Journal of Medicinal Chemistry Article

*J. Med. Chem.* **2010,** *53,* 61–76 **61**
DOI: 10.1021/jm900672t

# Structure−Activity Relationship and Mechanism of Action Studies of Manzamine Analogues for the Control of Neuroinflammation and Cerebral Infections

Jiangnan Peng,[†] Sucheta Kudrimoti,[†] Sivaprakasam Prasanna,[‡,○] Srinivas Odde,[‡] Robert J. Doerksen,[‡,§] Hari K Pennaka,[†] Yeun-Mun Choo,[†] Karumanchi V. Rao,[†] Babu L. Tekwani,[§] Vamsi Madgula,[§] Shabana I. Khan,[†,§] Bin Wang,[†] Alejandro M. S. Mayer,[∥] Melissa R. Jacob,[§] Lan Chun Tu,[⊥] Jürg Gertsch,[#,▽] and Mark T. Hamann*[,†,§]

[†]*Department of Pharmacognosy,* [‡]*Department of Medicinal Chemistry, and* [§]*National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, Oxford, Mississippi 38677,* [∥]*Department of Pharmacology, Chicago College of Osteopathic Medicine, Midwestern University, Downers Grove, Illinois 60515,* [⊥]*Institute of Biochemistry, National Yang-Ming University, Taipei, Taiwan, Republic of China,* [#]*Department of Chemistry and Applied Biosciences, ETH Zurich, Ch-8093 Zurich, Switzerland,* [▽]*Institute of Biochemistry and Molecular Medicine, University of Bern, CH-3012 Bern, Switzerland.* [○]*Current address: Computer-Aided Drug Design, Bristol-Myers Squibb Co., Wallingford, CT.*

*Received May 20, 2009*

Structure−activity relationship studies were carried out by chemical modification of manzamine A (**1**), 8-hydroxymanzamine A (**2**), manzamine F (**14**), and ircinal isolated from the sponge *Acanthostrongylophora*. The derived analogues were evaluated for antimalarial, antimicrobial, and antineuroinflammatory activities. Several modified products exhibited potent and improved in vitro antineuroinflammatory, antimicrobial, and antimalarial activity. **1** showed improved activity against malaria compared to chloroquine in both multi- and single-dose in vivo experiments. The significant antimalarial potential was revealed by a 100% cure rate of malaria in mice with one administration of 100 mg/kg of **1**. The potent antineuroinflammatory activity of the manzamines will provide great benefit for the prevention and treatment of cerebral infections (e.g., *Cryptococcus* and *Plasmodium*). In addition, **1** was shown to permeate across the blood−brain barrier (BBB) in an in vitro model using a MDR-MDCK monolayer. Docking studies support that **2** binds to the ATP-noncompetitive pocket of glycogen synthesis kinase-3$\beta$ (GSK-3$\beta$), which is a putative target of manzamines. On the basis of the results presented here, it will be possible to initiate rational drug design efforts around this natural product scaffold for the treatment of several different diseases.

## Introduction

Marine invertebrates have been recognized as an important source of pharmacologically unique bioactive natural products. Apart from human medicines, the research involving marine natural products in the last three decades has also generated significant discoveries that are now utilized routinely as pharmacological tools with unique cellular targets. Some of them have become indispensable tools in biochemical research and played significant roles in the recent advancements of life sciences. In our search for bioactive compounds against infectious and neurological diseases, we have focused on marine alkaloids isolated from sponges and in particular the manzamine alkaloids.

Manzamines are characterized by a unique 5-, 6-, 6-, 8-, 13-membered heterocyclic ring system coupled to a $\beta$-carboline moiety. The first representative of this class, manzamine A (**1**), was isolated from the marine sponge *Haliclona* sp. collected near Manzamo Island by Higa and co-workers in 1986 and its structure including absolute configuration was established by X-ray diffraction.[1] In recent years, the manzamines have been regarded as an interesting group of marine alkaloids with extraordinary biological activities, and as a result the molecules have received considerable attention for their chemistry and pharmacology. To date, over 80 manzamine related alkaloids have been isolated from more than 16 species of marine sponges belonging to five families distributed from the Red Sea to Indonesia, which suggested a potential microbial origin of manzamine alkaloids.[2,3] The manzamine alkaloids have shown a variety of bioactivities including antimicrobial,[4−7] antiparasitic,[8] cytotoxic,[1,9] antineuroinflammatory,[10−13] and pesticidal.[14]

The greatest potential for the manzamine alkaloids appears to be against malaria and neuroinflammation, which is an interesting combination and may make it possible to treat both the infection and the symptoms with a single drug. The most effective and widely used antimalarial drugs are those derived from quinine and artemisinin, which are natural products from terrestrial plants. Our previous studies showed that the marine natural products **1** and 8-hydroxymanzamine A (**2**) exhibited improved potency against the malarial parasites both in vitro and in vivo relative to chloroquine and artemisinin.[15] A remarkable aspect of **1** is its ability to prolong the survival of highly parasitemic mice with significant recovery after a single injection. Oral administration of **1** (2 × 100 $\mu$mol/kg) and **2** (2 × 100 $\mu$mol/kg) produced a significant reduction (90%) in parasitemia. All mice treated with a single dose (50 or 100 $\mu$mol/kg) of **1** or **2** showed significant improvements in survival times over mice treated with chloroquine or artemisinin.[16] Significant variation of antimalarial

*To whom correspondence should be addressed. Phone: 662-9155730. Fax: 662-915697. E-Mail: mthamann@olemiss.edu.



Published on Web 12/17/2009

activity among naturally isolated manzamines such as manzamine F (**14**) and ircinal A (**19**) provided some interesting information regarding the structure−activity relationship of these alkaloids.[4,7,12,15−17]

Manzamines have also been reported to have antimicrobial activities against a number of bacteria and fungi.[3] In particular, most naturally isolated manzamines were active against *Mycobacterium tuberculosis*.[15,16,18] Compounds **1** and **2** showed 98−99% inhibition of *Mycobacterium tuberculosis*, with MIC end points of 1.56 and 0.91 $\mu$g/mL, respectively.[7] **1** showed antimicrobial activity against *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus*,[7,12,17] with $IC_{50}$ values of 0.5 and 0.7 $\mu$g/mL, respectively.[19]

Several manzamines were reported to modulate the generation of $O_2^-$ and $TXB_2$[a] generated by activated rat neonatal microglia.[12,13] **1** was the most potent inhibitor and did not show in vitro toxicity to microglia, suggesting that it is a good candidate for treatment of neuroinflammatory diseases.[10,11]

Recently, **1** and related derivatives have been identified as a new class of GSK-3$\beta$ inhibitors.[20] Additionally, **1** was shown to be effective in decreasing tau hyperphosphorylation in human neuroblastoma cell lines, a demonstration of its ability to enter cells and to interfere with tau pathology. Inhibition studies of **1** against five different kinases related to GSK-3$\beta$ including CDK-1, PKA, CDK-5, MAPK, and GSK-3$\alpha$ showed that manzamine A specifically inhibited GSK-3$\beta$ and CDK-5,[20] the two kinases involved in tau pathological hyperphosphorylation.[21] These results suggest that the manzamines may represent a new class from which more potent and selective GSK-3$\beta$/CDK5 inhibitors could be designed as potential therapeutic agents for Alzheimer's disease. In addition, these results suggest that a group of closely related kinases may be responsible for the diverse activity seen for this class and that targeting *Mycobacterium tuberculosis* (Mtb) and plasmodial kinases may indeed be a viable approach to drug discovery and development for Mtb and malaria.

The unique structure and extraordinary bioactivity of manzamine alkaloids has attracted considerable interest from synthetic chemists due to a challenging structure for total synthesis.[22−24] Methodology studies toward the synthesis of manzamine structural units have also been reported,[25−28] and a group of simplified analogues of **1** and manzamine C were synthesized.[29−31] The simplified manzamine C products showed equal or more potent cytotoxicity than the natural manzamine C, while simplified **1** products showed significantly reduced antimalarial activity.

Modification of **1** using olefin metathesis has been carried out to generate novel structures with important biological properties.[32] To better understand the structure−activity relationship and to generate better leads as potential drug candidates, we synthesized a number of new analogues of manzamines and evaluated their biological activity. An evaluation of the activity was made using molecular docking to an ATP-noncompetitive binding site of human GSK-3$\beta$.

## Results and Discussion

**Chemistry.** To explore the structure−activity relationship, diversity is the first thought. Modifications were carried out on different positions and functional groups of manzamines to generate diverse analogues. For this reason, four major manzamine alkaloids, **1**, **2**, **14**, and **19**, were chosen as starting material for the synthesis of analogues, although **14** and **19** only show marginal biological activity. Large quantities of these alkaloids had been produced from a common Indonesian sponge *Acanthostrongylophora* sp. through an optimized isolation procedure. Because of the number of potential reactive functional groups and instability of large and complex molecules such as manzamines, only mild and highly efficient reactions were employed for modification of manzamines. Thirty-nine analogues were synthesized in total, using various synthetic strategies, including 16 analogues of **1**, four of **2**, 11 of **14**, and eight of **19**.

*N*-Alkylations (Scheme 1) (**6a**−**i**) of the $\beta$-carboline moiety with various alkyl halides were carried out starting with **1** in the presence of sodium hydride in dimethyl formamide at 0 °C. The alkylated products were obtained in good yield (81−87%). One- and two-dimensional NMR showed that alkylation occurred at the 9-*N* position.

Reductions of **1** and **2** were performed in the presence of hydrogen-10% Pd/C in ethanol at room temperature for 4 h. Manzamine A afforded a major product, 15,16,32,33-tetrahydromanzamine A (**3**), and a minor product, **5**, and **2** gave a major product, **4**, and a minor product, **12**.

**1** was nitrated by sodium nitrate using TFA as a solvent to yield two nitrated products **7** and **8**. The 6-nitromanzamine A (**8**) was generated in excess when compared to 8-nitromanzamine (**7**). These two products could be readily purified by flash column chromatography. Refluxing of **1** and **2** with acetic anhydride and potassium carbonate in dry acetone resulted in dehydration product **10** and **11**. Oxidation of **1** with *m*-chloroperoxybenzoic acid in the presence of a catalytic amount of sodium bicarbonate in dichloromethane (DCM) solvent gave the corresponding *N*-oxides (**13**) without disturbing the double bond.

Modification of **14** was focused on the carbonyl group in the bottom eight-membered ring (Scheme 2). The carbonyl functional group was first reduced when treated with sodium borohydride to give a mixture of hydroxylated products and only one isomer (**16**) was obtained after purification. **14** was treated with Grignard reagent in dry THF to give the 31-alkyl product (**18**). Manzamine F-31-hydrazone (**15**) was obtained by heating **14** with hydrazine hydrate in ethanol. Knovenagel condensation of **14** with different aromatic aldehydes in the presence of a catalytic amount of piperidine yielded $\alpha,\beta$-unsaturated products (**17a**−**h**).

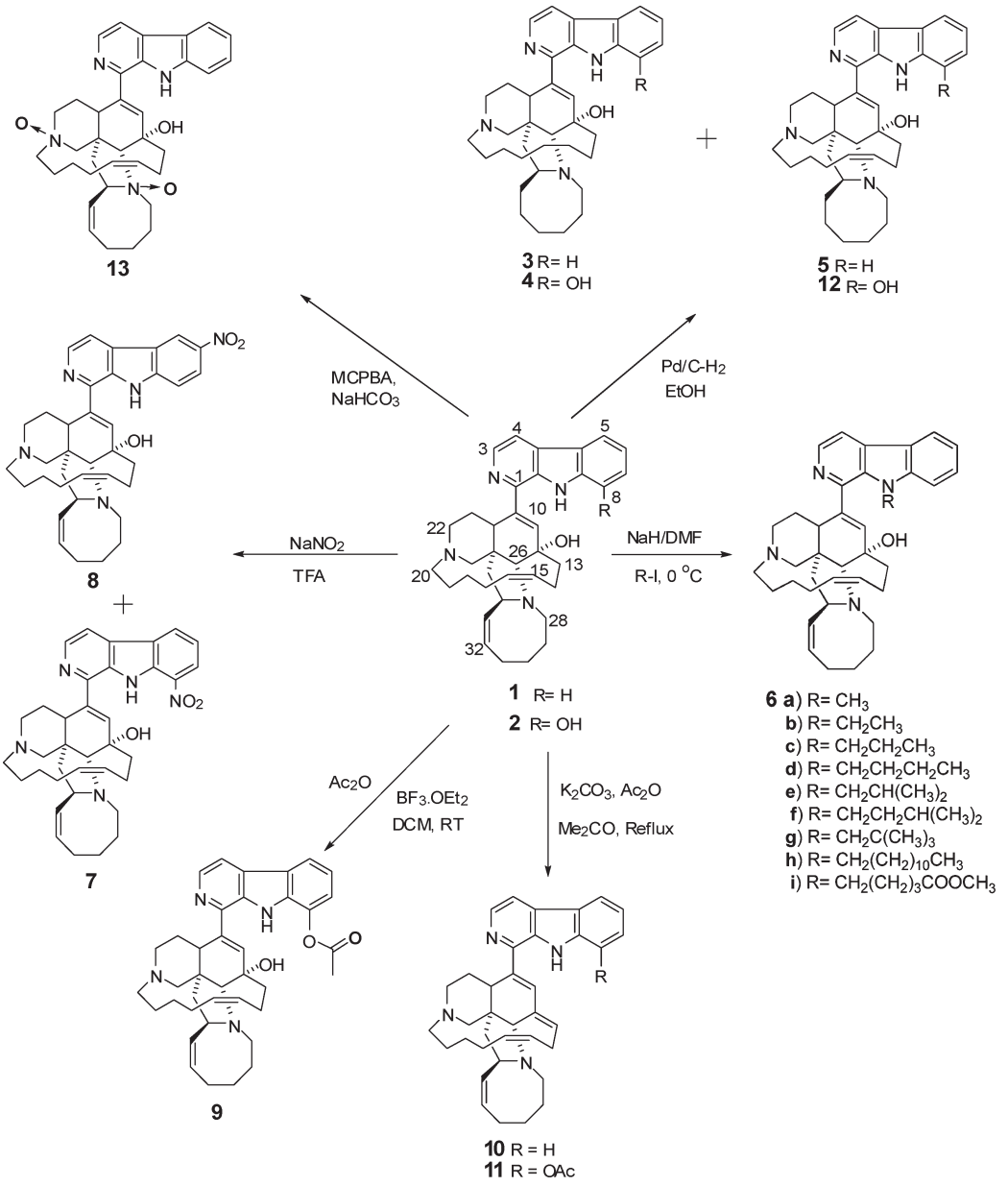
We next focused our efforts on converting **19** (Scheme 3) to analogues of **1** with a modified $\beta$-carboline moiety, which were otherwise difficult to synthesize directly from **1**. **19** was reacted with substituted tryptamines in the presence of catalytic amounts of trifluoroacetic acid and activated molecular sieves in DCM to yield products through Pictet−Spengler cyclization. The Pictet−Spengler products[33] were further aromatized with DDQ to yield substituted analogues of **1** (**21a**−**d**).

All of the above analogues were characterized by analysis of spectral data, provided in the Experimental Section, and were evaluated for antimalarial, antimicrobial, and anti-inflammatory activities.

**Biological Activity. In Vitro Antimalarial Activity.** All analogues were evaluated in vitro for antiprotozoal activity against *Plasmodium falciparum* (D6 and W2 clones). In addition, they were also tested for cytotoxicity against

[a] Abbreviations: $TXB_2$, thromboxane $B_2$; BBB, blood−brain barrier; Mtb, *Mycobacterium tuberculosis*; DCM, dichloromethane; PO, oral administration; GSK-3$\beta$, glycogen synthesis kinase-3$\beta$; HsGSK-3$\beta$, *Homo sapiens* GSK-3$\beta$; PfGSK-3, *Plasmodium falciparum* parasite GSK-3; AD, Alzheimer's disease; LPS, lipopolysaccharide; HBSS, Hanks' balanced salt solution; PMA, phorbol 12-myristate 13-acetate.

