manzamine amides **5–24** have been synthesized and were screened in vitro for antimalarial and antibacterial activities. Amidation at positions 6 and 8 was a good choice for eliminating the toxicity associated with manzamine A, since all the amides (**5–24**) did not show cytotoxicity in Vero assay. In general, the amides were less active than **1**. Some amides such as (**14**, **17** and **21**) showed the best antimalarial activities among the amide series with IC_{50} values of 34, 34, and 32 nM against *P. falciparum*, respectively. Our structure–activity relationship study showed that linear, short alkyl groups adjacent to the amide carbonyl at position 8 are favored for antimalarial activity, while bulky and cyclic groups at position 6 appear to be the best choice for achieving better activity. Two of the most active amides, **14** and **21**, were evaluated in vivo in a *P. berghei* mouse malaria model. Oral administration of **14** and **21** at the dose of 30 mg/kg (once daily for three days) caused parasitemia suppression of 24% and 62%, respectively, with no apparent toxicity.

Most of the amides showed potent activity against *M. intracellulare.* The activity profile for the position 8 series was similar to that for the antimalarial activity profile, in which linear, slightly short alkyl groups adjacent to the amide carbonyl showed improved activity. 8-*n*-Hexamidomanzamine A (**21**) was the most potent amide with an IC_{50} of 0.030 μM which is one order of magnitude more potent than the control. The potency of **21** against *M. intracellulare* will encourage us to continue investigating this amide in animals. Position 6 analogues **5–14** were less potent than those at position 8 against *M. intracellulare*. 6-Cyclohexamidomanzamine A (**14**) and **10** showed improved activities within position 6 series with an IC_{50} of 1.485 and 1.312 μM, respectively, which was similar to the activity profile of antimalarial activity for position 6 amides.

4. Experimental section

4.1. General experimental procedures

The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker DRX NMR spectrometer operating at 400 MHz for ^1H and 75 MHz for ^{13}C. Chemical shift (δ) values are expressed in parts per million (ppm) and are referenced to the residual solvent signals of $CDCl_3$. UV and IR spectra were respectively obtained using a Perkin–Elmer Lambda 3B UV–vis spectrophotometer and an AATI Mattson Genesis series FTIR instrument. Optical rotations were measured with a JASCO DIP-310 digital polarimeter. The high resolution ESI-MS spectra were measured using a Bruker Daltonic (GmbH, Germany) micro-TOF series with electrospray ionization. TLC analysis was carried out on precoated silica gel G254 aluminum plates.

4.1.1. Nitration of manzamine A

Manzamine A (**1**) (5 g, 9.12 mmol) was dissolved in trifluoroacetic acid (TFA) (133 mL, 1.79 mmol), and kept at 0 °C with stirring for 30 min. Sodium nitrite (1 g, 14.5 mmol) was added in one portion and allowed to stir at 0 °C for an additional 3 h. The reaction mixture was poured into water and neutralized by ammonium hydroxide producing a precipitate that was filtered and dried. The crude nitro products of manzamine A (4.50 g) were loaded onto a column packed with 450 g of silica gel. 6-Nitromanzamine A (**3a**) eluted first using 99:1 DCM/MeOH followed by 8-nitromanzamine A (**3b**) after the mobile phase polarity was increased with 95:5 DCM/MeOH.

4.1.1.1. 6-Nitromanzamine A (3a). Compound **3a** (2.50 g, 46%); yellow powder; ^1H NMR ($CDCl_3$) δ 9.04 (1H, d, J = 2.0), 8.50 (1H, d, J = 5.2), 8.40 (1H, dd, J = 9.2, 2.0), 7.89 (1H, d, J = 5.2), 7.77 (1H, d, J = 9.2), 6.50 (1H, s), 6.21 (1H, s), 5.62 (m), 5.42 (t, J = 10.8), 4.70 (br), 3.69 (s), 3.25 (1H, t, J = 11.0), 2.93 (d, J = 9.0), 2.80–2.20 (m), 2.10–1.20 (m); HRESIMS m/z calcd for $C_{36}H_{44}N_5O_3$ ($M+H^+$) 594.3444, found 594.3439.

4.1.1.2. 8-Nitromanzamine A (3b). Compound **3b** (2.35 g, 43%) yellow powder; ^1H NMR ($CDCl_3$) δ 10.40 (1H, s), 8.57 (1H, d, J = 5.2), 8.48 (1H, d, J = 8.0), 8.45 (1H, d, J = 8.1), 7.87 (1H, d, J = 5.2), 7.40 (1H, t, J = 8.0), 6.45 (1H, s), 5.96 (1H, m), 5.69 (1H, m), 5.55 (1H, m), 5.32 (1H, t, J = 10.0), 4.27 (1H, br), 3.58 (1H, s), 3.11 (1H, m), 2.61 (m), 2.50–1.6 (m), 1.4 (m); HRESIMS m/z calcd for $C_{36}H_{44}N_5O_3$ ($M+H^+$) 594.3444, found 594.3439.

4.1.1.3. Reduction of nitromanzamines. 6-Nitromanzamine A (**3**) or 8-nitromanzamine A (**4**) (118.6 mg, 0.21 mmol) were dissolved in methanol (5 mL). Zinc (50.0 mg) and 5% acetic acid in methanol (5 mL) were added to the nitromanzamines solution, and the reaction mixture was stirred for 10 min at room temperature. After complete conversion of the nitro products into the corresponding amine (monitored by TLC), concd HCl was added drop wise till no further precipitate was formed. The precipitate was collected by filtration and used for the following reactions without further purification.