**Scheme 1.** Modification of Manzamine A

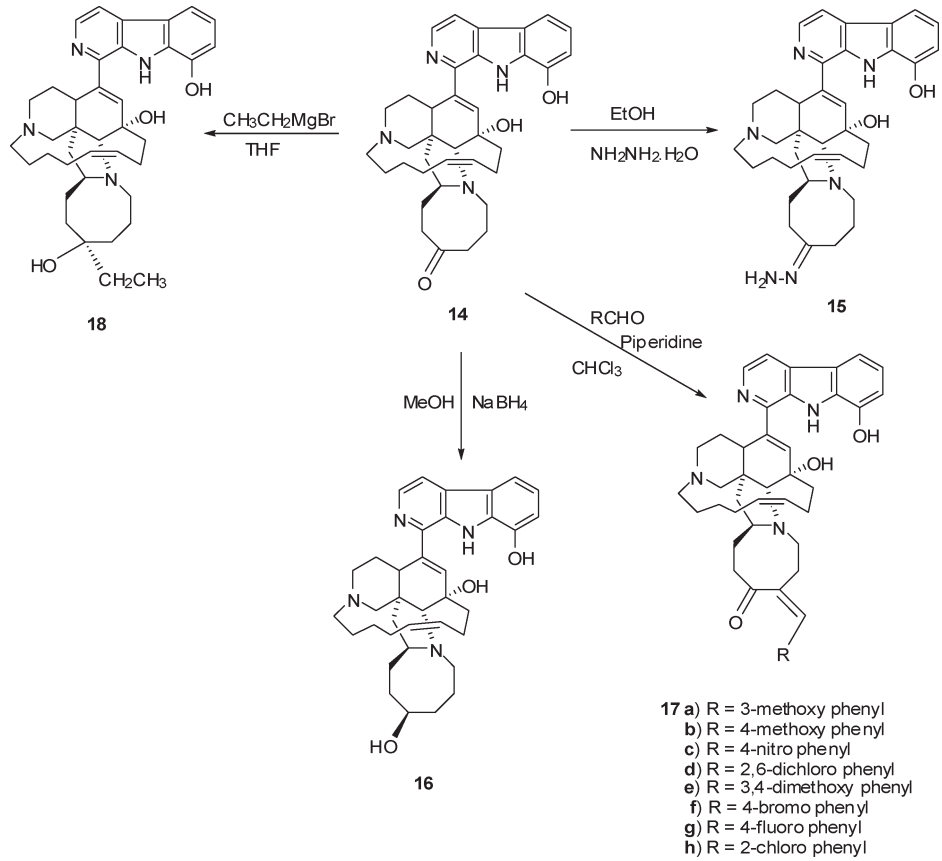



mammalian kidney fibroblasts (Vero cells) as shown in Table 1. The activity profile exhibited varied results for each analogue against *Plasmodium* strains. **1** still provided the highest activity with $IC_{50}$ of 4.5 ng/mL (D6 clone) and 8.0 ng/mL (W2 clone), which was comparable to the standard drugs chloroquine and artemisinin. Although toxicity to Vero cells was observed for **1** ($IC_{50}$ 200 ng/mL), the selectivity index (ratio of $IC_{50}$ for cytotoxicity to $IC_{50}$ for antimalarial activity) for antimalarial activity was high (44 and 25 for D6 and W2 clones, respectively). Introducing an alkyl substituent on 9-*N* (**6a**−**i**) decreased the antimalarial activity significantly in both D6 and W2 clones, showing that free 9-NH is required for antimalarial activity. Introducing lipophilic groups at 9*N* also diminished the antimalarial activity.

Substitution on other positions of the $\beta$-carboline moiety also decreased antimalarial activity but not as much as did the substitution at 9-NH. The hydroxyl group at position 8 (**2**) did not produce a significant change in antimalarial activity ($IC_{50}$ 6.0 ng/mL) but was less toxic ($IC_{50}$ 1100 ng/mL), yielding an improved selectivity index of 180 (D6 clone) and 140 (W2 clone). Nitration at the 6-position of **1** resulted in a slight decrease in the activity ($IC_{50}$ 18 ng/mL, **8**), while nitration at the 8-position decreased the activity significantly ($IC_{50}$ 310 ng/mL). Introduction of a 6-methoxyl group (**21a**) also decreased the activity ($IC_{50}$ 28 ng/mL). The methyl ester group in the 3-position in the $\beta$-carboline moiety (**21b**) retained good activity ($IC_{50}$ 11 ng/mL). Reduction of the pyridine ring in the $\beta$-carboline moiety (**20a**−**d**) also decreased the activity. The above results indicated that modification of the $\beta$-carboline ring is tolerated. Further modification may result in a lead with a better therapeutic index.

Reduction of the double bonds at 15 and 32 resulted in differences in activity. When just the double bond at C-32 in the bottom eight-membered ring of **1** or **2** was reduced (**5** and **6**), the activity was reduced significantly ($IC_{50}$ 200 ng/mL). A second reduction of the double bond at C-15 gave **3** and **4** and increased activities with $IC_{50}$ values of 82 and 90 ng/mL,

**Scheme 2.** Modifications Beginning with Manzamine F




**Scheme 3.** Modifications Beginning with Ircinal

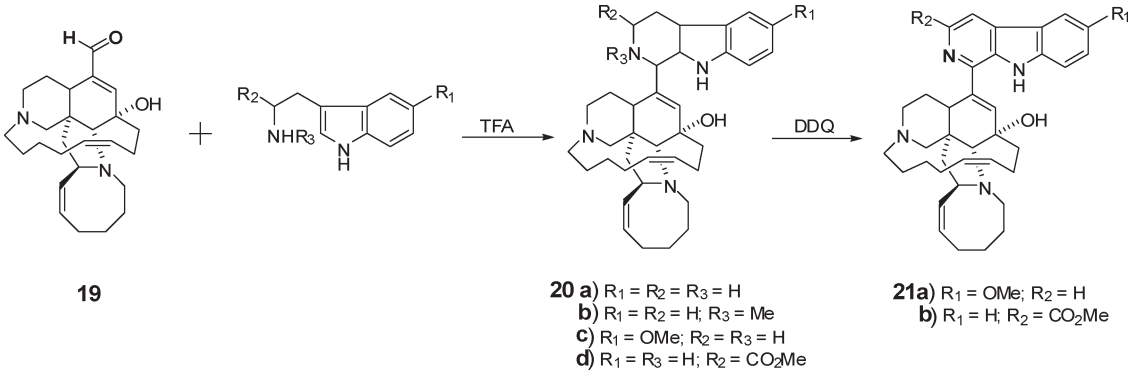



**Table 1.** In Vitro Antimalarial Activity against *Plasmodium falciparum* and Cytotoxicity to Mammalian Cells[a]

| compd | *P. falciparum* (D6 clone) $IC_{50}$ (ng/mL) | *P. falciparum* (W2 clone) $IC_{50}$ (ng/mL) | cytotoxicity (Vero) $IC_{50}$ ($\mu$g/mL) |
|---|---|---|---|
| **1** | 4.5 | 8.0 | 0.2 |
| **2** | 6.0 | 8.0 | 1.1 |
| **21b** | 11 | 15 | 0.2 |
| **21a** | 28 | 58 | 0.5 |
| **15** | 29 | 38 | NC |
| **18** | 77 | 86 | NC |
| **13** | 17 | 67 | NC |
| **8** | 18 | 28 | 270 |
| chloroquine | 16 | 155 | NC |
| artemisinin | 13 | 8 | NC |

[a] For a full list of compounds and activity data, please see Supporting Information. NC = no cytotoxicity up to 4.76 $\mu$g/mL.

respectively. These results indicate that the double bond at C-32 is important for the activity (either bonding or conformation), while the double bond at 15 is not required. Selectively reducing the double bond at C-15 in **1** and **2** may result in more potent antimalarial products.

Compound **14**, differing from **2** by an additional carbonyl functionality at position 31 and lack of a double bond at position 32 in the bottom eight-membered ring, only showed marginal antimalarial activity ($IC_{50}$ 780 ng/mL), a hundred-fold less active than **2**. Introduction of an $\alpha,\beta$-unsaturated system with carbonyl functionality intact (**17a**−**h**) did not improve the antimalarial activity, nor did reduction of the carbonyl group (**16**). Modification of the carbonyl functional group with hydrazone (**15**) or alkylation (**18**) resulted in significant improvement in antimalarial activity. Manzamine F-31-hydrazone (**15**) showed an $IC_{50}$ of 29 ng/mL

**Table 2.** In Vitro Antibacterial and Antifungal Activities[a]

| compd | *M. tuberculosis* (H37Rv) MIC ($\mu$g/mL) | *C. albicans* $IC_{50}$ ($\mu$g/mL) | *C. neoformans* $IC_{50}$ ($\mu$g/mL) | *M. intracellulare* $IC_{50}$ ($\mu$g/mL) |
|---|---|---|---|---|
| **1** | 1.5 | 2.0 | 1.5 | 0.35 |
| **2** | 0.9 | 3.5 | 2.0 | 0.1 |
| **3** | 50 | 7.5 | 0.9 | 0.45 |
| **4** | 24 | 6 | 1.5 | 0.1 |
| **20a** | 0.99 | 15 | 0.8 | 0.1 |
| **20c** | >64 | >20 | 2.0 | 0.2 |
| **20d** | >64 | >20 | 3.5 | 0.06 |
| **21b** | >64 | >20 | 2.0 | <0.02 |
| **15** | 1.9 | >20 | 1.0 | 0.09 |
| **18** | 1.9 | 2.5 | 6.0 | 0.25 |
| **9** | NT | 1.5 | 1.5 | 0.10 |
| **8** | 1.6 | >20 | 2.5 | 0.15 |
| **7** | NT | >20 | 4.0 | 0.25 |
| **14** | NT | 15 | 6.5 | NT |
| amphotericin B | NT | 0.20 | 0.75 | NT |
| ciprofloxacin | NT | NT | NT | 0.30 |
| rifampin | 0.09 | NT | NT | NT |

[a] For a full list of compounds and activity data, please see Supporting Information. NT = not tested.

against *P. falciparum* (D6 clone), which is a ca. 27-fold increase in activity over the mother compound. **18** showed an $IC_{50}$ of 77 ng/mL, a 1 order of magnitude increase. Neither compound showed toxicity against Vero cells at the highest tested concentration of 4.6 $\mu$g/mL. The improved activity of **14** analogues clearly showed that the carbonyl functional group is a liability for the bioactivity, and a lipophilic functionality is preferred for the antimalarial activity.

**Antibacterial and Antifungal Activity.** In addition to the antiprotozoal assays, in vitro antimicrobial activities of the manzamine analogues against *Mycobacterium tuberculosis*, *Candida albicans*, *Cryptococcus neoformans*, and *Mycobacterium intracellulare* were investigated. Manzamines showed moderate activity against *Mycobacterium tuberculosis* (H37Rv), of which **2** exhibited the best activity with an MIC of 0.9 $\mu$g/mL (see Table 2). In contrast, manzamine derivatives exhibited highly potent activity against *M. intracellulare*. Compounds **21b** and **20d** showed the most potent activity, with $IC_{50}$ values of <0.02 and 0.06 $\mu$g/mL, which are about 1 order of magnitude more potent than **1** and the positive control ciprofloxacin. **2**, tetrahydro-8-hydroxymanzamine A (**4**), manzamine D (**20a**), 8-acetoxymanzamine A (**9**), 6-methoxymanzamine D (**20c**), 6-nitromanzamine A (**8**), and 8-nitromanzamine A (**7**) showed slightly improved activity compared to **1** and ciprofloxacin with $IC_{50}$ values of 0.1−0.25 $\mu$g/mL, indicating that the $\beta$-carboline ring can be modified to yield improved activity against *M. intracellulare*. The decreased activity of the 9-*N* alkylation products of **1** (**6a**−**g**) revealed that the free NH is required for the activity. Modification of the carbonyl group of **14** gave varied results. While the $\alpha,\beta$-unsaturated derivatives of **14** (**17a**−**g**) diminished the activity, **15** and **18** exhibited potent activity, with $IC_{50}$ values of 0.09 and 0.25 $\mu$g/mL, respectively, more than 1 order of magnitude more potent than **14**.

All the analogues were evaluated for activity against *Cryptococcus neoformans*, and improved activities were observed with several of the analogues synthesized for this study. Compounds **3**, **20a**, and **15** showed potent activity ($IC_{50}$ 0.9, 0.8, and 1.0 $\mu$g/mL, respectively), comparable with that of amphotericin B ($IC_{50}$ 0.75 $\mu$g/mL), and can be considered as leads. The remaining compounds are less active than **1**.

**1** showed moderate activity against *Candida albicans*. Modification resulted in decreasing of activity for all products.

**Table 3.** In Vitro Antineuroinflammatory Activity[a]

| compd | $O_2^-$ $IC_{50}$ ($\mu$M) | $TXB_2$ $IC_{50}$ ($\mu$M) | LDH $LDH_{50}$ ($\mu$M) |
|---|---|---|---|
| **1** | 0.1 | <0.1 | >10 |
| **6d** | 0.12 | 0.4 | >10 |
| **6f** | 3.2 | 3.2 | >10 |
| **6g** | 0.07 | 0.07 | >10 |
| **21a** | 0.1 | 0.11 | >10 |
| **21b** | 0.03 | 0.11 | >10 |

[a] Antineuroinflammatory assay: effect of compounds on rat microglia PMA [1 $\mu$M]-stimulated release of $O_2^-$, $TXB_2$, and LDH. Data shown corresponds to 2−7 independent experiments and is expressed as $IC_{50}$ ($\mu$M) for $O_2^-$ and $TXB_2$. $LDH_{50}$ ($\mu$M) is the compound's concentration, causing 50% percent of maximal LDH release triggered by treating microglia with 0.1% Triton X-100. For a full list of compounds and activity data, please see Supporting Information.

**Antineuroinflammatory Activity.** The in vitro antineuroinflammatory activity of natural and synthetic analogues of manzamines was investigated on phorbol 12-myristate 13-acetate-stimulated generation of superoxide anion ($O_2^-$) and thromboxane $B_2$ ($TXB_2$) from activated rat neonatal microglia as previously described.[13,34] Lactate dehydrogenase (LDH) was used as a marker for cell cytotoxicity.[35] As shown in Table 3, **21b** potently inhibited $O_2^-$ ($IC_{50}$ = 0.03 $\mu$M) and $TXB_2$ ($IC_{50}$ = 0.11 $\mu$M) release from brain microglia, followed by **6g**, **21a**, and **6d**. Furthermore, minimal cytotoxicity, determined as lactate dehydrogenase release, was observed for these compounds and for **1** at the highest concentration tested in vitro (10 $\mu$M). This data thus supports the hypothesis that these manzamine compounds may be potential leads for the development of novel agents to modulate activated microglia cells in neuroinflammatory disorders such as Alzheimer's disease and malaria.[10,11,36−38] All other *N*-alkylation products (compounds **6a**,**b**,**c**,**d**,**e**,**f**) exhibited decreased activity on microglia $O_2^-$ and $TXB_2$ generation. Hydrogenation (**3**, **4**) also appeared to decrease the inhibitory activity on $O_2^-$ and $TXB_2$ generation. **20a** derivatives showed both less activity in the antineuroinflammatory assay and increased cytotoxicity to microglia cells.

**Blood−Brain Barrier Permeability.** Because of the efficacy of **1** and its analogues in neuroinflammation and against infectious agents that could cause cerebral infections (e.g., *Cryptococcus* and *Plasmodium*), the transport of **1** was evaluated across MDR-MDCK monolayer−a model for in vitro BBB transport study. The permeability of the compound was observed in both absorptive and secretory directions but the efflux ratio was <1, indicating no net efflux. The transport was linear with time (Figure 1A,B) in both directions but drug permeated at a higher rate from the apical side to the basolateral side (absorptive direction) of the monolayer. The permeability of **1** HCl salt in the absorptive direction (Papp $1.9 \times 10^{-6}$ cm/s) was much higher than that of its free base form (Papp $0.87 \times 10^{-6}$ cm/s) as shown in Figure 2. However, the permeability of **1** was much lower in comparison to caffeine (Papp $17 \times 10^{-6}$ cm/s), which is a highly permeable drug. That **1** can permeate across

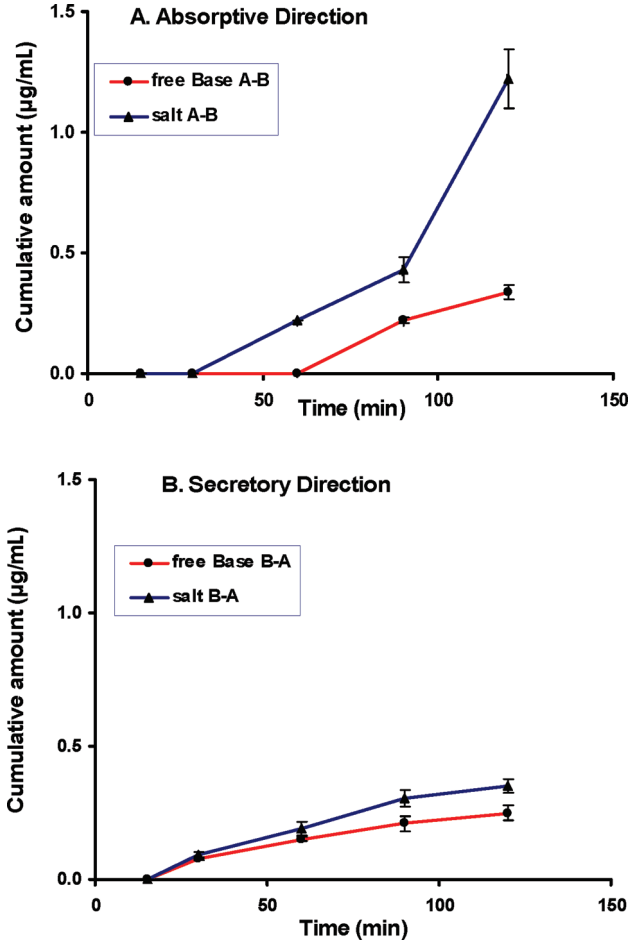



**Figure 1.** Time dependent transport of **1** salt and its free base (50 $\mu$M) across MDR-MDCK monolayer.

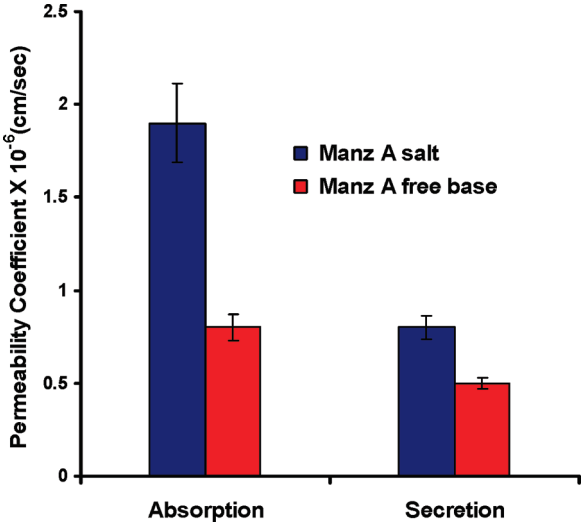



**Figure 2.** Permeability coefficient of **1** salt and its free base in absorptive and secretory directions of MDR-MDCK monolayer.

the BBB strengthens the therapeutic value of this class of compounds, which already have promising anti-infectious and antineuroinflammatory activities and significant oral bioavailability.

**In Vivo Antimalarial Evaluation of 1 and 82.** Initial in vivo evaluations of **1** were carried out with the assistance of Tropical Disease Research at the Swiss Tropical Institute in Basel and Northwick Park Institute for Medical Research, Harrow, UK, through a WHO contract. **1** has shown $ED_{50}/ED_{90}$ values of 0.57/1.7 mg/kg po in *Plasmodium yoelii* infected mice, which were significantly better than sodium artesunate, which had $ED_{50}/ED_{90}$ value of 4.1/17.0 mg/kg. Administration of 3 × 5 mg/kg of **1** subcutaneously or orally to mice inoculated with *P. berghei* resulted in prolonged mean survival times of 24.3 and 19.7 days in comparison with 14.3 days for chloroquine at 3 × 10 mg/kg po.

In our in vivo experiments, **1** was orally administered to mice from day one after the infection at three doses, 3 × 1.1 mg/kg, 3 × 3.3 mg/kg, and 3 × 10 mg/kg. **1** was able to produce a 75% reduction of the parasitemia level and significantly increase the survival time of mice infected with *P. berghei* with a dose of as little as 1.1 mg/kg (Table 4). A 75% cure rate of infected mice was achieved by a treatment with 3 × 10 mg/kg of **1**, while 25% cure of the infected animals was seen at 3 × 3.3 mg/kg. This antimalarial activity of **1** in mice was better than chloroquine at a similar dose.

We have shown that **1** has good oral bioavailability, a long half-life of 54 h, and low plasma clearance.[14] This facilitates the possible clearance of malaria with a single treatment of **1**. For this reason, single dose schedules of **1** in mice infected with malaria were evaluated. A single dose of 50 mg/kg showed complete clearance of parasitemia until day 14 post infection and was able to produce a 60% cure rate. Dosing with 100 mg/kg or higher was found to cure all the mice infected with *P. berghei* (Table 5). In contrast, treatment with quinine (200 mg/kg) failed to cure any mice while chloroquine (200 mg/kg) only produced an 80% cure rate. The untreated infected mice showed some initial weight gain followed by marked weight loss about 10 days after the inoculation, which correlated with the development of parasitemia. Treatment with **1** caused mild weight loss initially until day 5 followed by normal weight gain thereafter. Mice treated with 100 mg/kg of **1** did not show any behavioral signs of toxicity. Mice treated with higher doses of 200 or 300 mg/kg began to exhibit signs of toxicity beyond weight loss. A successful single dose treatment for malaria would be highly significant because of its potential to reduce the onset of drug-resistance associated with noncompliance of multiple dose schedules.

Intraperitoneal administration of **1** and **2** (10 × 3 mg/kg) in mice infected with malarial parasites was also evaluated (see Table 6). **1** possesses strong antimalarial activity in vivo with 100% suppression of the parasitemia at day 5, but it is toxic at 10 mg/kg. No immediate adverse/toxic reactions were noticed until day 3 post treatment with **1**. **2** produced a remarkable 80% cure with mild reversible weight loss. This result is consistent with the in vitro data, which indicated lower toxicity of **2** relative to **1** in Vero cells.

**GSK-3$\beta$ Docking.** Glycogen synthase kinase 3 (GSK-3) was originally identified as a protein kinase involved in the regulation of glycogen metabolism and now is known to function in a wide range of cellular processes including in the cell life cycle, apoptosis, and development. Aberrant regulation of GSK-3 has been implicated in a range of human pathologies such as Alzheimer's disease, type-2 diabetes, bipolar disorder, and cancer.[39−42] GSK-3 was originally identified in mammals, and homologues have been found in all eukaryotes. Because several of the manzamines were recently discovered to be inhibitors of *Homo sapiens* GSK-3$\beta$ (*Hs*GSK-3$\beta$),[20,43,44] we performed molecular docking studies[44] for the representative compound **2** with *Hs*GSK-3$\beta$.

**Table 4.** Multiple Dose Treatments of Manzamine A in *Plasmodium berghei* Infected Mice

| compd (dose) | inhibition of parasitemia (%)[a] | average % parasitemia (days post infection) 7 | 10 | 14 | 21 | 28 | MST days (%)[b] | cure (%)[c] |
|---|---|---|---|---|---|---|---|---|
| control | | 16.7 | 22.1 | | | | 11.8 (0) | 0 |
| **1** (3 × 1.1 mg/kg) | 75 | 5.5 | 11.5 | 21.7 | 39.6 | 58.6 | 26.5 (50) | 0 |
| **1** (3 × 3.3 mg/kg) | 88 | 2.8 | 4.3 | 11.9 | 21.6 | 53.9 | > 30 (100) | 25 |
| **1** (3 × 10 mg/kg) | 98 | 0.2 | 0.4 | 0.9 | 1.9 | 3.3 | > 30 (100) | 75 |
| chloroquine (3 × 5 mg/kg) | 88 | 4.5 | 7.8 | 13.5 | 21.7 | 39.3 | > 30 (100) | 0 |

[a] % Decrease in level of parasitemia in mice on day 5 post infection, as compared to untreated controls. [b] MST: mean survival time in days (% of mice remaining alive until day 28 post infection). [c] Mice without parasitemia until 28 days after the infection were considered as cure.

**Table 5.** Oral Single Dose Treatment of Manzamine A on *Plasmodium berghei* Infection in Mice

| compd (dose) | activity (%)[a] | average parasitemia (%) days post infection 5 | 7 | 10 | 14 | 21 | 28 | cure (%)[b] |
|---|---|---|---|---|---|---|---|---|
| control | | 9.8 | 15.9 | 21.7 | 39.8 | | | |
| **1** (50 mg/kg) | 100 | 0 | 0 | 0.4 | 1.0 | 5.2 | 6.25 | 60 |
| **1** (100 mg/kg) | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| **1** (200 mg/kg) | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| chloroquine (200 mg/kg) | 100 | 0 | 0 | 0 | 0.2 | 4.0 | 8.0 | 80 |
| quinine (200 mg/kg) | 26 | 7.3 | 11.6 | 14 | 32 | 45 | | 0 |

[a] % Decrease in level of parasitemia in mice on day 5 post infection, as compared to untreated controls. [b] Percentage of mice without parasitemia until day 28 post infection.

**Table 6.** Antimalarial Activity of Manzamine A and 8-Hydroxymanzamine A by ip

| treatments | % parasitemia suppression[a] day 5 | day 7 | survival[b] day of the death (MST)[c] | cure[d] |
|---|---|---|---|---|
| vehicle control | | | 0/5 12/16/12/16/12 (14) | 0/5 |
| **1** 10 × 3 mg/kg | 100 | | 0/5 5[e]/6[e]/5[e]/6[e]/6[e] 5.6[e] | 0/5 |
| **2** 10 × 3 mg/kg | 100 | 100 | 5/5 (> 28) | 4/5 |

[a] % Suppression in parasitemia is calculated by considering the mean parasitemia in control group as 100%. [b] Number of animal survived day 28/total animal in group (the day of the death-post infection). [c] MST: mean survival time (days). [d] Number of mice without parasitemia (cured) through day 28 post infection. [e] Death related to toxicity.

Building on the recently published evidence that **2** was an ATP-noncompetitive inhibitor of *Hs*GSK-3$\beta$,[20,43,45] we explored the ability of **2** to bind in a pocket that is located in the vicinity of the activation pocket formed by three basic residues, Arg96, Arg180, and Lys205. In the first X-ray crystal structures of *Hs*GSK-3$\beta$[46,47] a charged phosphate, sulfate, or sulfonate-containing moiety was found to be bound in that vicinity. This pocket was proposed to be the binding site for thiadiazolidinones by Martinez et al. in their reports on the first ATP-noncompetitive inhibitor.[48,49] For this work, we used an X-ray crystal structure of *Hs*GSK-3$\beta$ without a cocrystallized ligand in the ATP-binding site but which had a sulfate coordinated to Arg96, Arg180, and Lys205, with Val204 and Asn203 making fairly close interactions as well (pdb code: 1gng).[47]

The docking results showed that **2** fits well into the ATP-noncompetitive pocket (Figures 3 and 4). The three polar interactions exhibited by **2** are shown in Figure 4. The 8-hydroxyl group of **2** formed significant electrostatic interactions with Arg96 and Ala204. The acceptor oxygen of the 8-hydroxyl group interacted with a donor NH from Arg96, whereas the 8-hydroxyl donor hydrogen interacted with both the backbone carbonyl of Ala204 and with an intermediary water molecule, which in turn was hydrogen bonded to the NH of Arg96. The tertiary nitrogen of the $\beta$-carboline ring of **2** showed water mediated hydrogen bonding with the NH of Asp90. This may partly explain why **20a**, which has a reduced $\beta$-carboline ring, is less active than **2**. A key hydrophobic interaction between the 8-membered ring of **2** and Phe93 was found. This may partly explain why **5**, which has a reduced 8-membered ring, is less active than **2**. The $\beta$-carboline ring also showed hydrophobic interactions with the backbone of Arg96. Thus the combination of three polar interactions and several nonpolar interactions lead to a favorable docking pose for **2** in the ATP-noncompetitive binding site of *Hs*GSK-3$\beta$.

To date, the mechanism for the diverse bioactivities of manzamine alkaloids is still unclear. The above docking results and our recent discovery of **1** as a selective inhibitor of GSK-3$\beta$ and CDK-5 suggest that GSK-3$\beta$ and closely related kinases may represent common targets for the diversity of biological activity observed including the antimalarial activity. Recently, *Plasmodium falciparum* parasite GSK-3 (*Pf*GSK-3) was identified by Droucheau et al. and was suggested to be a possible antimalarial target. The whole protein sequence of *Pf*GSK-3 is 43.1% identical and 58.9% similar to *Hs*GSK-3$\beta$ (central catalytic domain is 58.2% identical and 76.0% similar).[50] Hence it is possible that the antimalarial activity exhibited by manzamine and its analogues is due to inhibition of *Pf*-kinases. However, further experimental studies are required to prove that the antimalarial activity of manzamines is through the inhibition of *Pf*-kinases. Although *Pf*GSK-3 shares similarity with human GSK-3, divergent sensitivity to a series of GSK-3$\beta$ inhibitors was observed, suggesting that selective inhibitors to either *Pf*GSK-3 or *Hs*GSK-3$\beta$ might be identified.[50] The high activity of **1** against *P. falciparum* parasites and the moderate inhibitory activity of *Hs*GSK-3$\beta$ by **1** may indicate that **1** is a

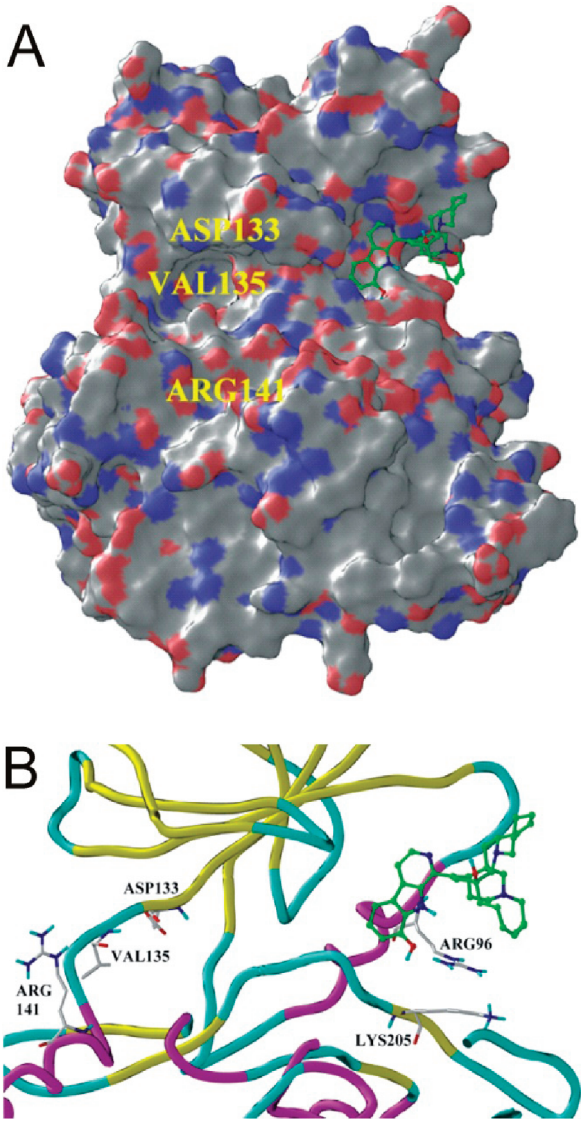



**Figure 3.** Binding mode of docked **2** (green C, red O, blue N, cyan H) within the ATP-noncompetitive pocket found in the vicinity of Arg96, Arg180, and Lys205. Key amino acids in the binding pocket for ATP-competitive inhibitors (left side of the enzyme) are labeled. (A) Connolly molecular surface of GSK-3$\beta$, with surface red and blue regions indicating the presence of hydrogen bond donors and acceptors, respectively, in the protein. (B) Tube model (colors) of the two active sites of GSK-3$\beta$ (magenta, yellow and cyan indicate $\alpha$-helices, $\beta$-sheets and loops, respectively; protein side-chain gray C, red O, blue N, cyan H).

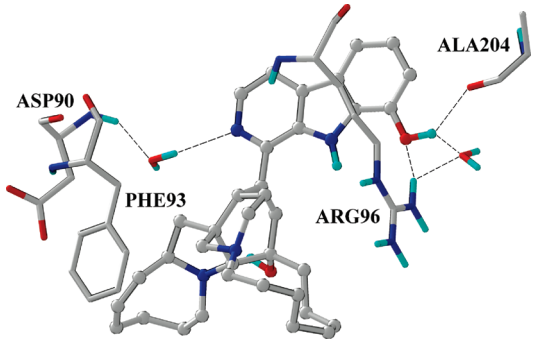



**Figure 4.** Key interactions of **2** within the ATP-noncompetitive pocket of GSK-3$\beta$ (gray C, red O, blue N, cyan H).

highly selective inhibitor of *Pf*GSK-3. A new function has been found recently for GSK-3 as a vital factor in the inflammation process.[51,52] Thus, the antineuroinflammatory activity of manzamines may be also caused by inhibition of GSK-3$\beta$. There is no published information about GSK-3 as a target for antibacterial agents, but broad-spectrum kinase inhibitors like staurosporine do indeed show antibiotic activity.[53,54]

## Conclusion

This structure−activity relationship study has allowed us to refine the structural requirements for the unique manzamine alkaloids to act against neuroinflammation and infectious disease including malaria. A number of derivatives exhibited highly promising activity and can be considered as potential drug leads.

Both in vitro and in vivo data clearly revealed significant potential for **1** and **2** analogues in the treatment of malaria. Although toxicity has been seen in high doses and daily dosing schedules of **1**, the successful development of commonly utilized antimalarial agents from quinine and quinolines, despite their significant toxicity at higher doses, bodes well for the development of a novel class of antimalarial drugs from manzamines by optimization of both structure and drug administration. This was evidenced by the significantly reduced toxicity of **2** compared to **1** in intraperitoneal administration to mice (Table 6). Highly encouraging results have been found by modification of **14**. Two derivatives with modification of the carbonyl group of **14** (**15** and **18**) showed 10- to 27-fold increase in antimalarial activity without showing toxicity to Vero cells. This suggests that further modification along this direction may result in an optimized candidate with suitably potent antimalarial activity and a better therapeutic window.

One of the most deadly complications of malaria is cerebral malaria, which is fatal in about 30−50% of the cases.[36] Although the immunopathogenesis of cerebral malaria remains incompletely understood,[55] it appears that one of the earliest events is an increase in the permeability of the blood−brain barrier, probably mediated by $CD8^+$ T cell damage to the microvascular endothelium, an event that is followed by leakage of cytokines, malaria antigens, and other potentially harmful molecules into the cerebral parenchyma, with concomitant activation of microglia cells.[36] Thus a significant pathophysiological alteration observed in human cerebral malaria is widespread activation and proliferation of microglial cells.[56,57] The antineuroinflammatory and immunosuppressive activity of manzamines[13,58] along with their BBB permeation is exceptional for the development of novel treatments of cerebral malaria because currently there is no specific treatment for cerebral malaria.[59]

Manzamine analogues exhibited selective activity against *Mycobacterium intracellulare*. Eleven derivatives (**2**, **4**, **7**, **8**, **9**, **15**, **18**, **20a**, **20c**, **20d**, and **21b**) exhibited improved activity compared to manzamine A and the positive control ciprofloxacin. The two derivatives with a 3-methyl carboxylate, **21b** and **20d**, were 5- to 15-fold more potent than ciprofloxacin. Compounds **20a**, **3**, and **15** showed potent activity against *Cryptococcus neoformans*, which are comparable with amphotericin B. These manzamine analogues clearly have great potential as antibiotics or antifungals, which are selectively active against *M. intracellulare* and *C. neoformans*.

With regard to antineuroinflammatory activity, the most effective manzamine derivatives were **21b** and **6g**, with **21b** the

best for $O^{2-}$ and **6g** best for $TXB_2$ release from brain microglia. **21a** and **6d** were also highly active. The four compounds showed no toxicity at 10 $\mu$M, suggesting they are potentially good leads for the development of novel agents for the control of neuroinflammatory diseases.