4.1.2. General preparation of the amide products

6-Aminomanzamine A or 8-aminomanzamine A (100 mg, 0.17 mmol) and catalytic amount of DMAP were dissolved in anhydrous THF (3 mL) under nitrogen atmosphere. Triethylamine Et_3N (25 μL, 0.17 mmol) was then added, and the mixture was stirred at room temperature for 10 min. The desired acid chloride was added in excess, and the reaction mixture was stirred for 1 h. The completion of the reaction was monitored by TLC, then the reaction was quenched with water, and the product(s) were extracted by DCM (3 × 10 mL). The organic layer was dried over anhydrous sodium sulfate, and then evaporated under reduced pressure. The crude amide derivatives were first purified by silica column chromatography using hexane/acetone (9:1). Further purification was carried out on a Phenomenex Luna C8 250 × 10 mm, 5 μm Luna reverse-phase HPLC column using gradient CH_3CN (0.1% TFA)/water (0.1% TFA) with flow rate of 6 mL/min to gave the pure amide derivative.

4.1.2.1. 6-Acetamidomanzamine A (5). Compound **5** (15 mg, 14%); $[\alpha]_D^{25}$ 12.9 (*c* 0.11, MeOH); UV λ_{max} (MeOH) 260, 310, 375 nm; IR neat: 3232 (br), 2935, 2580, 1978, 1703, 1665,1538, 1470, 1437, 1365, 1290, 1178, 1130, 1018 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.32 (1H, s), 8.65 (1H, s), 8.50 (1H, s), 8.32 (1H, d, J = 7.6), 7.79 (1H, d, J = 7.5), 7.66 (1H, d, J = 7.6), 7.36 (1H, d, J = 7.6), 6.64 (1H, s), 6.28 (1H, m), 5.57 (1H, m), 5.42 (1H, t, J = 4.0), 4.96 (1H, t, J = 8.1), 4.04 (1H, dd, J = 16.1, 8.0), 3.7 (1H, s), 3.37 (1H, t, J = 12.1), 3.04 (1H, d, J = 8.1), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.31 (m), 2.26 (m) 2.08 (s), 2.03 (m), 1.96 (m), 1.74 (m), 1.48 (m); ^{13}C NMR ($CDCl_3$) δ 175.04, 166.03, 143.57, 142.05, 141.99,

138.60, 135.78, 134.48, 133.85, 133.42, 130.20, 128.08, 125.35, 122.38, 122.12, 114.63, 112.93, 77.85, 71.70, 70.50, 57.09, 53.49, 49.32, 47.07, 44.82, 31.21, 39.16, 33.62, 28.45, 26.52, 26.36, 24.99, 24.58, 24.32, 23.97, 20.73; HRESIMS *m*/*z* calcd for $C_{38}H_{49}N_5O_2$ $(M+H)^+$ 606.3743, found 606.3784.

4.1.2.2. 6-*n*-Propamidomanzamine A (6). Compound **6** (16 mg, 14%); $[\alpha]_D^{25}$ 17.1 (*c* 0.15, MeOH); ^{1}H NMR ($CDCl_3$) δ 11.33 (1H, s), 8.66 (1H, s), 8.50 (1H, s), 8.31 (1H, d, *J* = 7.6), 7.80 (1H, d, *J* = 7.5), 7.67 (1H, d, *J* = 7.6), 7.35 (1H, d, *J* = 7.6), 6.64 (1H, s), 6.29 (1H, m), 5.56 (1H, m), 5.43 (1H, t, *J* = 4.2), 4.96 (1H, t, *J* = 8.0), 4.04 (1H, dd, *J* = 16.1, 8.0), 3.69 (1H, s), 3.37 (1H, t, *J* = 12.1), 3.04 (1H, d, *J* = 8.0), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.41 (2H, q, *J* = 7.2), 2.31 (m), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.48 (m), 1.14 (3H, t, *J* = 7.2); ^{13}C NMR ($CDCl_3$) δ 175.04, 166.03, 143.57, 142.05, 141.99, 138.60, 135.78, 134.48, 133.85, 133.42, 130.20, 128.08, 125.35, 122.38, 122.12, 114.63, 112.93, 77.85, 71.70, 70.50, 57.09, 53.49, 49.32, 47.07, 44.82, 31.21, 39.16, 33.62, 30.36, 28.45, 26.52, 26.36, 24.99, 24.58, 24.32, 20.73, 9.86; HRESIMS *m*/*z* calcd for $C_{39}H_{50}N_5O_2$ $(M+H)^+$ 620.3936, found 620.3942.