Evidence shows that GSK-3 plays a key role in the neurodegenerative diseases including Alzheimer's (AD), Parkinson's, and related neurodegenerative diseases, and GSK-3 has been considered as one of the most important potential targets for neurodegenerative disease.[40−42] It is also well established that inflammation occurs in AD and contributes to the pathological progression of AD.[60−62] Leads like manzamines, which have both antineuroinflammatory and GSK-3$\beta$ inhibitory activity, would have increased benefits for the treatment of neurodegenerative diseases. Moreover, because GSK-3 is involved in so many pathways for cell function, selective and moderate inhibition is the best way to reach the therapeutic goal and avoid or decrease potential side effects. Manzamines exhibited selective, moderate inhibition to GSK-3$\beta$, which for **1** was shown to be in a specific ATP-noncompetitive manner,[34] suggesting that they are appropriate candidates for GSK-3$\beta$ inhibition.

Molecular docking of **2** to *Hs*GSK-3$\beta$ was used to find a binding pose in the previously identified ATP-noncompetitive binding site, which was stabilized by a combination of three polar interactions and several nonpolar interactions. This opens up the exciting possibility of rational structure-based optimization of manzamines as GSK-3$\beta$ inhibitors.

Despite the diverse bioactivities which have been shown for manzamine alkaloids, our results clearly show that promising drug leads for treatment of malarial, bacterial, neuroinflammatory, and neurodegenerative diseases are achievable by modification and optimization of derivatives of **1**, which also shows moderate BBB permeation in an in vitro model. **1** has been shown to be highly stable at room temperature in aqueous solution. No significant decomposition of the compound was observed for over 24 months during exposure to light at room temperature in a 0.05 N HCl aqueous solution (20 mg/mL). A putative *Micromonospora* (*Actinomycetes* sp.) isolated from the manzamine producing sponge has been patented for manzamine production, but validation through incorporation experiments remains a challenge.[63] Although considerable effort remains to optimize the culture condition to reach a high yield of manzamines, access to a reliable microbial source for the production of the manzamines may provide potential cost-effective production of manzamines without disruption to fragile marine ecosystem by excessive sponge harvest. As a result, there is significant potential for analogues of the manzamines class as antiparasitic-antibiotics and in the control of neuroinflammation. This unique combination of activities could be exploited to control both the pathogen as well as the associated inflammation.

## Experimental Section

**General Experimental Procedures.** The NMR data were recorded in $CDCl_3$ on a Bruker DRX NMR spectrometer operating at 400 MHz for $^1$H and 75 MHz for $^{13}$C. Chemical shift ($\delta$) values are expressed in parts per million (ppm) and are referenced to the residual solvent signals of $CDCl_3$. The HRMS spectra were measured using a Bruker MicroTOF with electrospray ionization. TLC analysis was carried out on silica gel $G_{254}$ or aluminum oxide ALOX-100 $UV_{254}$ $\mu$m. The purity of each compound were tested to be ≥95% by LC/MS using an Agilent HPLC connected to a Bruker MicroTOF with electrospray ionization (Phenomenex Luna C18 4.6 mm × 150 mm 5 $\mu$m column, eluted with gradient acetonitrile:$H_2O$:0.1% formic acid, detected at 235 and 282 nm).

**General Preparation of Compounds 6a−i. 1** (54.8 mg, 0.1 mmol) was dissolved in 5 mL of dry *N*,*N*-dimethylformamide and stirred for 15 min in a Dewar flask at 0 °C. To this 95% NaH (2.33 mg, 0.12 mmol) was added with care and stirred for an additional 5 min; methyl iodide (MeI, 15 $\mu$L, 0.24 mmol) was added to the reaction mixture at 0 °C. After 1 h, the mixture was allowed to warm to room temperature and was stirred continuously for 2−3 h. The completion of the reaction was monitored by TLC, and the reaction mixture was slowly added to a mixture of ice-cold water and stirred for 15 min.

The aqueous layer was extracted with $Et_2O$ (2 × 50 mL), and the combined organic layers were washed with brine and dried over anhydrous $Na_2SO_4$, with the solvent being removed under reduced pressure. The crude product was chromatographed on a 250 mm × 10 mm, 5 m Luna C8 Phenomenex HPLC column using a water and acetonitrile gradient with the flow rate of 8 mL/min. The remainder of the manzamine A analogues (**6b−i**) were synthesized using the same reaction conditions.

**9*N*-Methylmanzamine A (6a):** Yield 84%. $^1$H NMR ($CDCl_3$) $\delta$ 8.40 (d, $J$ = 5.2), 7.78 (d, $J$ = 5.2), 7.51 (d, $J$ = 7.8), 7.36 (t, $J$ = 8.0), 7.56 (d, $J$ = 7.4), 7.59 (d, J = 8.0), 5.78 (s), 1.59 (m), 2.23 (m), 2.42 (m), 2.79 (m), 5.54 (brs), 5.30 (brs), 1.55 (m) 1.81(m), 1.30 (m), 1.56 (m), 1.42 (m), 1.50 (m), 2.31 (m), 2.82 (m), 2.07 (m), 2.94 (m), 2.45 (m), 2.71 (m), 2.29 (m) 3.76 (s), 2.79 (m), 3.19 (m), 1.70 (m), 1.79 (m), 1.23 (m), 1.70 (m), 1.58 (m), 1.79 (m), 5.90 (brs), 5.66 (brs), 3.05 (m), 2.30 (m), 2.43 (m), 2.64 (d, $J$ = 11.3), 2.21 (d, $J$ = 11.3), 3.87 (s). $^{13}$C NMR ($CDCl_3$) $\delta$ 142.9, 142.8, 139.5, 138.1, 136.5, 134.8, 130.5, 130.0, 129.2, 128.4, 128.1, 127.4, 123.2, 120.7, 120.5, 113.8, 113.4, 75.0, 70.1, 69.5, 63.8, 58.9, 53.6, 52.3, 51.8, 44.1, 37.9, 37.6, 33.6, 32.7, 31.7, 29.6, 28.0, 27.1, 26.7, 24.6, 20.3. HRESIMS $m/z$ calcd for $C_{37}H_{47}N_4O$ $[M + H]^+$ 563.3750, found 563.3764.

**9*N*-Ethylmanzamine A (6b):** Yield 87%. $^1$H NMR ($CDCl_3$) $\delta$ 8.38 (d, $J$ = 5.2) 7.72 (d, $J$ = 5.2), 7.57 (d, $J$ = 7.8), 7.38 (t, $J$ = 8.0) 7.51 (d, $J$ = 7.4), 7.59 (d, $J$ = 8.0), 5.73 (s), 1.56 (m), 2.24 (m), 2.47 (m), 2.76 (m) 5.55 (brs), 5.23 (brs), 1.52 (m), 1.78 (m), 1.54 (m), 1.67 (m), 1.76 (m), 1.56 (m), 2.67 (m), 2.78 (m), 2.02 (m), 2.95 (m), 2.44 (m), 2.77 (m), 2.26 (m), 3.75 (s),2.74 (m), 3.23 (m), 1.67 (m), 1.76 (m), 1.26 (m), 1.75 (m), 1.54 (m), 1.75 (m), 5.94 (brs), 5.67 (brs), 3.04 (m), 2.36 (m), 2.46 (m), 2.63 (d, $J$ = 11.3), 2.27 (d, $J$ = 11.3), 3.84 (m), 1.53 (m). $^{13}$C NMR ($CDCl_3$) $\delta$ 142.7, 142.6, 139.7, 138.4, 136.5, 134.6, 130.3, 130.1, 129.1, 128.2, 128.2, 127.5, 123.3, 120.7, 120.4, 113.6, 113.2, 75.2, 70.4, 69.4, 63.7, 58.9, 53.5, 52.4, 51.7, 47.8, 44.3, 37.6, 37.5, 32.5, 31.5, 29.7, 28.1, 27.3, 26.4, 24.5, 20.2, 14.1. HRESIMS $m/z$ calcd for $C_{38}H_{49}N_4O$ $[M + H]^+$ 577.3906, found 577.3911.

**9*N*-Propylmanzamine A (6c):** Yield 82%. $^1$H NMR ($CDCl_3$) $\delta$ 8.42 (d, $J$ = 5.2), 7.81 (d $J$ = 5.2), 7.69 (d, $J$ = 7.8), 7.34 (t, $J$ = 8.0), 7.53 (d, $J$ = 7.4), 7.57 (d, $J$ = 8.0), 5.83 (s), 1.42 (m), 2.13 (m), 2.30(m), 2.61 (m), 5.59 (brs), 5.34 (brs), 1.57 (m), 1.77 (m), 1.37 (m), 1.62 (m), 1.48 (m), 1.51 (m), 2.38 (m), 2.73 (m), 2.01 (m), 2.99 (m), 2.30 (m), 2.62 (m), 2.14 (m), 3.78 (s), 2.68 (m), 3.15 (m), 1.82 (m), 1.95 (m), 1.35 (m), 1.67 (m), 1.51 (m), 1.66 (m), 5.92(brs), 5.63 (brs), 3.11 (m), 2.25 (m), 2.56 (m), 2.62 (d, $J$ = 11.3), 2.30 (d, $J$ = 11.3), 3.83 (m), 1.81 (m), 1.27 (m). $^{13}$C NMR ($CDCl_3$) $\delta$ 142.7, 142.6, 139.4, 138.3, 136.8, 134.8, 130.3, 130.2, 129.6, 128.4, 128.1, 127.3, 123.1, 120.8, 120.6, 113.7, 113.5, 75.0, 70.6, 69.5, 63.4, 58.6, 54.2, 53.3, 52.7, 51.4, 44.4, 37.5, 37.3, 32.8, 31.4, 29.6, 28.4, 27.5, 26.6, 24.3, 21.3, 20.7, 13.8. HRESIMS $m/z$ calcd for $C_{39}H_{51}N_4O$ $[M + H]^+$ 591.4063, found 591.4067.

**9*N*-butylmanzamine A (6d):** Yield 87%. $^1$H NMR ($CDCl_3$) $\delta$ 8.48 (d, $J$ = 5.2), 7.78 (d, $J$ = 5.2), 7.51 (d, $J$ = 7.8), 7.34 (t, $J$ = 8.0), 7.58 (d, $J$ = 7.4), 7.59 (d, $J$ = 8.0), 5.72 (s), 1.56 (m), 2.23 (m), 2.47 (m), 2.79 (m), 5.54 (brs), 5.30 (brs), 1.59 (m), 1.81 (m), 1.30 (m), 1.56 (m), 1.43(m), 1.50 (m), 2.31 (m), 2.86 (m), 2.08 (m), 2.94 (m), 2.45 (m), 2.71 (m), 2.25 (m), 3.77(s), 2.77 (m), 3.19 (m), 1.70(m), 1.79 (m), 1.29 (m), 1.70(m), 1.54 (m), 1.75 (m), 5.90 (brs), 5.66 (brs), 3.09 (m), 2.30 (m), 2.43 (m), 2.64 (d, $J$ = 11.3), 2.21 (d,

$J$ = 11.3), 3.87 (m), 1.79 (m), 1.42 (m), 1.18 (m). $^{13}$C NMR ($CDCl_3$) $\delta$ 142.7, 142.5, 139.3, 138.2, 136.7, 134.5, 130.1, 130.1, 129.3, 128.4, 128.2, 127.5, 123.4, 120.6, 120.4, 113.5, 113.4, 75.2, 70.3, 69.6, 63.8, 58.7, 57.1, 53.6, 52.4, 51.2, 44.6, 37.7, 37.5, 32.5, 31.3, 29.8, 29.8, 28.2, 27.6, 26.7, 24.6, 20.3, 19.1, 13.3. HRESIMS $m/z$ calcd for $C_{40}H_{53}N_4O$ $[M + H]^+$ 605.4219, found 605.4222.

**9*N*-isobutylmanzamine A (6e):** Yield 82%. $^1$H NMR ($CDCl_3$) $\delta$ 8.47 (d, $J$ = 5.2), 7.74 (d, $J$ = 5.2), 7.57 (d, $J$ = 7.8), 7.35 (t, $J$ = 8.0), 7.57 (d, $J$ = 7.4), 7.54 (d, $J$ = 8.0), 5.75 (s), 1.56 (m), 2.24 (m), 2.46 (m), 2.74 (m), 5.55 (brs), 5.30 (brs), 1.56 (m), 1.85 (m), 1.33 (m) 1.55 (m), 1.47 (m), 1.54 (m), 2.36(m), 2.84 (m), 2.07 (m), 2.93 (m), 2.46 (m), 2.74 (m), 2.26 (m), 3.75 (s), 2.74 (m), 3.16 (m), 1.73 (m), 1.73 (m), 1.26 (m), 1.74 (m), 1.58 (m), 1.74 (m), 5.94 (brs), 5.64 (brs), 3.06 (m), 2.34 (m), 2.43 (m), 2.64 (d, $J$ = 11.3), 2.23 (d, $J$ = 11.3), 3.82 (m), 1.76 (m), 1.23 (m), 1.23(m). $^{13}$C NMR ($CDCl_3$) $\delta$ 142.4, 142.2, 139.2, 138.1, 136.8, 134.7, 130.5, 130.4, 129.1, 128.4, 128.2, 127.7, 123.2, 120.6, 120.6, 113.6, 113.3, 75.8, 70.5, 69.3, 63.6, 61.5, 58.4, 53.5, 52.2, 51.8, 44.2, 37.3, 37.2, 32.6, 31.6, 29.5, 28.3, 27.4. 27.2, 26.8, 24.8, 20.1, 18.6 (2C). HRESIMS $m/z$ calcd for $C_{40}H_{53}N_4O$ $[M + H]^+$ 605.4219, found 605.4225.

**9*N*-isopentylmanzamine A (6f):** Yield 84%. $^1$H NMR ($CDCl_3$) $\delta$ 8.40 (d, $J$ = 5.2), 7.79 (d, $J$ = 5.2), 7.51 (d, $J$ = 7.8), 7.30 (t, $J$ = 8.0), 7.56 (d, $J$ = 7.4), 7.59 (d, $J$ = 8.0), 5.78 (s), 1.53 (m), 2.21 (m), 2.47 (m) 2.79 (m), 5.54 (brs), 5.33 (brs), 1.55 (m), 1.85 (m), 1.33 (m), 1.59 (m), 1.44 (m), 1.53 (m), 2.36 (m), 2.82 (m), 2.07 (m), 2.94 (m), 2.46 (m), 2.75 (m), 2.28 (m), 3.72 (m), 2.75 (m), 3.16 (m), 1.78 (m), 1.77 (m), 1.23 (m), 1.70 (m), 1.58 (m), 1.79 (m), 5.92 (brs), 5.64 (brs), 3.05 (m), 2.33 (m), 2.43 (m), 2.62 (d, $J$ = 11.3), 2.23 (d, $J$ = 11.3), 3.85 (m), 1.72 (m), 1.85 (m), 1.09 (m), 1.09 (m). $^{13}$C NMR ($CDCl_3$) $\delta$ 142.8, 142.2, 139.6, 138.0, 136.7, 134.5, 130.3, 130.2, 129.7, 128.3, 128.2, 127.3, 123.6, 120.5, 120.4, 113.6, 113.6, 75.5, 70.6, 69.7, 63.8, 59.3, 58.2, 53.8, 52.1, 51.6, 44.1, 37.6, 37.3, 33.5, 32.4, 31.8, 29.7, 28.6, 27.4, 26.7, 26.6, 24.6, 21.8, 21.8, 20.4. HRESIMS $m/z$ calcd for $C_{41}H_{55}N_4O$ $[M + H]^+$ 619.4376, found 619.4381.

**9*N*-neopentylmanzamine A (6g):** Yield 85%. $^1$H NMR ($CDCl_3$) $\delta$ 8.45 (d, $J$ = 5.2), 7.82 (d, $J$ = 5.2), 7.66 (d, $J$ = 7.8), 7.34 (t, $J$ = 8.0), 7.52 (d, $J$ = 7.4), 7.56 (d, $J$ = 8.0), 5.83 (s), 1.42 (m), 2.17 (m), 2.32 (m), 2.61 (m), 5.58 (brs), 5.34 (brs), 1.56 (m), 1.77 (m), 1.38 (m), 1.63 (m), 1.45 (m), 1.51 (m), 2.37 (m), 2.73 (m), 2.04 (m), 2.97 (m), 2.30 (m), 2.63 (m), 2.14 (m), 3.76 (s), 2.65 (m), 3.13 (m), 1.82 (m), 1.97 (m), 1.32 (m), 1.67 (m), 1.53 (m), 1.66 (m), 5.92 (brs), 5.63 (brs), 3.14 (m), 2.25 (m), 2.55 (m), 2.61 (d, $J$ = 11.3), 2.33 (d, $J$ = 11.3), 3.81 (m), 1.05 (s), 1.05 (s) 1.05 (s). $^{13}$C NMR ($CDCl_3$) $\delta$ 142.9, 142.2, 139.9, 138.5, 136.4, 134.4, 130.7, 130.6, 129.3, 128.9, 128.3, 127.2, 123.4, 120.9, 120.7, 113.9, 113.3, 75.1, 70.2, 69.5, 68.3, 63.6, 58.5, 53.5, 52.7, 51.9, 44.6, 37.9, 37.0, 32.7, 31.4, 29.1, 28.9, 27.6, 27.2, 27.1(3C), 26.8, 24.3, 20.6. RESIMS $m/z$ calcd for $C_{41}H_{55}N_4O$ $[M + H]^+$ 619.4376, found 618.4382.