4.1.2.3. 6-*n*-Butamidomanzamine A (7). Compound **7** (16 mg, 14%); $[\alpha]_D^{25}$ 11.9 (*c* 0.11, MeOH); ^{1}H NMR ($CDCl_3$) δ 11.25 (1H, s), 8.70 (1H, s), 8.50 (1H, s), 8.30 (1H, d, *J* = 7.6), 7.75 (1H, d, *J* = 7.5), 7.66 (1H, d, *J* = 7.6), 7.36 (1H, d, *J* = 7.6), 6.62 (1H, s), 6.25 (1H, m), 5.54 (1H, m), 5.39 (1H, t, *J* = 4.0), 4.92 (1H, t, *J* = 8.0), 4.00 (1H, dd, *J* = 16.1, 8.0), 3.66 (1H, s), 3.37 (1H, t, *J* = 12.0), 3.04 (1H, d, *J* = 8.1), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.37 (2H, t, *J* = 7.0), 2.31 (m), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.48 (m), 1.00 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$) δ 171.45, 166.03, 143.69, 141.99, 141.16, 138.32, 137.62, 134.48, 133.85, 133.42, 130.20, 128.08, 125.35, 122.38, 122.12, 114.63, 112.93, 77.85, 71.70, 70.50, 57.09, 53.49, 49.32, 47.07, 44.82, 40.09, 31.21, 39.16, 33.62, 30.36, 26.52, 26.36, 24.99, 24.58, 24.32, 20.73, 19.72, 14.52; HRESIMS *m*/*z* calcd for $C_{40}H_{52}N_5O_2$ $(M+H)^+$ 634.4129, found 634.4425.

4.1.2.4. 6-Isobutamidomanzamine A (8). Compound **8** (14 mg, 12%); $[\alpha]_D^{25}$ 20.5 (*c* 0.21, MeOH); ^{1}H NMR ($CDCl_3$) δ 11.41 (1H, s), 8.57 (1H, s), 8.50 (1H, s), 8.30 (1H, d, *J* = 7.6), 7.80 (1H, d, *J* = 7.5), 7.69 (1H, d, *J* = 7.6), 7.35 (1H, d, *J* = 7.6), 6.59 (1H, s), 6.30 (1H, m), 5.57 (1H, m), 5.42 (1H, t, *J* = 4.0), 4.96 (1H, t, *J* = 8.1), 4.04 (1H, dd, *J* = 16.1, 8.0), 3.7 (1H, s), 3.37 (1H, t, *J* = 12.0), 3.04 (1H, d, *J* = 8.1), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.63 (m), 2.31 (m), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.48 (m), 1.29 (6H, d, *J* = 7.2); ^{13}C NMR ($CDCl_3$) δ 175.04, 166.03, 143.57, 142.05, 141.99, 138.60, 135.78, 134.48, 133.85, 133.42, 130.20, 128.08, 125.35, 122.38, 122.12, 114.63, 112.93, 77.85, 71.70, 70.50, 57.09, 53.49, 49.32, 47.07, 44.82, 40.9, 31.21, 39.16, 35.64, 33.62, 26.52, 26.36, 24.99, 24.58, 24.32, 20.73, 20.06, 19.86; HRESIMS *m*/*z* calcd for $C_{40}H_{52}N_5O_2$ $(M+H)^+$ 634.4129, found 634.4045.

4.1.2.5. 6-*n*-Pentamidomanzamine A (9). Compound **9** (15 mg, 13%); $[\alpha]_D^{25}$ 9.9 (*c* 0.15, MeOH); ^{1}H NMR ($CDCl_3$) δ 11.32 (1H, s), 8.65 (1H, s), 8.50 (1H, s), 8.32 (1H, d, *J* = 7.6), 7.79 (1H, d, *J* = 7.5), 7.66 (1H, d, *J* = 7.6), 7.36 (1H, d, *J* = 7.6), 6.64 (1H, s), 6.28 (1H, m), 5.57 (1H, m), 5.42 (1H, t, *J* = 4.1), 4.96 (1H, t, *J* = 8.0), 4.04 (1H, dd, *J* = 16.0, 8.1), 3.7 (1H, s), 3.37 (1H, t, *J* = 12.1), 3.04 (1H, d, *J* = 8.0), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.39 (2H, t, *J* = 7.2), 2.31 (m), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.48 (m), 0.99 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$) δ 175.04, 166.03, 143.57, 142.05, 141.99, 138.60, 135.78, 134.48, 133.85, 133.42, 130.20, 128.08, 125.35, 122.38, 122.12, 114.63, 112.93, 77.85, 71.70, 70.50, 57.09, 53.49, 49.32, 47.07, 44.82, 31.21, 39.16, 37.47, 27.83, 33.62, 30.36, 29.68, 26.52, 26.36, 24.99, 24.58, 24.32, 22.44, 20.87, 13.84; HRESIMS *m*/*z* calcd for $C_{41}H_{54}N_5O_2$ $(M+H)^+$ 648.4277, found 648.4550.

4.1.2.6. 6-Pivalamidomanzamine A (10). Compound **10** (14 mg, 12%); $[\alpha]_D^{25}$ 31.6 (*c* 0.21, MeOH); ^{1}H NMR ($CDCl_3$) δ 11.32 (1H, s), 8.65 (1H, s), 8.50 (1H, s), 8.32 (1H, d, *J* = 7.6), 7.79 (1H, d, *J* = 7.5), 7.66 (1H, d, *J* = 7.6), 7.36 (1H, d, *J* = 7.6), 6.64 (1H, s), 6.28 (1H, m), 5.57 (1H, m), 5.42 (1H, t, *J* = 4.0), 4.96 (1H, t, *J* = 8.1), 4.04 (1H, dd, *J* = 16.0, 8.0), 3.7 (1H, s), 3.37 (1H, t, *J* = 12.1), 3.04 (1H, d, *J* = 8.0), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.31 (m), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.49 (9H, s), 1.48 (m); ^{13}C NMR ($CDCl_3$) δ 175.04, 166.03, 143.57, 142.05, 141.99, 138.60, 135.78, 134.48, 133.85, 133.42, 130.20, 128.08, 125.35, 122.38, 122.12, 114.63, 112.93, 77.85, 71.70, 70.50, 57.09, 53.49, 49.32, 47.07, 44.82, 39.31, 39.16, 33.62, 29.69, 28.45, 26.52, 26.36, 24.99, 24.58, 24.32, 20.73; HRESIMS *m*/*z* calcd for $C_{41}H_{54}N_5O_2$ $(M+H)^+$ 648.4277, found 648.4550.

4.1.2.7. 6-*n*-Hexamidomanzamine A (11). Compound **11** (17 mg, 14%); $[\alpha]_D^{25}$ 36.1 (*c* 0.25, MeOH); ^{1}H NMR ($CDCl_3$) δ 11.32 (1H, s), 8.65 (1H, s), 8.50 (1H, s), 8.32 (1H, d, *J* = 7.6), 7.79 (1H, d, *J* = 7.5), 7.66 (1H, d, *J* = 7.6), 7.36 (1H, d, *J* = 7.6), 6.64 (1H, s), 6.28 (1H, m), 5.57 (1H, m), 5.42 (1H, t, *J* = 4.0), 4.96 (1H, t, *J* = 8.1), 4.04 (1H, dd, *J* = 16.0, 8.0), 3.7 (1H, s), 3.37 (1H, t, *J* = 12.1), 3.04 (1H, d, *J* = 8.0), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.56 (2H, t, *J* = 7.1), 2.31 (m), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.48 (m), 0.94 (3H, t, *J* = 7.0); ^{13}C NMR ($CDCl_3$) δ 175.39, 166.03, 143.57, 142.05, 141.99, 138.60, 135.78, 134.48, 133.85, 133.42, 130.20, 128.08, 125.35, 122.38, 122.12, 114.63, 112.93, 77.85, 71.70, 70.50, 57.09, 53.49, 49.32, 47.07, 44.82, 31.21, 39.16, 37.47, 32.11, 29.68, 27.12, 26.78, 26.40, 25.54, 25.28, 24.90, 23.05, 21.34, 14.22; HRESIMS *m*/*z* calcd for $C_{42}H_{56}N_5O_2$ $(M+H)^+$ 662.4488, found 662.4448.

4.1.2.8. 6-*n*-Octamidomanzamine A (12). Compound **12** (15 mg, 12%); $[\alpha]_D^{25}$ 25.4 (*c* 0.12, MeOH); ^{1}H NMR ($CDCl_3$) δ 11.32 (1H, s), 8.65 (1H, s), 8.50 (1H, s), 8.32 (1H, d, *J* = 7.6), 7.79 (1H, d, *J* = 7.5), 7.66 (1H, d, *J* = 7.6), 7.36 (1H, d, *J* = 7.6), 6.64 (1H, s), 6.28 (1H, m), 5.57 (1H, m), 5.42 (1H, t, *J* = 4.0), 4.96 (1H, t, *J* = 8.1), 4.04 (1H, dd, *J* = 16.0, 8.1), 3.7 (1H, s), 3.37 (1H, t, *J* = 12.0), 3.04 (1H, d, *J* = 8.1), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.46 (2H, t, *J* = 7.2), 2.31 (m), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.48 (m), 0.90 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$) δ 176.14, 166.69, 143.57, 142.05, 141.99, 138.60, 135.78, 134.48, 133.85, 133.42, 130.20, 128.08, 125.35, 122.38, 122.12, 114.63, 112.93, 77.85, 71.70, 70.50, 57.09, 53.49, 49.32, 47.07, 44.82, 38.60, 37.47, 31.61, 29.68, 28.17, 26.78, 26.40, 25.54, 25.28, 24.90, 22.82, 21.34, 14.47; HRESIMS *m*/*z* calcd for $C_{44}H_{60}N_5O_2$ $(M+H)^+$ 690.4732, found 690.4785.

4.1.2.9. 6-(*t*-Butyl)-acetamidomanzamine A (13). Compound **13** (18 mg, 15%); $[\alpha]_D^{25}$ 21.5 (*c* 0.10, MeOH); ^{1}H NMR ($CDCl_3$) δ 11.32 (1H, s), 8.65 (1H, s), 8.50 (1H, s), 8.32 (1H, d, *J* = 7.6), 7.79 (1H, d, *J* = 7.5), 7.66 (1H, d, *J* = 7.6), 7.36 (1H, d, *J* = 7.6, 6.64 (1H, s), 6.28 (1H, m), 5.57 (1H, m), 5.42 (t, *J* = 4.0), 4.96 (1H, t, *J* =8.0), 4.04 (1H, dd, *J* = 16.0, 8.1), 3.7 (1H, s), 3.37 (1H, t, *J* = 12.1), 3.04 (1H, d, *J* = 8.0), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.24 (2H, s), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.02 (9H, s); ^{13}C NMR ($CDCl_3$) δ 175.89, 166.03, 143.51, 142.09, 142.01, 138.60, 135.78, 134.48, 133.85, 133.42, 130.20, 128.08, 125.35, 122.38, 122.12, 114.63, 112.93, 77.85, 71.70, 70.50, 57.09, 53.49, 49.32, 47.07, 44.82, 39.3, 33.62, 31.65, 29.69, 28.80, 24.99, 24.58, 24.32, 20.73; HRESIMS *m*/*z* calcd for $C_{42}H_{56}N_5O_2$ $(M+H)^+$ 662.4425, found 662.4451.