**9*N*-dodecylmanzamine A (6h):** Yield 81%. $^1$H NMR ($CDCl_3$) $\delta$ 8.40 (d, $J$ = 5.2), 7.78 (d, $J$ = 5.2), 7.51 (d, $J$ = 7.8), 7.36 (t, $J$ = 8.0), 7.56 (d, $J$ = 7.4), 7.59 (d, $J$ = 8.0), 5.78 (s), 1.59 (m), 2.23 (m), 2.42 (m), 2.79 (m), 5.54 (br s), 5.30 (br s), 1.55 (m), 1.81 (m), 1.30 (m), 1.56 (m), 1.42 (m), 1.50 (m), 2.31 (m), 2.82 (m), 2.07 (m), 2.94 (m), 2.45 (m), 2.71 (m), 2.29 (m), 3.76 (s), 2.79 (m), 3.19 (m), 1.70 (m), 1.79 (m), 1.23 (m), 1.70 (m), 1.58 (m), 1.79 (m), 5.90 (brs), 5.66, (brs), 3.05 (m), 2.30 (m), 2.43 (m), 2.64 (d, $J$ = 11.3), 2.21 (d, $J$ = 11.3), 3.87 (s). $^{13}$C NMR ($CDCl_3$) $\delta$ 142.7, 142.6, 139.2, 138.3, 136.8, 134.7, 130.5, 130.4, 129.8, 128.7, 128.5, 127.5, 123.5, 120.9, 120.8, 113.8, 113.7, 75.4, 70.5, 69.4, 63.5, 58.8, 57.8, 53.5, 52.2, 51.6, 44.4, 37.5, 37.4, 32.9, 31.8, 31.4 (4), 31.1, 30.3, 29.8, 29.3, 28.4, 27.8, 26.5, 26.3, 24.6, 24.4, 22.8, 20.8, 13.8. HRESIMS $m/z$ calcd for $C_{48}H_{69}N_4O$ $[M + H]^+$ 717.5471, found 717.5478.

**9*N*-(4-methylcarboxybutyl)manzamine A (6i):** Yield 76%. $^1$H NMR ($CDCl_3$) $\delta$ 8.42 (d, $J$ = 5.2), 7.81 (d, $J$ = 5.2), 7.69 (d, $J$ = 7.8), 7.34 (t, $J$ = 8.0), 7.53 (d, $J$ = 7.4) 7.57 (d, $J$ = 8.0), 5.83 (s), 1.42 (m), 2.13 (m), 2.30 (m), 2.61 (m), 5.59 (brs), 5.34 (brs), 1.57 (m), 1.77 (m), 1.37 (m), 1.62 (m), 1.48 (m), 1.51 (m), 2.38 (m), 2.73 (m), 2.01 (m), 2.99 (m), 2.30 (d, $J$ = 11.3), 3.85 (t, $J$ = 6.1), 1.68 (m), 1.57 (m), 2.31 (m), 3.92 (s). $^{13}$C NMR ($CDCl_3$) $\delta$ 174.8, 144.8, 144.2, 142.3, 138.6, 137.3, 134.1, 132.7, 129.9, 129.6, 128.9, 128.3(2), 122.0, 121.8, 120.0, 113.6, 112.0, 75.2, 69.2, 68.7, 63.6, 53.1, 52.7, 49.9, 49.3, 48.2, 46.0, 44.8, 42.0, 41.2, 35.8, 33.8, 29.7, 28.5, 26.9, 25.9, 25.8, 25.6, 25.4, 21.3. HRESIMS $m/z$ calcd for $C_{42}H_{55}N_4O_3$ $[M + H]^+$ 663.4274, found 663.4291.

**General Preparation of Compounds 3, 4, 5, and 12.** **1** (54.8 mg, 0.1 mmol) was dissolved in 5 mL of ethanol, to which was added 5 mg of palladium charcoal, and stirred for 4 h under $H_2$ gas. The completion of the reaction was monitored by TLC, and the solvent was removed in vacuo. The crude reaction mixture was chromatographed on a silica gel column using hexane−ethyl acetate gradient, yielding **3** as a major compound (68.3%) and **5** as a minor compound (6.5%). The similar reaction procedure has been applied to 8-hydroxymanzamine A, yielding **4** as a major compound (51.7%) and **12** as a minor compound (1.6%).

**Tetrahydromanzamine A (3):** Yield 68.3%. $^1$H NMR ($CDCl_3$) $\delta$ 8.64 (d, $J$ = 5.0), 7.83 (d, $J$ = 5.1), 8.09 (d, $J$ = 7.7), 7.26 (dd, $J$ = 7.6, 7.5), 7.53 (dd, $J$ = 7.6, 7.5), 7.58 (d $J$ = 7.5), 6.61 (s), 2.02 (m), 1.83 (m), 2.30 (m), 2.21 (m), 1.53 (m), 1.68 (m), 2.57 (m), 1.57, (m), 1.70 (m), 1.72 (m), 2.68 (m), 2.43 (m), 2.80 (m), 1.99 (m), 2.30 (m), 3.14 (m), 3.78 (s) 3.38 (m), 2.95 (m), 1.80 (m), 1.65 (m), 1.55 (m), 1.77 (m), 2.15 (m), 1.55 (m), 1.65 (m), 3.11 (m), 1.85 (m), 1.56 (m), 2.62 (brd, $J$ = 11.9), 2.30 (d, $J$ = 12.0). $^{13}$C NMR ($CDCl_3$) $\delta$ 142.3, 142.1, 139.4, 138.6, 136.9, 134.7, 131.4, 127.6, 123.4, 120.9, 120.7, 113.2, 112.9, 75.6, 70.4, 69.3, 63.3, 58.5, 53.3, 52.7, 51.6, 44.7, 37.6, 37.4, 34.1, 32.5, 32.3, 31.7, 31.7, 29.2, 28.0, 27.8, 27.2, 26.2, 24.9, 20.2. HRESIMS $m/z$ calcd for $C_{36}H_{49}N_4O$ $[M + H]^+$ 553.3906, found 553.3922.

**Dihydromanzamine A (5):** Yield 6.5%. $^1$H NMR ($CDCl_3$) $\delta$ 8.56 (d, $J$ = 5.0), 7.87 (d, $J$ = 5.1), 8.25 (d, $J$ = 7.7), 7.21 (dd, $J$ = 7.6, 7.5), 7.49 (dd, $J$ = 7.6, 7.5), 7.46 (d, $J$ = 7.5), 6.59 (s), 2.10 (m), 1.87 (m), 2.36 (m), 2.22 (m), 1.52 (m), 1.63 (m), 2.52 (m), 1.53 (m), 1.79 (m), 1.79 (m), 2.61 (m), 2.47 (m), 2.87 (m), 1.93 (m), 2.33 (m), 3.18 (m), 3.72 (s), 3.31 (m), 2.97 (m), 1.84 (m), 1.67 (m), 1.53 (m), 1.73 (m), 5.89 (m), 5.61 (m), 3.19 (m) 1.81 (m), 1.53 (m), 2.61 (brd, $J$ = 11.9), 2.36 (d, $J$ = 12.0). $^{13}$C NMR ($CDCl_3$) $\delta$ 142.9, 142.8, 139.8, 137.8, 136.2, 134.2, 130.6, 130.2, 129.7, 127.8, 123.7, 120.7, 120.2, 113.5, 113.2, 75.2, 70.8, 69.8, 63.8, 58.3, 53.9, 52.1, 51.3, 44.4, 37.8, 37.3, 32.9, 32.6, 31.8, 31.6, 28.6, 28.4, 27.6, 26.2, 24.4, 20.9. HRESIMS $m/z$ calcd for $C_{36}H_{47}N_4O$ $[M + H]^+$ 551.3750, found 551.3758.

**Tetrahydro-8-hydroxymanzamine A (4):** Yield 51.7%. $^1$H NMR ($CDCl_3$) $\delta$ 8.40 (d, $J$ = 5.2), 7.78 (d, $J$ = 5.2), 7.56 (d, $J$ = 7.7), 7.03 (dd, $J$ = 7.7, 7.5), 6.96 (d, $J$ = 7.5), 6.78 (s), 2.07 (m), 1.60 (m), 1.42 (m), 1.73 (m), 1.58 (m), 1.55 (m), 1.64 (m), 1.43 (m) 2.45 (dd, $J$ = 12.0, 5.3), 2.32 (m), 2.97 (m), 2.34 (m), 2.05 (m), 1.74 (m), 3.36 (m), 3.76 (s), 3.44 (m), 2.97 (m), 1.70 (m), 2.05 (m), 1.75 (m), 1.58 (m), 1.75 (m), 1.72 (m), 2.99 (m), 2.40 (m), 2.62 (d, $J$ = 11.2), 2.18 (d, $J$ = 11.2). $^{13}$C NMR ($CDCl_3$) $\delta$ 142.1, 139.1, 138.2, 136.6, 135.4, 132.7, 130.1, 129.7, 123.5, 120.5, 117.5, 113.5, 110.4, 75.3, 70.2, 69.8, 63.9, 58.2, 53.7, 52.4, 51.6, 44.9, 37.7, 37.7, 33.5, 32.7, 32.1, 31.9, 30.9, 29.3, 28.8, 28.6, 27.9, 26.2, 24.7, 20.3. HRESIMS $m/z$ calcd for $C_{36}H_{49}N_4O_2$ $[M + H]^+$ 569.3856, found 569.3961.

**Dihydro-8-hydroxymanzamine A (12):** Yield 16%. $^1$H NMR ($CDCl_3$) $\delta$ 8.31 (d, $J$ = 5.2), 7.81 (d, $J$ = 5.2), 7.58 (d, $J$ = 7.6), 7.12 (dd, $J$ = 7.7, 7.6), 7.05 (d, $J$ = 7.6), 6.18, (s), 1.55 (m), 1.45 (m), 1.46 (m), 1.55 (m), 1.57 (m), 1.66 (m), 1.70 (m), 2.58 (m), 2.25 (m) 3.11 (m), 2.03 (m), 2.34 (m), 1.67 (m), 1.85 (m), 4.93 (s), 4.65 (brd, $J$ = 4.9), 4.24 (m), 1.70, (m), 2.02 (m), 1.65 (m), 1.50 (m), 5.92 (m), 5.67 (m), 2.99 (m), 2.60 (m), 2.52 (d, $J$ = 11.3) 2.20 (m). $^{13}$C NMR ($CDCl_3$) $\delta$ 142.6, 142.3, 139.4, 138.7, 136.9, 134.7, 133.9, 130.8, 130.6, 129.6, 127.8, 123.7, 120.3, 120.2, 113.2, 75.2, 70.9, 69.8, 63.5, 58.4, 53.4, 52.7, 51.7, 44.4, 37.9, 37.3, 32.3, 32.2, 31.8, 31.4, 29.4, 28.6, 27.2, 26.9, 24.3, 20.9. HRESIMS $m/z$ calcd for $C_{36}H_{47}N_4O_2$ $[M + H]^+$ 567.3699, found 567.3708.

**General Preparation of Compounds 10 and 11. 1** (54.8 mg, 0.1 mmol) was dissolved in 15 mL of dry acetone, with 16.4 mg (1.2 mmol) of anhydrous $K_2CO_3$ and 11 $\mu$L of anhydrous acetic anhydride added. The reaction was refluxed for 9 h under nitrogen. The completion of the reaction was monitored by TLC, and the solvent was removed in vacuo. Then 100 mL of ethyl acetate was added and washed with an equal amount of brine solution. This was repeated twice and the organic layer was dried on $Na_2SO_4$ (200 g), and the solvent was removed in vacuo. The reaction mixture was chromatographed on a silica gel column using hexane−ethyl acetate gradient yielded compound **10** (49.8%). The similar reaction procedure has been applied to 8-hydroxymanzamine A (**2**) to yield the compound **11** (46.4%).

**12,13-Dehydromanzamine A (10):** Yield 49.8%. $^1$H NMR ($CDCl_3$) $\delta$ 8.41 (d, $J$ = 5.3), 7.81 (d, $J$ = 5.3), 7.96 (d, $J$ = 7.5), 7.14 (dd, $J$ = 7.6, 7.5), 7.30 (d, $J$ = 7.6), 7.68 (d, $J$ = 7.6), 6.60 (s), 6.45 (dd, $J$ = 8.5, 7.0), 2.12 (m), 5.43 (m), 5.35 (m), 1.89 (m), 1.73 (m) 1.38 (m), 1.92 (m), 2.62 (m), 2.51 (m), 2.71 (m), 1.98 (m), 2.73 (m), 2.41 (m), 3.96 (m), 4.88 (s), 3.85 (dd, $J$ = 14.7, 4.7), 3.34 (dd, $J$ = 14.7, 10.8), 1.38 (m), 1.88 (m), 1.60 (m), 2.15 (m), 5.87 (dd, $J$ = 7.7, 7.6), 5.24 (m), 2.41 (m), 1.46 (d, $J$ = 12.9), 2.62 (d, $J$ = 10.9), 2.51 (d, $J$ = 10.9). $^{13}$C NMR ($CDCl_3$) $\delta$ 142.9, 142.6, 142.4, 139.7, 138.5, 134.3, 130.8, 130.7, 129.7, 129.3, 127.8, 127.6, 123.6, 120.9, 120.8, 118.3, 115.8, 113.7, 113.7, 75.9, 69.6, 63.4, 58.7, 53.3, 52.9, 51.3, 44.8, 37.3, 32.3, 31.5, 29.3, 28.8, 27.8, 26.5, 24.8, 21.1. HRESIMS $m/z$ calcd for $C_{36}H_{43}N_4$ $[M + H]^+$ 531.3488, found 531.3495.

**12,13-Dehydro-8-*O*-acetylmanzamine A (11):** Yield 46.4%. $^1$H NMR ($CDCl_3$) $\delta$ 8.38 (d, $J$ = 5.6), 7.82 (d, $J$ = 5.6), 7.97 (d, $J$ = 7.8), 7.23 (dd, $J$ = 7.8, 7.6), 7.29 (d, $J$ = 7.6), 6.50 (s) 6.43 (dd, $J$ = 8.5, 7.0), 2.21 (m), 1.80 (m), 5.56 (m), 5.50 (m), 1.65 (m), 1.42 (m), 1.30 (m), 1.40 (m), 1.32 (m), 2.60 (m), 2.43 (m), 2.83 (m), 1.95 (m), 2.81 (m), 2.44 (m), 3.50 (s), 3.14 (dd, $J$ = 11.9, 8.7), 2.99 (dd, $J$ = 11.8, 4.7), 1.76 (m), 2.15 (m), 2.05 (m), 2.42 (m), 5.84 (dd, $J$ = 7.7, 7.6), 5.21 (m), 3.99 (dd, $J$ = 8.3, 8.2), 2.42 (m), 1.49 (d, $J$ = 12.9), 2.76 (d, $J$ = 11.5), 2.30 (m), 2.53 (s). $^{13}$C NMR ($CDCl_3$) $\delta$ 167.8, 142.8, 142.8, 142.6, 139.2, 138.7, 136.9, 134.5, 134.4, 130.6, 130.6, 129.4, 128.3, 128.1, 127.8, 123.9, 120.2, 120.1, 117.4, 113.3, 75.8, 69.5, 63.5, 58.6, 53.9, 52.7, 51.2, 44.7, 37.2, 32.2, 31.4, 29.3, 28.2, 27.8, 26.4, 24.3, 20.2, 17.8.; HRESIMS $m/z$ calcd for $C_{38}H_{45}N_4O_2$ $[M + H]^+$ 589.3543, found 589.3548.

**8-Acetoxymanzamine A (9):** see ref 64.

**6-Nitro-manzamine A (8) and 8-nitromanzamine A (7):** Manzamine A (**1**) (55 mg) was dissolved in 3 mL of trifluoroacetic acid and cooled to 0 °C in ice bath. $NaNO_2$ (11 mg) was added in one portion and stirred at 0 °C for 1.5 h. The mixture was poured into water and the precipitate was collected by centrifuge. The precipitate was separated using silica gel preparative TLC (hexane:acetone:triethylamine 8:2:0.1) to yield **8** (6 mg) and **7** (6 mg). 6-Nitro-manzamine A (**8**): yellow powder. $^1$H NMR ($CDCl_3$) $\delta$ 9.04 (d, $J$ = 2.0), 8.49 (d, $J$ = 5.2), 8.41 (dd, $J$ = 9.2, 2.0), 7.89 (d, $J$ = 5.2), 7.77 (d, $J$ = 9.2), 6.50 (s), 6.19 (s), 5.58 (m), 5.38 (t, 10.8), 4.70 (br), 3.69 (s), 3.25 (t, 11.0), 2.93 (d, $J$ = 9.0), 2.80−2.20 (m), 2.10−1.20 (m). $^{13}$C NMR ($CDCl_3$) $\delta$ 144.5, 144.2, 142.7, 141.2, 141.1, 139.4, 135.7, 134.5, 133.2, 129.7, 127.0, 123.7, 123.5, 121.0, 118.4, 114.2, 112.9, 77.9, 71.2, 70.5, 57.6, 53.7 (2C), 49.4, 47.1, 44.9, 40.8, 39.5, 33.9, 28.7, 26.6, 26.6, 25.2, 24.8, 24.3, 20.9. HRESIMS $m/z$ calcd for $C_{36}H_{44}N_5O_3$. $[M + H]^+$ 594.3444, found 594.3435. 8-Nitromanzamine A (**7**): yellow powder. $^1$H NMR ($CDCl_3$) $\delta$ 10.4 (s), 8.57 (d, $J$ = 5.2), 8.48 (d, $J$ = 8.0), 8.45 (d, $J$ = 8.0), 7.87 (d, $J$ = 5.2), 7.40 (t, 8.0), 6.45 (s), 5.96 (m), 5.69 (m), 5.55(m), 5.32 (t, $J$ = 10), 4.27 (br), 3.58 (s), 3.11 (m), 2.61 (m), 2.50−1.6 (m), 1.4 (m). $^{13}$C NMR ($CDCl_3$) $\delta$ 144.6, 1443.6, 142.7, 141.4, 141.2, 139.4, 135.3, 134.6, 133.2, 130.0, 127.1, 123.8, 123.7, 121.1, 118.5, 114.4, 113.0, 77.8, 71.2, 70.6, 57.7, 53.7, 53.72, 53.68, 49.5, 47.1, 44.8, 40.5, 39.8, 34.1, 28.8, 26.7, 26.6, 25.3, 24.9, 24.3, 21.0. HRESIMS $m/z$ calcd for $C_{36}H_{44}N_5O_3$. $[M + H]^+$ 594.3444, found 594.3426.