4.1.2.10. 6-Cyclohexamidomanzamine A (14). Compound **14** (20 mg, 17%); $[\alpha]_D^{25}$ 18.5 (*c* 0.13, MeOH); ^{1}H NMR ($CDCl_3$) δ 11.33 (1H, s), 9.02 (1H, s), 8.53 (1H, s), 8.22 (1H, d, *J* = 7.6), 7.76 (1H, d,

J =7.5), 7.56 (1H, d, *J* = 7.6), 7.46 (1H, d, *J* = 7.6), 6.68 (1H, s), 6.08 (1H, m), 5.51 (1H, m), 5.42 (1H, t, *J* = 4.0), 4.77 (1H, t, *J* = 8.0), 3.84 (1H, dd, *J* = 16.0, 8.1), 3.66 (1H, s), 3.26 (1H, t, *J* = 12.0), 2.91 (1H, d, *J* = 8.1), 2.78 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.31 (m), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.48 (m); ^{13}C NMR ($CDCl_3$) δ 175.04, 166.03, 143.57, 142.05, 141.99, 138.60, 135.78, 134.48, 133.85, 133.42, 130.20, 128.08, 125.35, 122.38, 122.12, 114.63, 112.93, 77.85, 71.70, 70.50, 57.09, 53.49, 49.32, 47.07, 44.82, 39.16, 33.62, 28.45, 26.52, 26.36, 24.99, 24.58, 24.32, 23.97, 20.73; HRESIMS *m*/*z* calcd for $C_{43}H_{56}N_5O_2$ $(M+H)^+$ 674.4434, found 674.4464.

4.1.2.11. 8-Acetamidomanzamine A (15). Compound **15** (14 mg, 13%); $[\alpha]_D^{25}$ 16.2 (*c* 0.15, MeOH); UV λ_{max} (MeOH) 260, 310, 375 nm; IR neat: 3232, 2935, 2580, 1703, 1665, 1538, 1470, 1437, 1365, 1290, 1178, 1130, 1018 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 11.37 (1H, s), 8.52 (1H, d, *J* = 8.0), 8.33 (1H, d, *J* = 7.6), 7.82 (1H, d, *J* = 7.5), 7.79 (1H, d, *J* = 7.6), 7.23 (1H, d, *J* = 7.6), 6.64 (1H, s), 6.28 (1H, m), 5.57 (1H, m), 5.42 (1H, t, *J* = 4.0), 4.96 (1H, t, *J* = 8.0), 4.04 (1H, dd, *J* = 16.0, 8.1) 3.7 (1H, s), 3.37 (1H, t, *J* = 12.1), 3.04 (1H, d, *J* = 8.0), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.31 (m), 2.26 (m) 2.08 (3H, s), 2.03 (m), 1.96 (m), 1.74 (m), 1.48 (m); ^{13}C NMR ($CDCl_3$) δ 174.04, 166.01, 143.27, 141.95, 141.80, 138.64, 136.00, 134.49, 133.85, 133.42, 130.20, 128.08, 125.35, 122.38, 122.12, 114.63, 112.93, 77.85, 71.70, 70.50, 57.09, 53.49, 49.32, 47.07, 44.82, 31.21, 39.16, 33.62, 28.45, 26.52, 26.36, 24.99, 24.58, 24.32, 23.97, 20.73; HRESIMS *m*/*z* calcd for $C_{38}H_{49}N_5O_2$ $(M+H)^+$ 606.3843, found 606.3811.

4.1.2.12. 8-*n*-Propamidomanzamine A (16). Compound **16** (15 mg, 14%); $[\alpha]_D^{25}$ 6.1 (*c* 0.09, MeOH);); ^{1}H NMR ($CDCl_3$) δ 11.38 (1H, s), 8.51 (1H, d, *J* = 8.0), 8.36 (1H, d, *J* = 7.6), 7.80 (1H, d, *J* = 7.5), 7.78 (1H, d, *J* = 7.6), 7.26 (1H, d, *J* = 7.6), 6.68 (1H, s), 6.29 (1H, m), 5.56 (1H, m), 5.43 (1H, t, *J* = 4.0), 4.96 (1H, t, *J* = 8.1), 4.04 (1H, dd, *J* = 16.1, 8.0) 3.69 (1H, s), 3.37 (1H, t, *J* = 12.0), 3.04 (1H, d, *J* = 8.0), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.41 (2H, q, *J* = 7.0), 2.31 (m), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.48 (m), 1.14 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$) δ 174.84, 166.09, 143.51, 142.25, 142.12, 138.62, 135.78, 134.48, 133.85, 133.42, 130.20, 128.08, 125.35, 122.38, 122.12, 114.63, 112.93, 77.85, 71.70, 70.50, 57.09, 53.49, 49.32, 47.07, 44.82, 31.21, 39.16, 33.62, 30.36, 28.45, 26.52, 26.36, 24.99, 24.58, 24.32, 20.78, 10.01; HRESIMS *m*/*z* calcd for $C_{39}H_{50}N_5O_2$ $(M+H)^+$ 620.3936, found 620.3981.

4.1.2.13. 8-*n*-Butamidomanzamine A (17). Compound **17** (16 mg, 14%); $[\alpha]_D^{25}$ 13.2 (*c* 0.12, MeOH); ^{1}H NMR ($CDCl_3$) δ 11.25 (1H, s), 8.70 (1H, s), 8.51 (1H, d, *J* = 8.2), 8.36 (1H, d, *J* = 7.6), 7.80 (1H, d, *J* = 7.5), 7.78 (1H, d, *J* = 7.6), 7.26 (1H, d, *J* = 7.6), 6.62 (1H, s), 6.25 (1H, m), 5.54 (1H, m), 5.39 (1H, t, *J* = 4.0), 4.92 (1H, t, *J* = 8.0), 4.00 (1H, dd, *J* = 16.1, 8.0), 3.66 (1H, s), 3.37 (1H, t, *J* = 12.0), 3.04 (1H, d, *J* = 8.1), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.37 (2H, t, *J* = 7.2), 2.31 (m), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.48 (m), 1.00 (3H, t, *J* = 7.2); ^{13}C NMR ($CDCl_3$) δ 173.41, 143.82, 142.97, 141.90, 138.48, 135.17, 133.45, 133.29, 132.45, 130.42, 130.01, 127.30, 125.15, 123.99, 122.87, 120.67, 116.50, 114.32, 78.41, 71.70, 70.74, 63.42, 57.67, 53.88, 49.65, 47.45, 45.03, 41.44, 39.48, 34.05, 32.26, 30.36, 27.41, 26.36, 24.99, 24.58, 24.32, 20.73, 19.76, 14.42; HRESIMS *m*/*z* calcd for $C_{40}H_{52}N_5O_2$ $(M+H)^+$ 634.4011, found 634.4101.

4.1.2.14. 8-Isobutamidomanzamine A (18). Compound **18** (16 mg, 14%); $[\alpha]_D^{25}$ 14.2 (*c* 0.10, MeOH); ^{1}H NMR ($CDCl_3$) δ 11.41 (1H, s), 8.50 (1H, d, *J* = 8.0), 8.32 (1H, d, *J* = 8.1), 7.78 (1H, d, *J* = 7.9), 7.77 (1H, d, *J* = 7.8), 7.29 (1H, d, *J* = 7.6), 6.67 (1H, s), 6.30 (1H, m), 5.57 (1H, m), 5.42 (1H, t, *J* = 4.2), 4.96 (1H, t, *J* = 8.1), 4.04 (1H, dd, *J* = 16.0, 8.1), 3.7 (1H, s), 3.37 (1H, t, *J* = 12.1), 3.04 (1H, d, *J* = 8.0), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.63 (m), 2.31 (m), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.48 (m), 1.29 (6H, d, *J* = 7.2); ^{13}C NMR ($CDCl_3$) δ 172.35, 143.57, 142.57, 142.14, 138.19, 135.87, 133.91, 133.78, 133.04, 129.86, 128.13, 126.75, 123.60, 120.36, 116.20, 114.02, 77.97, 71.70, 70.33, 57.35, 53.50, 49.25, 47.07, 44.63, 40.9, 39.16, 35.64, 33.75, 29.68, 28.36, 25.00, 24.34, 20.74, 20.06, 19.86; HRESIMS *m*/*z* calcd for $C_{40}H_{52}N_5O_2$ $(M+H)^+$ 634.4011, found 634.4095.

4.1.2.15. 8-*n*-Pentamidomanzamine A (19). Compound **19** (17 mg,15%); $[\alpha]_D^{25}$ 17.2 (*c* 0.16, MeOH); ^{1}H NMR ($CDCl_3$) δ 11.29 (1H, s), 8.51 (1H, d, *J* = 8.0), 8.34 (1H, d, *J* = 8.2), 7.80 (1H, d, *J* = 7.9), 7.77 (1H, d, *J* = 7.8), 7.24 (1H, d, *J* = 7.6), 6.54 (1H, s), 6.28 (1H, m), 5.57 (1H, m), 5.42 (1H, t, *J* = 4.1), 4.96 (1H, t, *J* = 8.0), 4.04 (1H, dd, *J* = 16.1, 8.0), 3.7 (1H, s), 3.37 (1H, t, *J* = 12.0), 3.04 (1H, d, *J* = 8.1), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.39 (2H, t, *J* = 7.1), 2.31 (m), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.48 (m), 0.99 (3H, t, *J* = 7.2); ^{13}C NMR ($CDCl_3$) δ 172.97, 143.57, 142.60, 141.99, 138.18, 135.78, 134.88, 133.85, 133.42, 130.02, 127.22, 125.35, 123.63, 119.65, 116.18, 114.01, 78.07, 71.70, 70.40, 57.33, 53.53, 49.30, 47.07, 44.72, 41.08, 39.08, 37.07, 33.72, 31.91, 30.36, 29.68, 26.51, 26.36, 25.01, 24.59, 24.34, 22.67, 22.48, 20.75, 13.97; HRESIMS *m*/*z* calcd for $C_{41}H_{54}N_5O_2$ $(M+H)^+$ 648.4277, found 648.4281.