**21,27-*N*-oxamanzamine A (13):** Manzamine A (**1**) (56 mg) and 50 mg of 3-chloroperoxybenzoic acid were dissolved in 1 mL of $CH_2Cl_2$. After 20 mg of sodium bicarbonate was added, the solution was stirred overnight at room temperature. The reaction mixture was washed with 5% sodium bicarbonate solution and extracted with chloroform. The chloroform layer was evaporated, and the residue was purified using silica gel preparative TLC ($CHCl_3$:MeOH 85:15) to get **13** (20 mg). 21,27-*N*-oximanzamine A (**13**): yellow powder. $^1$H NMR ($CDCl_3$−$CD_3OD$) $\delta$ 9.21 (s), 8.35 (d, $J$ = 5.2), 8.08 (d, $J$ = 7.6), 7.82 (d, $J$ = 5.2), 7.49 (m), 7.26 (t, $J$ = 7.6), 6.21 (dt, $J$ = 10, 7.6), 6.12 (s), 5.71 (t, $J$ = 10), 5.60 (br), 5.44 (br), 4.49 (m), 3.91 (d, $J$ = 13.6), 3.84 (s), 3.35 (m), 3.11 (d, $J$ = 10.4), 2.97 (s), 2.72 (m), 2.51 (m), 2.29 (m), 2.10−1.39 (m). $^{13}$C NMR ($CDCl_3$) $\delta$ 142.2, 140.7, 139.7, 137.6, 136.7, 133.4, 133.3, 133.2, 132.3, 130.7, 129.7, 128.8, 126.4, 121.5, 121.1, 120.1, 114.3, 112.0, 74.5, 73.7, 70.4, 61.9, 61.4, 49.6, 46.5, 43.1, 38.8, 37.5, 27.7, 25.7, 23.1, 23.1, 22.2, 19.4. HRESIMS $m/z$ calcd for $C_{36}H_{45}N_4O_3$ 581.3492 $[M + H]^+$ found 581.3474 $[M + H]^+$, 603.3301 $[M + Na]^+$, 563.3359 $[M - H_2O]^+$, 291.1766 $[M + 2H]^{2+}$, 282.1719 $[M + 2H - H_2O]^{2+}$.

**General Preparation of Compounds 20a−d and 21a−d.** Method A: **19**, tryptamine derivatives, and molecular sieves were stirred at room temperature in $CH_2Cl_2$ or EtOH (3 mL) for 2 h. TFA (50 $\mu$L) was then added to the reaction mixture and further stirred for another 3 h at 50 °C. The reaction mixture was chromatographed over silica gel immediately to give the Pictet−Spengler product.

Method B: To **19** and tryptamine derivatives in 1 mL anhydrous EtOH were added 0.2 mL of trifluoroacetic acid (TFA). The mixture was then absorbed onto 1.5 g of silica gel. The silica gel mixture was placed in a glass vessel and was irradiated in a microwave oven at 390 W for 10 min. The crude mixture was then eluted from the silica gel using acetone, concentrated, and chromatographed over silica gel to furnish the Pictet−Spengler product.

**Manzamine D (20a): 19** (46.3 mg) and tryptamine (31 mg) in $CH_2Cl_2$ were reacted as described in method A to give manzamine D (**20a**, 52.7 mg, 86%). Manzamine D (**20a**): white amorphous. $^1$H NMR ($CDCl_3$) $\delta$ 7.78 (br s), 7.51 (d, $J$ = 7.5), 7.35 (brd, $J$ = 7.5), 7.17 (t, $J$ = 7.5), 7.12(t, $J$ = 7.5), 5.95 (dd, $J$ = 18.7), 5.77 (brs), 5.67 (q, $J$ = 9), 5.55 (td, $J$ = 10, 5), 5.30 (t, $J$ = 10), 4.60 (s), 4.17 (t, $J$ = 7), 3.42 (s), 3.35 (m), 3.04 (m), 2.83(m), 2.75(m), 2.51 (m), and 1.20−2.40 (complex). $^{13}$C NMR ($CDCl_3$) $\delta$ 141.2, 136.8, 135.6, 134.5, 133.9, 132.2, 129.9, 128.8, 127.8, 121.4, 119.2, 118.0, 111.0, 109.4, 75.3, 69.6, 68.5, 59.7, 54.9, 53.4, 50.8, 49.7, 47.3, 44.6, 43.2, 40.7, 37.8, 33.0, 31.8, 28.2, 26.9, 26.0, 25.7, 22.4, 21.7. HRESIMS $m/z$ calcd for $C_{36}H_{49}N_4O$ $[M + H]^+$ 553.3906, found 553.3898.

**2*N*-Methylmanzamine D (20b): 19** (50.2 mg) and (2*N*)-methyltryptamine (30.2 mg) in $CH_2Cl_2$ were reacted as described in method A to give *N*(2)-methylmanzamine D (**20b**, 22.5 mg, 33%) and unreacted ircinal A (**19**, 27.9 mg). *N*(2)-methylmanzamine D (**20b**): yellowish oil. $^1$H NMR ($CDCl_3$) $\delta$ 7.47 (br s), 7.46 (d, $J$ = 7.6), 7.31 (brd, $J$ = 7.6), 7.12 (t, $J$ = 7.2), 7.07 (t, $J$ = 7.2), 5.88 (q, $J$ = 8), 5.80 (s), 5.61 (q, $J$ = 9), 5.48 (td, $J$ = 10.5), 5.23 (t, $J$ = 9), 4.16 (brs), 3.82 (s), 3.40 (s), 3.11 (m), 2.99 (m), 2.88 (m), 2.40 (s), and 1.2−2.7 (complex). $^{13}$C NMR ($CDCl_3$) $\delta$ 141.2, 136.5, 135.9, 134.8, 134.1, 132.3, 129.8, 128.9, 121.5, 119.5, 118.2, 111.3, 108.6, 75.5, 69.5, 68.5, 68.1, 55.3, 53.9, 53.6, 51.2, 49.9, 47.2, 44.6, 41.7, 37.1, 32.4, 32.2, 30.0, 29.5, 28.3, 27.1, 26.3, 25.9, 21.9, 21.6. HRESIMS $m/z$ calcd for $C_{37}H_{51}N_4O$ $[M + H]^+$ 567.4063, found 567.4042.

**6-Methoxymanzamine D (20c): 19** (38.8 mg) and 5-methoxytryptamine (**6**, 56.1 mg) in $CH_2Cl_2$ were reacted as described in method A to give 6-methoxymanzamine D (**20c**, 48.6 mg, 89%). Ircinal A (**19**, 29.5 mg) and 5-methoxytryptamine (26.4 mg) in anhydrous EtOH were reacted as described in method B to give 6-methoxymanzamine D (**20c**, 35.1 mg, 83%). 6-Methoxymanzamine D (**20c**): white amorphous. $^1$H NMR ($CDCl_3$) $\delta$ 7.52

(brs), 7.21 (d, $J$ = 8.8), 6.93 (d, $J$ = 1.6), 6.79 (dd, $J$ = 8.8, 1.6), 5.90 (q, $J$ = 8.3), 5.73 (brs), 5.62 (q, $J$ = 8.5), 5.50 (td, $J$ = 10.4, 4.4), 5.22 (t, $J$ = 9.2), 4.14 (brs), 3.85 (s), 3.33 (m), and 1.00−3.20 (complex). $^{13}$C NMR ($CDCl_3$) $\delta$ 153.4, 141.1, 136.8, 134.8, 134.5, 132.2, 130.6, 129.9, 128.7, 128.2, 111.6, 111.1, 109.2, 100.4, 75.3, 69.5, 68.5, 59.8, 55.9, 54.8, 53.4, 50.7, 49.6, 47.3, 44.5, 43.2, 40.6, 37.7, 33.0, 31.8, 28.5, 26.8, 25.9, 25.7, 22.5, 21.7. HRESIMS $m/z$ calcd for $C_{37}H_{51}N_4O_2$ $[M + H]^+$ 583.4012, found 583.4033.

**Methyl Manzamine D-3-carboxylate (20d): 19** (83.2 mg) and D-tryptophan methyl ester HCl (67.1 mg) in EtOH were reacted as described in method A to give **20d** (87.6 mg, 70%). Ircinal A (**19**, 105.6 mg) and D-tryptophan methyl ester HCl (67.7 mg) in anhydrous EtOH were reacted as described in method B to give **20d** (95.4 mg, 61%). **20d**: white amorphous. $^1$H NMR ($CDCl_3$) $\delta$ 7.60 (brs), 7.48 (d, $J$ = 7), 7.35 (d, $J$ = 7), 7.16 (td, $J$ = 7.1), 7.10 (t, $J$ = 7), 5.90 (q, $J$ = 10), 5.86 (brs), 5.64 (q, $J$ = 8), 5.52 (td, $J$ = 10.4), 5.22 (t, $J$ = 9), 4.75 (brs), 4.17 (t, $J$ = 6), 3.82 (s), 3.37 (br s), 3.12 (brd, $J$ = 15), 3.02 (m), 2.88 (brt, $J$ = 12), 2.65 (d, $J$ = 11), and 1.20−2.55 (complex). $^{13}$C NMR ($CDCl_3$) $\delta$ 173.4, 171.1, 142.8, 136.2, 132.7, 132.6, 127.5, 127.1, 121.7, 119.3, 117.8, 111.4, 108.7, 70.5, 70.1, 60.4, 59.7, 56.8, 56.2, 53.4, 52.5, 52.1, 49.3, 47.1, 44.2, 40.0, 37.7, 32.9, 28.3, 26.5, 25.7, 25.1, 24.8, 24.6, 21.0, 21.0, 14.2. HRESIMS $m/z$ calcd for $C_{38}H_{51}N_4O_3$ $[M + H]^+$ 611.3961, found 611.3947.

**General Preparation of Compounds (21a) and (21b).** To a solution of manzamine D derivatives in $CH_2Cl_2$ (2 mL) at room temperature was added DDQ (2,3-dichloro-5,6-dicyanobenzoquinone) in benzene (2 mL) and the mixture was stirred for 1 h. The mixture was then washed with saturated $NaHCO_3$ (5 mL), extracted with $CH_2C_{l2}$ (2 × 5 mL), dried ($MgSO_4$), and evaporated to give brownish oil. Chromatography of the reaction products over silica gel gave the products.

**6-Methoxymanzamine A (21a): 20c** (141.52 mg) and DDQ (69.5 mg) were reacted as described above to give 6-methoxymanzamine A (**21a**, 46.4 mg, 33%). 6-Methoxymanzamine A (**21a**): yellowish oil. $^1$H NMR ($CDCl_3$) $\delta$ 8.63 (brs), 8.40 (d, $J$ = 5), 7.78 (d, $J$ = 5), 7.53 (d, $J$ = 2), 7.40 (dd, $J$ = 8, 2), 6.40 (s), 5.91 (brs), 5.62 (q, $J$ = 8.5), 5.52 (td, $J$ = 10.5), 5.26 (t, $J$ = 9), 4.31 (brs), 3.92 (s), 3.50 (d, $J$ = 4), 3.08 (m), 2.78 (m), 2.56 (m), and 1.2−2.5 (complex). $^{13}$C NMR ($CDCl_3$) $\delta$ 154.2, 143.5, 139.9, 138.2, 137.4, 135.1, 134.5, 134.2, 132.3, 129.9, 129.2, 128.5, 122.2, 118.3, 117.2, 113.3, 112.5, 103.5, 75.1, 70.0, 68.7, 56.0, 55.0, 53.5, 50.9, 49.6, 47.1, 44.7, 40.9, 40.3, 32.7, 31.7, 28.1, 26.8, 26.0, 25.7, 21.7. HRESIMS $m/z$ cacld for $C_{37}H_{47}N_4O_2$. $[M + H]^+$ 579.3699, found 579.3704.

**Methyl Manzamine A-3-carboxylate (21b): 20d** (26.9 mg) and DDQ (17.3 mg) were reacted as described above to gave **21b** (18.1 mg, 68%). **21b**: yellowish oil. $^1$H NMR ($CDCl_3$) $\delta$ 9.25 (brs), 8.92 (s), 8.32 (d, $J$ = 8), 7.77 (d, $J$ = 8), 7.74 (t, $J$ = 8), 7.50 (t, $J$ = 8), 6.59 (s), 6.11 (br s), 5.79 (q, $J$ = 8), 5.70 (td, $J$ = 10.5), 5.44 (t, $J$ = 9), 4.20 (s), 3.72 (brs), and 1.4−3.4 (complex). $^{13}$C NMR ($CDCl_3$) $\delta$ 166.8, 143.4, 140.4, 139.5, 138.2, 137.2, 134.8, 132.3, 129.7, 129.3, 128.7, 128.4, 122.1, 121.6, 120.9, 116.5, 112.1, 74.9, 69.9, 68.6, 55.1, 53.6, 52.4, 50.9, 49.6, 47.2, 46.9, 44.8, 40.8, 40.5, 34.7, 33.1, 31.6, 28.2, 26.8, 25.9, 25.7, 21.6. HRESIMS $m/z$ cacld for $C_{38}H_{47}N_4O_3$. $[M + H]^+$ 607.3648, found 607.3655.

**General Preparation of Compounds 17a−g. 14** (54.8 mg, 0.1 mmol) was dissolved in 15 mL of chloroform and a catalytic amount of piperidine was added to the aromatic aldehyde (2.33 mg, 0.12 mmol) and stirred for an additional 5 min and then refluxed for 8 h. The completion of the reaction was monitored by TLC, and the chloroform in reaction mixture was evaporated and the residue was washed with water and extracted with chloroform. The crude product was chromatographed on a silica gel column using a hexane/acetone (7:3). The analogues of **14** (**17a−g**) were synthesized using the same reaction conditions.

**31-(3-Methoxyphenyl)manzamine F (17a):** Yield 55%. $^1$H NMR ($CDCl_3$) $\delta$ 8.21 (d, $J$ = 5.2), 7.71 (d, $J$ = 5.2), 7.50 (d, $J$ = 7.6), 7.00 (t, $J$ = 7.6), 6.92 (s) 6.89 (d, $J$ = 7.6), 6.39 (s), 5.02 (m), 5.40 (ddd, $J$ = 10.8, 10.8, 4), 3.90 (brs), 3.80 (s), 3.64 (s), 3.23 (brt, $J$ = 4.5), 2.84 (brs), 2.64 (brd, $J$ = 10.8), 2.44 (m), 2.16 (m), 2.16 (m), 1.99 (m), 1.77−1.50 (m), 1.45 (m), 1.31 (m), 1.06 (m). HRESIMS $m/z$ calcd for $C_{44}H_{51}N_4O_4$ $[M + H]^+$ 699.3910, found 699.3888. $[M + 2H]^{+2}$ 350.2013.

**31-(4-Methoxyphenyl)manzamine F (17b):** Yield 50%. $^1$H NMR ($CDCl_3$) $\delta$ 8.22 (d, $J$ = 5.2), 7.71 (d, $J$ = 5.2), 7.50 (d, $J$ = 7.6), 7.00 (t, $J$ = 7.6), 6.92 (s) 6.89 (d, $J$ = 7.6), 6.39 (s), 5.01 (m), 5.40 (ddd, $J$ = 10.8, 10.8, 4), 3.90 (brs), 3.80 (s), 3.64 (s), 3.23 (brt, $J$ = 4.5), 2.84 (brs), 2.64 (brd, $J$ = 10.8), 2.44 (m), 2.16 (m), 2.16 (m), 1.99 (m), 1.77−1.50 (m), 1.45 (m), 1.31 (m), 1.06 (m). $^{13}$C NMR ($CDCl_3$) $\delta$ 197.3, 159.3, 143.5, 143.3, 143.3, 140.2, 138.6, 138.2, 133.6, 132.3, 132.0, 131.8, 130.9, 129.7, 128.6, 123.4, 121.7, 121.2, 114.1, 113.6, 81.3, 76.2, 68.7, 63.6, 55.2, 53.1, 52.8, 49.7, 47.2, 46.6, 41.8, 41.5, 39.0, 33.3, 33.1, 26.7, 25.4, 24.9, 24.1, 21.8. HRESIMS $m/z$ calcd for $C_{44}H_{50}N_4O_4$ $[M + H]^+$ 699.3910, found 699.3887. $[M + 2H]^{+2}$ 350.1977.

**31-(4-Nitrophenyl)manzamine F (17c):** Yield 45%. $^1$H NMR ($CDCl_3$) $\delta$ 8.21 (d, $J$ = 5.2), 7.7 (d, $J$ = 5.2), 7.50 (d, $J$ = 7.6), 7.00 (t, $J$ = 7.6), 6.92 (s) 6.89 (d, $J$ = 7.6), 6.39 (s), 5.01 (m), 5.40 (ddd, $J$ = 10.8, 10.8, 4), 3.90 (brs), 3.64 (s), 3.23 (brt, $J$ = 4.5), 2.84 (brs), 2.64 (brd, $J$ = 10.8), 2.44 (m), 2.16 (m), 2.16 (m), 1.99 (m), 1.77−1.50 (m), 1.45 (m), 1.31 (m), 1.06 (m). HRESIMS $m/z$ calcd for $C_{43}H_{48}N_5O_5$ $[M + H]^+$ 714.3655, found 714.3687. $[M + 2H]^{+2}$ 357.6822.