4.1.2.16. 8-Pivalamidomanzamine A (20). Compound **20** (15 mg, 12%); $[\alpha]_D^{25}$ 17.2 (*c* 0.11, MeOH); ^{1}H NMR ($CDCl_3$) δ 11.40 (1H, s), 8.40 (1H, d, *J* = 8.1), 8.38 (1H, d, *J* = 8.0), 7.84 (1H, d, *J* = 7.9), 7.82 (1H, d, *J* = 7.8), 7.24 (1H, d, *J* = 7.6), 6.78 (1H, s), 6.26 (1H, m), 5.58 (1H, m), 5.36 (1H, t, *J* = 4.0), 4.85 (1H, t, *J* = 8.1), 4.04 (1H, dd, *J* = 16.0, 8.1), 3.67 (1H, s), 3.21 (1H, t, *J* = 12.1), 3.04 (1H, d, *J* = 8.1), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.31 (m), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.49 (9H, s), 1.48 (m); ^{13}C NMR ($CDCl_3$) δ 175.04, 166.03, 143.57, 142.05, 141.99, 138.27, 135.04, 134.48, 133.85, 133.86, 131.87, 127.11, 125.35, 123.99, 121.91, 116.86, 113.96, 77.23, 71.70, 70.52, 58.36, 57.11, 53.56, 49.37, 47.07, 44.59, 39.29, 38.74, 33.77, 32.76, 29.69, 28.45, 26.71, 26.36, 25.61, 24.58, 24.44, 22.68, 20.80, 14.03; HRESIMS *m*/*z* calcd for $C_{41}H_{54}N_5O_2$ $(M+H)^+$ 648.4277, found 648.4268.

4.1.2.17. 8-*n*-Hexamidomanzamine A (21). Compound **21** (16 mg, 14%); $[\alpha]_D^{25}$ 21.1 (*c* 0.11, MeOH); ^{1}H NMR ($CDCl_3$) δ 11.22 (1H, s), 8.72 (1H, d, *J* = 8.0), 8.45 (1H, d, *J* = 8.0), 7.99 (1H, d, *J* = 7.9), 7.91 (1H, d, *J* = 7.8), 7.27 (1H, d, *J* = 7.6), 6.78 (1H, s), 6.12 (1H, m), 5.53 (1H, m), 5.16 (1H, t, *J* = 4.0), 4.88 (1H, t, *J* = 8.1), 4.04 (1H, dd, *J* = 16.1, 8.0) 3.79 (1H, s), 3.41 (1H, t, *J* = 12.0), 2.94 (1H, d, *J* = 8.0), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.56 (2H, t, *J* = 7.4), 2.31 (m), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.48 (m), 0.94 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$) δ 173.50, 164.75, 142.69, 142.28, 142.10, 139.23, 133.93, 132.38, 130.65, 126.01, 124.70, 123.46, 119.50, 117.24, 114.89, 77.47, 71.57, 70.07, 58.06, 53.77, 53.38, 49.61, 47.18, 44.99, 40.87, 40.26, 37.56, 34.60, 30.11, 29.73, 29.53, 28.73, 27.12, 26.78, 26.40, 25.54, 24.90, 23.05, 21.34, 14.22; HRESIMS *m*/*z* calcd for $C_{42}H_{56}N_5O_2$ $(M+H)^+$ 662.4488, found 662.4521.

4.1.2.18. 8-*n*-Octamidomanzamine A (22). Compound **22** (18 mg, 15%); $[\alpha]_D^{25}$ 19.9 (*c* 0.11, MeOH); ^{1}H NMR ($CDCl_3$) δ 11.32 (1H, s), 8.61 (1H, d, *J* = 8.1), 8.52 (1H, d, *J* = 8.0), 7.87 (1H, d, *J* = 7.9), 7.84 (1H, d, *J* = 7.8), 7.23 (1H, d, *J* = 7.6), 6.69 (1H, s), 6.28 (1H, m), 5.57 (1H, m), 5.42 (1H, t, *J* = 4.0), 4.96 (1H, t, *J* = 8.1), 4.04 (1H, dd, *J* = 16.1, 8.0), 3.7 (1H, s), 3.37 (1H, t, *J* = 12.1), 3.04 (1H, d, *J* = 8.0), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.46 (2H, t, *J* = 7.1), 2.31 (m), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.48 (m), 0.90 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$) δ 173.54, 164.54, 142.87, 142.25, 142.04, 138.60, 136.12, 134.48, 133.85, 133.42, 130.20,

128.08, 125.35, 122.38, 122.12, 116.26, 114.33, 77.85, 71.70, 70.50, 57.09, 53.49, 49.32, 47.07, 44.82, 38.60, 37.47, 31.61, 29.68, 28.17, 26.78, 26.40, 25.54, 25.28, 24.94, 22.45, 21.34, 14.51; HRESIMS *m*/*z* calcd for $C_{44}H_{60}N_5O_2$ $(M+H)^+$ 690.4732, found 690.4772.

4.1.2.19. 8-(*t*-Butyl)-acetamidomanzamine A (23). Compound **23** (16 mg, 14%); $[\alpha]_D^{25}$ 25.7 (*c* 0.12, MeOH ^{1}H NMR ($CDCl_3$) δ 11.32 (1H, s), 8.63 (1H, d, *J* = 8.0), 8.51 (1H, d, *J* = 8.1), 7.88 (1H, d, *J* = 7.9), 7.86 (1H, d, *J* = 7.8), 7.27 (1H, d, *J* = 7.6), 6.71 (1H, s), 6.28 (1H, m), 5.57 (1H, m), 5.42 (1H, t, *J* = 4.0), 4.96 (1H, t, *J* = 8.1), 4.04 (1H, dd, *J* = 16.0, 8.1), 3.7 (1H, s), 3.37 (1H, t, *J* = 12).1, 3.04 (d, *J* = 8), 2.95 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.24 (2H, s), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.02 (9H, s); ^{13}C NMR ($CDCl_3$) δ 174.15, 164.43, 143.59, 142.41, 142.01, 138.27, 135.04, 134.48, 133.85, 132.86, 131.87, 127.11, 123.99, 122.38, 121.91, 116.86, 113.96, 77.23, 70.52, 58.35, 57.11, 53.56, 52.92, 49.37, 45.52, 44.59, 39.29, 33.77, 32.76, 29.69, 27.86, 26.71, 26.48, 25.10, 24.72, 24.44, 22.68, 20.80; HRESIMS *m*/*z* calcd for $C_{42}H_{56}N_5O_2$ $(M+H)^+$ 662.4425, found 662.4478.

4.1.2.20. 8-Cyclohexamidomanzamine A (24). Compound **24** (19 mg, 16%); $[\alpha]_D^{25}$ 19.5 (*c* 0.17, MeOH); ^{1}H NMR ($CDCl_3$) δ 11.30 (s), 9.52 (s), 8.49 (1H, d, *J* = 8.0), 8.33 (1H, d, *J* = 8.1), 7.79 (1H, d, *J* = 7.9), 7.76 (1H, d, *J* = 7.8), 7.20 (1H, d, *J* = 7.6), 6.55 (s), 6.28 (m), 5.55 (m), 5.39 (1H, t, *J* = 4.0), 4.90 (1H, t, *J* = 8.0), 4.02 (1H, dd, *J* = 16.1, 8.0), 3.72 (s), 3.26 (1H, t, *J* = 12.0), 2.91 (1H, d, *J* = 8.0), 2.78 (m), 2.77 (m), 2.61 (m), 2.43 (m), 2.31 (m), 2.26 (m), 2.03 (m), 1.96 (m), 1.74 (m), 1.48 (m); ^{13}C NMR ($CDCl_3$) δ 174.12, 164.03, 143.57, 142.52, 141.99, 138.13, 134.92, 134.48, 133.85, 133.00, 130.86, 128.78, 126.79, 123.64, 120.29, 119.60, 116.05, 113.97, 77.96, 77.23, 71.70, 70.48, 58.32, 57.34, 53.54, 53.39, 49.31, 45.70, 44.78, 40.98, 39.11, 33.73, 31.90, 29.81, 29.75, 29.67, 29.33, 28.48, 26.48, 26.41, 25.68, 25.64, 25.00, 24.59, 24.28, 22.66, 20.75; HRESIMS *m*/*z* calcd for $C_{43}H_{56}N_5O_2$ $(M+H)^+$ 674.4434, found 674.4432.

4.2. In vitro antimalarial and antimicrobial activities

The detailed materials and methods used for in vitro antimalarial and in vitro antimicrobial assays were reported elsewhere.[19]

4.3. In vivo antimalarial activity

The in vivo antimalarial activity of the compounds was determined in mice infected with *P. berghei* (NK-65 strain). Male mice (Swiss Webster strain) weighing 18–20 g were intraperitoneally inoculated with 2×10^7 parasitized red blood cells obtained from a highly infected donor mouse. Mice were divided into different groups with 5 mice in each group. Test compounds were prepared in DMSO/0.1 N HCl/Tween-80/PEG-400/water (5:1:0.5:40:53.50) and administered orally to the mice about 2 h after the infection (day 0). The compounds were tested at three doses of 3.3, 10 and 30 mg/kg body weight. The test compounds were administered to the mice once a day for three consecutive days (days 0–3). A control group was treated with equal volume of vehicle and another control group was treated with chloroquine (10 mg/kg). The mice were closely observed after every dose for any apparent signs of toxicity. Blood smears were prepared on different days (till day 28 post infection) by clipping the tail end, stained with Giemsa and observed under microscope for determination of the parasitemia. Suppression in development of parasitemia was monitored on day 5 and day 7 post infection. Mice without parasitemia until day 28 post infection were considered as cured. Treatment of mice with three doses of chloroquine caused 100% suppression of the parasitemia.

Acknowledgments

This work was supported by the NIH (NCRR P20 RR021929, C06 RR1450301, NIAID AI 27094, R-01 AI 36596-12). We would like to thank Shabana Khan and John Trott for in vitro antimalarial evaluation. Also, Melissa R. Jacob and Marsha Wright for in vitro antimicrobial evaluation, and Rajnish Sahu for in vivo antimalarial evaluation. The biological screening was partially supported by the United States Department of Agriculture, Agricultural Research Service Specific Cooperative Agreement No. 58-6408-2-0009, and NIH, NIAID, Division of AIDS, Grant No. AI 27094. A.E.W. would like to thank The Ministry of Higher Education of Egypt for a predoctoral fellowship and Triton BioPharma AG for a Triton fellowship.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.09.026.

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