**31-(2,6-Dichlorophenyl)manzamine F (17d):** Yield 40%. $^1$H NMR ($CDCl_3$) $\delta$ 8.20 (d, $J$ = 5.2), 7.71 (d, $J$ = 5.2), 7.52 (d, $J$ = 7.6), 7.00 (t, $J$ = 7.6), 6.92 (s) 6.89 (d, $J$ = 7.6), 6.40 (s), 5.01 (m), 5.41 (ddd, $J$ = 10.8, 10.8, 4), 3.90 (brs), 3.64 (s), 3.23 (brt, 4.5), 2.84 (brs), 2.64 (brd, $J$ = 10.8), 2.44 (m), 2.16 (m), 2.16 (m), 1.99 (m), 1.77−1.50 (m), 1.45 (m), 1.31 (m), 1.06 (m). HRESIMS $m/z$ calcd for $C_{43}H_{47}Cl_2N_4O_3$ $[M + H]^+$ 737.3025, found 737.3028. $[M + 2H]^{+2}$ 369.1557.

**31-(3,4-Dimethoxyphenyl)manzamine F (17e):** Yield 47%. H NMR ($CDCl_3$) $\delta$ 8.20 (d, $J$ = 5.2), 7.71 (d, $J$ = 5.2), 7.50 (d, $J$ = 7.6), 7.00 (t, $J$ = 7.6), 6.91 (s) 6.89 (d, $J$ = 7.6), 6.39 (s), 5.01 (m), 5.41 (ddd, $J$ = 10.8, 10.8, 4), 3.90 (brs), 3.80 (s), 3.64 (s), 3.23 (brt, $J$ = 4.5), 2.84 (brs), 2.64 (brd, $J$ = 10.8), 2.44 (m), 2.16 (m), 2.16 (m), 1.99 (m), 1.77−1.50 (m), 1.45 (m), 1.31 (m), 1.06 (m). HRESIMS $m/z$ calcd for $C_{45}H_{53}N_4O_5$ $[M + H]^+$ 729.4016, found 729.4018. $[M + 2H]^{+2}$ 365.2025.

**31-(4-Bromophenyl)manzamine F (17f):** Yield 40%. $^1$H NMR ($CDCl_3$) $\delta$ 8.23 (d, $J$ = 5.2), 7.72 (d, $J$ = 5.2), 7.50 (d, $J$ = 7.6), 7.00 (t, $J$ = 7.6), 6.94 (s), 6.89 (d, $J$ = 7.6), 6.39 (s), 5.01 (m), 5.40 (ddd, $J$ = 10.8, 10.8, 4), 3.91 (brs), 3.80 (s), 3.64 (s), 3.23 (brt, $J$ = 4.5), 2.84 (brs), 2.64 (brd, $J$ = 10.8), 2.44 (m), 2.16 (m), 2.16 (m), 1.99 (m), 1.77−1.50(m), 1.45 (m), 1.31 (m), 1.06 (m). HRESIMS $m/z$ calcd for $C_{43}H_{48}BrN_4O_3$ $[M + H]^+$ 747.2910, found 747.2930. $[M + 2H]^{+2}$ 374.1516.

**31-(4-Fluorophenyl)manzamine F (17g):** Yield 45%. $^1$H NMR ($CDCl_3$) $\delta$ 8.21 (d, $J$ = 5.2), 7.71 (d, $J$ = 5.2), 7.50 (d, $J$ = 7.6), 7.00 (t, $J$ = 7.6), 6.92 (s) 6.89 (d, $J$ = 7.6), 6.39 (s), 5.01 (m), 5.40 (ddd, $J$ = 10.8, 10.8, 4), 3.90 (brs), 3.80 (s), 3.64 (s), 3.23 (brt, $J$ = 4.5), 2.84 (brs), 2.64 (brd, $J$ = 10.8), 2.44 (m), 2.16 (m), 2.16 (m), 1.99 (m), 1.77−1.50 (m), 1.45 (m), 1.31 (m), 1.06 (m). HRESIMS $m/z$ calcd for $C_{43}H_{48}FN_4O_3$ $[M + H]^+$ 687.3710, found 687.3713. $[M + 2H]^{+2}$ 344.1875.

**31-(2-Chlorophenyl)manzamine F (17h):** Yield 45%. $^1$H NMR ($CDCl_3$) $\delta$ 8.22 (d, $J$ = 5.2), 7.73 (d, $J$ = 5.2), 7.51 (d, $J$ = 7.6), 7.02 (t, $J$ = 7.6), 6.93 (s) 6.89 (d, $J$ = 7.6), 6.39 (s), 5.03 (m), 5.42 (ddd, $J$ = 10.8, 10.8, 4), 3.92 (brs), 3.82 (s), 3.64 (s), 3.23 (brt, $J$ = 4.5), 2.84 (brs), 2.64 (brd, $J$ = 10.8), 2.44 (m), 2.16 (m), 2.16 (m), 1.99 (m), 1.77−1.50 (m), 1.45 (m), 1.31 (m), 1.06 (m). HRESIMS $m/z$ calcd for $C_{43}H_{48}ClN_4O_3$ $[M + H]^+$ 703.3415, found 703.3445. $[M + 2H]^{+2}$ 352.1750.

**Manzamine F-31-hydrazone (15): 14** (58 mg) 2 mL of anhydrous ethanol. Hydrazine hydrate (200 $\mu$L) was added and refluxed for 2 h. The temperature was allowed to cool down to room temperature and **15** crystals formed. The mother liquid

was purified using silica gel preparative TLC ($CHCl_3$:MeOH 95:5), which gave additional amount of **15**. **15**: pale yellow crystals. $^1$H NMR $\delta$ 9.13 (s), 8.43 (d, $J = 4.4$), 8.09 (d, $J = 7.6$), 7.82 (d, $J = 4.4$), 7.53 (br), 7.25 (s), 6.42 (s), 5.90 (m), 5.62 (m), 5.52 (m), 5.26 (t, $J = 9.6$), 4.35 (br), 3.52 (s), 3.05 (m), 2.78 (m), 2.58 (m), 2.43−1.86 (m), 1.80−1.3 (m). HRESIMS $m/z$ calcd for $C_{36}H_{47}N_6O_2$ $[M + H]^+$ 595.3761, found 595.3782.

**31$\beta$-Hydroxymanzamine F (16):** First, 58 mg of **14** was dissolved in 1 mL of methanol and cooled to 0 °C. Then 20 mg of $NaBH_4$ was added and stirred for 10 min. Then 10 mL of water was added and extracted with ether 5 mL × 3. Ether was evaporated and the product was purified by silica gel preparative TLC ($CHCl_3$:MeOH 9:1) to yield **16** (24 mg). **16**: yellow powder. $^1$H NMR ($CDCl_3$) $\delta$ 8.21 (d, $J = 5.2$), 7.71 (d, $J = 5.2$), 7.50 (d, $J = 7.6$), 7.00 (t, $J = 7.6$), 6.89 (d, $J = 7.6$), 6.39 (s), 5.01 (m), 5.40 (ddd, $J = 10.8$, 10.8, 4), 3.90 (brs), 3.64 (s), 3.23 (brt, $J = 4.5$), 2.84 (brs), 2.64 (brd, $J = 10.8$), 2.44 (m), 2.16 (m), 2.16 (m), 1.99 (m), 1.77−1.50 (m), 1.45 (m), 1.31 (m), 1.06 (m). $^{13}$C NMR ($CDCl_3$) $\delta$ 137.8, 136.7, 132.6, 127.6, 120.9, 113.8, 113.2, 112.5, 81.8, 70.3, 68.5, 63.2, 53.3, 52.6, 49.4, 48.0, 41.7, 40.4, 36.5, 35.5, 33.0, 30.8, 26.5, 25.5, 25.1, 24.9, 21.5. HRESIMS $m/z$ calcd for $C_{36}H_{47}N_4O_3$ $[M + H]^+$ 583.3648, found 583.3664.

**31-Ethylmanzamine F (18): 14** (58 mg) was dissolved in 2 mL of tetrahydrofuran and cooled in dry ice acetone bath, then 10 equiv of 3 M EtMgBr in ether was added. The solution was warmed up to room temperature and stirred for 24 h. The mixture was poured into 5% $NH_4Cl$ under stirring and extracted using chloroform. The chloroform extract was purified using silica gel preparative TLC ($CHCl_3$:MeOH 85:15) twice to yield **18** (18 mg). **18**: yellow powder. $^1$H NMR ($CDCl_3$) $\delta$ 11.15 (s), 10.1 (s), 8.80 (s), 8.35 (d, $J = 4.8$), 7.82 (d, $J = 4.2$), 7.59 (d, $J = 7.6$), 7.13 (t, $J = 7.6$), 7.05 (d, $J = 7.6$), 6.88 (s), 4.19 (s), 3.31 (t, $J = 12.8$), 2.98 (d, $J = 12.8$), 2.83 (m), 2.55 (m), 2.26−2.40 (m), 2.16 (d, $J = 11.6$), 2.00 (t, $J = 10.8$), 1.80 (m), 1.53−1.22 (m), 0.88 (t, $J = 7.2$). $^{13}$C NMR (DEPT 135) $\delta$ 137.9, 134.1, 132.8, 127.6, 121.1, 114.3, 114.2, 112.0, 82.4, 67.7, 64.4, 54.2, 53.2, 49.8, 45.2, 43.4, 40.5, 38.8, 37.1, 34.3, 33.5, 26.9, 25.5, 24.9, 24.6, 23.7, 21.3, 7.87. HRESIMS $m/z$ calcd for $C_{38}H_{51}N_4O_3$ $[M + H]^+$ 611.3961, found 611.3953.

**In vitro Antimalarial Assay.** Antimalarial activity was determined in vitro against chloroquine sensitive (D6, Sierra Leone) and resistant (W2, Indo China) strains of *Plasmodium falciparum* by measuring plasmodial LDH activity as described earlier.[43,65] Test compounds were dissolved in DMSO (2 mg/mL). A 200 $\mu$L suspension of *P. falciparum culture* (2% parasitemia and 2% hematocrit in RPMI 1640 medium supplemented with 10% human serum and 60 $\mu$g/mL amikacin) was added to the wells of a 96-well plate containing 10 $\mu$L of serially diluted samples. The plate was flushed with a gas mixture of 90% $N_2$, 5% $O_2$, and 5% $CO_2$ and incubated at 37 °C for 72 h in a modular incubation chamber. Plasmodial LDH activity was determined by using Malstat reagent (Flow Inc., Portland, OR).[66] Briefly, 20 $\mu$L of the incubation mixture was mixed with 100 $\mu$L of the Malstat reagent and incubated for 30 min. Then 20 $\mu$L of a 1:1 mixture of NBT/PES (Sigma, St. Louis, MO) was added and the plate is further incubated for 1 h in dark. The reaction was stopped by adding 100 $\mu$L of a 5% acetic acid solution. The plate was read at 650 nm. Chloroquine and artemisinin were used as the positive controls. To determine the selectivity index of antimalarial activity of compounds, their in vitro cytotoxicity to mammalian cells (Vero cells) was also determined. Cells were seeded at a density of 25000 cells/well and incubated for 24 h. Serially diluted samples were added and again incubated for 48 h. The number of viable cells was determined by Neutral Red assay.[67] Doxorubicin was used as a positive control. $IC_{50}$ values were obtained from dose response curves.

**In Vitro Antimicrobial Assay.** All organisms were obtained from the American Type Culture Collection (Manassas, VA) and include the fungi *Candida albicans* ATCC 90028 and *Cryptococcus neoformans* ATCC 90113 and the bacterium *Mycobacterium intracellulare* ATCC 23068. Susceptibility testing was performed using a modified version of the CLSI (formerly NCCLS) methods.[68−70] *M. intracellulare* is tested using a modified method of Franzblau, et al.[71] Samples were serially diluted in 20% DMSO/saline and transferred in duplicate to 96-well flat bottom microplates. Microbial inocula were prepared by correcting the $OD_{630}$ of microbe suspensions in incubation broth to afford final target inocula. Drug controls (ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and amphotericin B (ICN Biomedicals, Ohio) for fungi) were included in each assay. All organisms were read at either 630 nm using the EL-340 Biokinetics Reader (Bio-Tek Instruments, Vermont) or 544ex/590em, (*M. intracellulare*) using the Polarstar galaxy plate reader (BMG LabTechnologies, Germany) prior to and after incubation.

**Antineuroinflammatory Assay.** Rat neonatal microglia ($2 \times 10^5$ cells) were seeded into each well of 24-well flat-bottom culture clusters and stimulated with *Escherichia coli* lipopolysaccharide (LPS) (0.3 ng/mL) in Dulbecco's modified Eagle medium + 10% fetal bovine serum + penicillin + streptomycin for 17 h in a humidified 5% $CO_2$ incubator at 35.9 °C as described.[13,34] Media were then removed, microglia washed with warm (37 °C) Hanks' balanced salt solution (HBSS), and then incubated with compounds (0.01−10 $\mu$M) or vehicle (DMSO) for 15 min prior to stimulation with phorbol 12-myristate 13-acetate (PMA) (1 $\mu$M). All experimental treatments were run in duplicate and in a final volume of 1 mL. Then 70 min after PMA stimulation, HBSS was aspirated from each well and superoxide anion ($O_2^-$), thromboxane $B_2$ ($TXB_2$), and lactated dehydrogenase (LDH) release were determined as described.[13,34] Table 3 shows data from 1−3 experiments and is expressed as the compound's inhibitory concentration 50% ($IC_{50}$) for either $O_2^-$ or $TXB_2$. LDH release from microglia was determined spectrophotometrically as described.[35] Microglia LDH release was expressed as the compound concentration ($LDH_{50}$) that yielded 50% percent of LDH release observed after treatment of control microglia with Triton X-100 (0.1%).

**Assay for Transport across BBB.** MDR-MDCK cell line was grown in Dulbecco's Modified Eagles Medium as described earlier.[72] For the transport experiment, cells with passage number 12−40 were seeded at a density of 75000 cells/cm$^2$ and grown for eight days in 12-well Transwell plates to form a monolayer. The bidirectional transport assay was conducted in HBSS, pH 7.4, as the transport medium. Caffeine (5 $\mu$M) was used as a standard drug for BBB transport. The transport of manzamine A free base and its salt (50 $\mu$M) was monitored in absorptive and secretory directions across MDR-MDCK monolayer for two hours. The concentration of manzamine A in the donor chamber as well as in the samples collected at different time intervals from the receiver chamber was determined by LC-MS method. The concentration of caffeine was determined by an HPLC method as reported previously.[72] The cumulative amount of the transported drug was plotted against time to obtain the rate of transport (as shown in Figure 1), and the apparent permeability coefficient (as shown in Figure 2) was calculated from the equation:

$$P_{app} = (dq/dt) \times 1/C_o \times 1/A$$

with $dq/dt$ = rate of transport, $C_o$ = initial concentration in the donor chamber, $A$ = surface area of the filter.

**LC/MS Analytical.** A stock solution of manzamine A was prepared by dissolving in DMSO. The standard solution was diluted with 4% DMSO and 2% cyclodextrin in HBSS at a level of 0.05, 0.1, 0.25, 0.5, 1.0, 2.0, and 5.0 $\mu$g/mL. Agilent HP1100 with Bruker micro-TOF was used. The analysis was performed on a $C_8$ column (4.6 mm × 150 mm, 5 $\mu$m, Phenomenex Luna) using a gradient of MeOH − 2 mM $NH_4Ac$ (0.05% formic acid) from 40:60 to 80:20 over 8 min at a flow rate of 0.8 mL/min. An after column splitter at ratio of 1:5 was utilized. The mass spectrometer was operated in positive ESI mode. The nebulizer

pressure was 2 bar, source temperature was 200 °C, and drying gas was 4 L/min. Extracted ion at $m/z$ 549.4 was integrated and used for quantification. The standard curve was linear over the range of 0.05−5 $\mu$g/mL ($R^2 = 0.9975$). The precision at level of 0.05, 0.5, 2.0 $\mu$g/mL were 3.85%, 3.21%, and 2.85%, respectively. The recovery at level of 0.05, 0.5, and 2.0 $\mu$g/mL were 109.0%, 94.71%, and 101.2%, respectively. Samples collected from donor or receiver side of the monolayer were directly injected into LC/MS.

**In Vivo Antimalarial Assay.** The in vivo antimalarial activity of **1** was determined in mice infected with *Plasmodium berghei* (NK-65 strain), originally obtained from the Walter Read Army Institute of Research, Silver Spring, MD. For multiple dose studies, the four days Peter's suppressive test was modified to a three days treatment schedule as recommended by Medicines for Malaria Venture (www.mmv.org) for evaluation of suppressive and curative activities. Male mice (Swiss Webster strain) weighing 18−20 g were intraperitoneally inoculated with $2 \times 10^7$ parasitized red blood cells obtained from a highly infected donor mouse. Mice were divided into different groups with at least five mice in each group. **1** stock was prepared in SSV or 0.1 M HCl, as specified in respective studies. Further dilutions of **1** were prepared in SSV or 0.01 M HCl. The mice infected with *P. berghei* were orally administered 100 $\mu$L of the compound. The animals were closely observed for at least 2 h after every dose for any apparent signs of toxicity. Blood smears were prepared on different days starting from 5 days post infection (through 28 days) by clipping the tail end, stained with Giemsa, and the slides were observed under microscope for determination of parasitemia. Mice without parasitemia through day 28 postinfection were considered cured.

**Docking Studies.** For the docking studies, we used GOLD 3.1.1 software.[45] The X-ray crystal structure of GSK-3$\beta$ (pdb code: 1gng)[47,49] was retrieved from the Protein Data Bank. The cocrystallized sulfates were removed before the docking studies. For docking, the active site was defined as any atom that lay within a 15 Å radius of the $\delta$N of Arg96. The geometry of **2** was optimized using MMFF94 in Sybyl 7.2 (Tripos Asociates, St. Louis, MO), starting from the published X-ray crystal structure of manzamine A.[1] The top ranked binding pose was identified based on the GOLD fitness score and used for further analysis.

**Acknowledgment.** We gratefully acknowledge the National Institutes of Health (1R01A136596 and 5K02A-101502) and the Medicines for Malaria Venture (MMV) for financial support. We thank Dr. Abbas G. Shilabin, Marsha Wright and John Trott for scale up preparation, biological testing, which was supported by the NIH, NIAID, Division of AIDS, grant no. AI 27094 and the USDA Agricultural Research Service Specific Cooperative Agreement no. 58-6408-2-0009. Additional support to AMSM from Midwestern University and technical assistance by Mary Hall are gratefully acknowledged. R.J.D. acknowledges funding from National Science Foundation (EPS-0556308), Centers for Disease Control and Prevention National Center for Zoonotic, Vector-Borne, and Enteric Diseases (NCZVED), American Association of Colleges of Pharmacy New Investigator Program, NIH National Center for Research Resources (NCRR) grants P20 RR021929 and C06 RR-14503-01, and University of Mississippi's Office of Research and Sponsored Programs, Partners and Provost's Associates Grants. S.P. is a CORE-NPN Natural Products Neuroscience fellow.

**Supporting Information Available:** Complete references 20 and 58, full list of Tables 1−3, and cytotoxicity studies. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) Sakai, R.; Higa, T.; Jefford, C. W.; Bernardinelli, G. Manzamine A, a novel antitumor alkaloid from a sponge. *J. Am. Chem. Soc.* **1986**, *108*, 6404–6405.
(2) Hu, J. F.; Hamann, M. T.; Hill, R.; Kelly, M. The manzamine alkaloids. *Alkaloids Chem. Biol.* **2003**, *60*, 207–285.
(3) Peng, J.; Rao, K. V.; Choo, Y. -M.; Hamann, M. T. In, *Moden Alakloids*; Fattorusso, E. T., S. O., Ed.; Wiley-VCH: Weinheim, Germany: 2008; pp 189−232.
(4) Rao, K. V.; Santarsiero, B. D.; Mesecar, A. D.; Schinazi, R. F.; Tekwani, B. L.; Hamann, M. T. New manzamine alkaloids with activity against infectious and tropical parasitic diseases from an Indonesian sponge. *J. Nat. Prod.* **2003**, *66*, 823–828.
(5) Hamann, M. T.; El-Sayed, K. A. Methods of treating drug-resistant infections through administration of pharmaceutical compositions containing manzamine alkaloids. U.S. Patent US27035, **2002**.
(6) Nakamura, H.; Deng, S.; Kobayashi, J.; Ohizumi, Y.; Tomotake, Y.; Matsuzaki, T.; Hirata, Y. Physiologically active marine natural products from Porifera. XV. Keramamine-A and -B, novel antimicrobial alkaloids from the Okinawan marine sponge Pellina sp. *Tetrahedron Lett.* **1987**, *28*, 621–624.
(7) El Sayed, K. A.; Kelly, M.; Kara, U. A.; Ang, K. K.; Katsuyama, I.; Dunbar, D. C.; Khan, A. A.; Hamann, M. T. New manzamine alkaloids with potent activity against infectious diseases. *J. Am. Chem. Soc.* **2001**, *123*, 1804–1808.
(8) Ang, K. K.; Holmes, M. J.; Higa, T.; Hamann, M. T.; Kara, U. A. In vivo antimalarial activity of the beta-carboline alkaloid manzamine A. *Antimicrob. Agents Chemother.* **2000**, *44*, 1645–1649.
(9) Longley, R. E.; McConnell, O. J.; Essich, E.; Harmody, D. Evaluation of marine sponge metabolites for cytotoxicity and signal transduction activity. *J. Nat. Prod.* **1993**, *56*, 915–920.
(10) Mayer, A. M. S.; Gunasekera, S. P.; Pomponi, S. A.; Sennett, S. H. Anti-inflammatory uses of manzamine. U.S. Patent US 20020187999, **2002**.
(11) Mayer, A. M. S.; Gunasekera, S. P.; Pomponi, S. A.; Sennett, S. H. Anti-inflammatory uses of manzamines. U.S. Patent US 6,602,881, **2003**.
(12) Yousaf, M.; Hammond, N. L.; Peng, J.; Wahyuono, S.; McIntosh, K. A.; Charman, W. N.; Mayer, A. M.; Hamann, M. T. New manzamine alkaloids from an Indo-Pacific sponge. Pharmacokinetics, oral availability, and the significant activity of several manzamines against HIV-I, AIDS opportunistic infections, and inflammatory diseases. *J. Med. Chem.* **2004**, *47*, 3512–3517.
(13) Mayer, A. M.; Hall, M. L.; Lynch, S. M.; Gunasekera, S. P.; Sennett, S. H.; Pomponi, S. A. Differential modulation of microglia superoxide anion and thromboxane B2 generation by the marine manzamines. *BMC Pharmacol.* **2005**, *5*, 6.
(14) Peng, J.; Shen, X.; El Sayed, K. A.; Dunbar, D. C.; Perry, T. L.; Wilkins, S. P.; Hamann, M. T.; Bobzin, S.; Huesing, J.; Camp, R.; Prinsen, M.; Krupa, D.; Wideman, M. A. Marine natural products as prototype agrochemical agents. *J. Agric. Food Chem.* **2003**, *51*, 2246–2252.
(15) Rao, K. V.; Kasanah, N.; Wahyuono, S.; Tekwani, B. L.; Schinazi, R. F.; Hamann, M. T. Three new manzamine alkaloids from a common Indonesian sponge and their activity against infectious and tropical parasitic diseases. *J. Nat. Prod.* **2004**, *67*, 1314–1318.
(16) Peng, J.; Hu, J. F.; Kazi, A. B.; Li, Z.; Avery, M.; Peraud, O.; Hill, R. T.; Franzblau, S. G.; Zhang, F.; Schinazi, R. F.; Wirtz, S. S.; Tharnish, P.; Kelly, M.; Wahyuono, S.; Hamann, M. T. Manadomanzamines A and B: a novel alkaloid ring system with potent activity against mycobacteria and HIV-1. *J. Am. Chem. Soc.* **2003**, *125*, 13382–13386.
(17) Yousaf, M.; El Sayed, K. A.; Rao, K. V.; Lim, C. W.; Hu, J.-F.; Kelly, M.; Franzblau, S. G.; Zhang, F.; Peraud, O.; Hill, R. T.; Hamann, M. T. 12,34-Oxamanzamines, novel biocatalytic and natural products from manzamine producing Indo-Pacific sponges. *Tetrahedron* **2002**, *58*, 7397–7402.
(18) El Sayed, K. A.; Bartyzel, P.; Shen, X.; Perry, T. L.; Zjawiony, J. K.; Hamann, M. T. Marine natural products as antituberculosis agents. *Tetrahedron* **2000**, *56*, 949–953.
(19) Kondo, K.; Ohnishi, N.; Takemoto, K.; Yoshida, H.; Yoshida, K. Synthesis of optically quadratic nonlinear phenylpyridylacetylenes. *J. Org. Chem.* **1992**, *57*, 1622–1625.
(20) Hamann, M.; Alonso, D.; Martin-Aparicio, E.; Fuertes, A.; Perez-Puerto, M. J.; Castro, A.; Morales, S.; Navarro, M. L.; Del Monte-Millan, M.; Medina, M.; Pennaka, H.; Balaiah, A.; Peng, J.; Cook, J.; Wahyuono, S.; Martinez, A. Glycogen synthase kinase-3 (GSK-3) inhibitory activity and structure−activity relationship (SAR) studies of the manzamine alkaloids. Potential for Alzheimer's disease. *J. Nat. Prod.* **2007**, *70*, 1397–1405.

(21) Liu, F.; Liang, Z.; Shi, J.; Yin, D.; El-Akkad, E.; Grundke-Iqbal, I.; Iqbal, K.; Gong, C. X. PKA modulates GSK-3beta- and cdk5-catalyzed phosphorylation of tau in site- and kinase-specific manners. *FEBS Lett*. **2006**, *580*, 6269–6274.
(22) Winkler, J. D.; Axten, J. M. The First Total Syntheses of Ircinol A, Ircinal A, and Manzamines A and D. *J. Am. Chem. Soc*. **1998**, *120*, 6425–6426.
(23) Martin, S. F.; Humphrey, J. M.; Ali, A.; Hillier, M. C. Enantioselective Total Syntheses of Ircinal A and Related Manzamine Alkaloids. *J. Am. Chem. Soc*. **1999**, *121*, 866–867.
(24) Humphrey, J. M.; Liao, Y.; Ali, A.; Rein, T.; Wong, Y. L.; Chen, H. J.; Courtney, A. K.; Martin, S. F. Enantioselective total syntheses of manzamine a and related alkaloids. *J. Am. Chem. Soc*. **2002**, *124*, 8584–8592.
(25) Pandit, U. K.; Overkleeft, H. S.; Borer, B. C.; Bieraugel, H. Synthesis mediated by ring-closing metathesis. Applications in the synthesis of azasugars and alkaloids. *Eur. J. Org. Chem*. **1999**, *5*, 959–968.
(26) Uchida, H.; Nishida, A.; Nakagawa, M. An efficient access to the optically active manzamine tetracyclic ring system. *Tetrahedron Lett*. **1999**, *40*, 113–116.
(27) Coldham, I.; Crapnell, K. M.; Fernandez, J. C.; Moseley, J. D.; Rabot, R. Synthesis of the ABC ring system of manzamine A. *J. Org. Chem*. **2002**, *67*, 6181–6187.
(28) Baldwin, J. E.; Claridge, T. D. W.; Culshaw, A. J.; Heupel, F. A.; Lee, V.; Spring, D. R.; Whitehead, R. C.; Boughtflower, R. J.; Mutton, I. M.; Upton, R. J. Investigations into the manzamine alkaloid biosynthetic hypothesis. *Angew. Chem., Int. Ed*. **1998**, *37*, 2661–2663.
(29) Winkler, J. D.; Londregan, A. T.; Ragains, J. R.; Hamann, M. T. Synthesis and biological evaluation of manzamine analogues. *Org. Lett*. **2006**, *8*, 3407–3409.
(30) Winkler, J. D.; Londregan, A. T.; Hamann, M. T. Antimalarial activity of a new family of analogues of manzamine A. *Org. Lett*. **2006**, *8*, 2591–2594.
(31) Torisawa, Y.; Hashimoto, A.; Okouchi, M.; Iimori, T.; Nagasawa, M.; Hino, T.; Nakagawa, M. Manzamine C congeners with modified azacyclic rings: synthesis and biological evaluation. *Bioorg. Med. Chem. Lett*. **1996**, *6*, 2565–2570.
(32) Winkler, J. D.; Londregan, A. T.; Hamann, M. T. Synthetic modification of manzamine A via Grubbs metathesis. Novel structures with enhanced antibacterial and antiprotozoal properties. *Org. Lett*. **2007**, *9*, 4467–4469.
(33) Choo, Y.-M.; Hamann, M. T. An improved Pictet−Spengler condensation: a convenient synthetic route to bioactive manzamine derivatives. *Heterocycles* **2007**, *71*, 245–252.
(34) Mayer, A. M.; Oh, S.; Ramsey, K. H.; Jacobson, P. B.; Glaser, K. B.; Romanic, A. M. *Escherichia coli* lipopolysaccharide potentiation and inhibition of rat neonatal microglia superoxide anion generation: correlation with prior lactic dehydrogenase, nitric oxide, tumor necrosis factor-alpha, thromboxane B2, and metalloprotease release. *Shock* (*Augusta, GA*) **1999**, *11*, 180–186.
(35) Morgenstern, S.; Flor, R.; Kessler, G.; Klein, B. The automated determination of NAD-coupled enzymes. II. Serum lactate dehydrogenase. *Clin. Chem*. **1966**, *12*, 274–281.
(36) Deininger, M. H.; Kremsner, P. G.; Meyermann, R.; Schluesener, H. Macrophages/microglial cells in patients with cerebral malaria. *Eur. Cytokine Network* **2002**, *13*, 173–185.
(37) Van der Heyde, H. C.; Nolan, J.; Combes, V.; Gramaglia, I.; Grau, G. E. A unified hypothesis for the genesis of cerebral malaria: sequestration, inflammation and hemostasis leading to microcirculatory dysfunction. *Trends Parasitol*. **2006**, *22*, 503–508.
(38) Schluesener, H. J.; Kremsner, P. G.; Meyermann, R. Widespread expression of MRP8 and MRP14 in human cerebral malaria by microglial cells. *Acta Neuropathol*. **1998**, *96*, 575–580.
(39) Woodgett, J. R. In *Glycogen synthase Kinase 3* (*GSK-3*) *and its Inhibitors*; Martinez, A., Castro, A., Medina, M., Eds.; John Wiley & Sons: Hoboken, NJ 2006; pp 3−23.
(40) Forde, J. E.; Dale, T. C. Glycogen synthase kinase 3: a key regulator of cellular fate. *Cell. Mol. Life Sci*. **2007**, *64*, 1930–1944.
(41) Cohen, P.; Frame, S. The renaissance of GSK3. *Nat. Rev. Mol. Cell Biol*. **2001**, *2*, 769–776.
(42) Meijer, L.; Flajolet, M.; Greengard, P. Pharmacological inhibitors of glycogen synthase kinase 3. *Trends Pharmacol. Sci*. **2004**, *25*, 471–480.
(43) Rao, K. V.; Donia, M. S.; Peng, J.; Garcia-Palomero, E.; Alonso, D.; Martinez, A.; Medina, M.; Franzblau, S. G.; Tekwani, B. L.; Khan, S. I.; Wahyuono, S.; Willett, K. L.; Hamann, M. T. Manzamine B and E and ircinal A related alkaloids from an Indonesian Acanthostrongylophora sponge and their activity against infectious, tropical parasitic, and Alzheimer's diseases. *J. Nat. Prod*. **2006**, *69*, 1034–1040.
(44) Tu, L. C.; Chou, C. K.; Chen, C. Y.; Chang, Y. T.; Shen, Y. C.; Yeh, S. F. Characterization of the cytotoxic mechanism of Mana-Hox, an analog of manzamine alkaloids. *Biochim. Biophys. Acta* **2004**, *1672*, 148–156.
(45) Verdonk, M. L.; Cole, J. C.; Hartshorn, M. J.; Murray, C. W.; Taylor, R. D. Improved protein-ligand docking using GOLD. *Proteins* **2003**, *52*, 609–623.
(46) Dajani, R.; Fraser, E.; Roe, S. M.; Young, N.; Good, V.; Dale, T. C.; Pearl, L. H. Crystal structure of glycogen synthase kinase 3 beta: structural basis for phosphate-primed substrate specificity and autoinhibition. *Cell* **2001**, *105*, 721–732.
(47) Bax, B.; Carter, P. S.; Lewis, C.; Guy, A. R.; Bridges, A.; Tanner, R.; Pettman, G.; Mannix, C.; Culbert, A. A.; Brown, M. J.; Smith, D. G.; Reith, A. D. The structure of phosphorylated GSK-3beta complexed with a peptide, FRATtide, that inhibits beta-catenin phosphorylation. *Structure* **2001**, *9*, 1143–1152.
(48) Martinez, A.; Alonso, M.; Castro, A.; Perez, C.; Moreno, F. J. First non-ATP competitive glycogen synthase kinase 3 beta (GSK-3beta) inhibitors: thiadiazolidinones (TDZD) as potential drugs for the treatment of Alzheimer's disease. *J. Med. Chem*. **2002**, *45*, 1292–1299.
(49) Martinez, A.; Alonso, M.; Castro, A.; Dorronsoro, I.; Gelpi, J. L.; Luque, F. J.; Perez, C.; Moreno, F. J. SAR and 3D-QSAR studies on thiadiazolidinone derivatives: exploration of structural requirements for glycogen synthase kinase 3 inhibitors. *J. Med. Chem*. **2005**, *48*, 7103–7112.
(50) Droucheau, E.; Primot, A.; Thomas, V.; Mattei, D.; Knockaert, M.; Richardson, C.; Sallicandro, P.; Alano, P.; Jafarshad, A.; Baratte, B.; Kunick, C.; Parzy, D.; Pearl, L.; Doerig, C.; Meijer, L. *Plasmodium falciparum* glycogen synthase kinase-3: molecular model, expression, intracellular localization and selective inhibitors. *Biochim. Biophys. Acta, Proteins Proteomics* **2004**, *1697*, 181–196.
(51) Martin, M.; Rehani, K.; Jope, R. S.; Michalek, S. M. Toll-like receptor-mediated cytokine production is differentially regulated by glycogen synthase kinase 3. *Nat. Immunol*. **2005**, *6*, 777–784.
(52) Jope, R. S.; Yuskaitis, C. J.; Beurel, E. Glycogen synthase kinase-3 (GSK3): inflammation, diseases, and therapeutics. *Neurochem. Res*. **2007**, *32*, 577–595.
(53) Magae, Y.; Magae, J. Effect of staurosporine on growth and hyphal morphology of *Pleurotus ostreatus*. *J. Gen. Microbiol*. **1993**, *139*, 161–164.
(54) Park, H. J.; Lee, J. Y.; Hwang, I. S.; Yun, B. S.; Kim, B. S.; Hwang, B. K. Isolation and antifungal and antioomycete activities of staurosporine from *Streptomyces roseoflavus* strain LS-A24. *J. Agric. Food Chem*. **2006**, *54*, 3041–3046.
(55) Hunt, N. H.; Golenser, J.; Chan-Ling, T.; Parekh, S.; Rae, C.; Potter, S.; Medana, I. M.; Miu, J.; Ball, H. J. Immunopathogenesis of cerebral malaria. *Int. J. Parasitol*. **2006**, *36*, 569–582.
(56) Szklarczyk, A.; Stins, M.; Milward, E. A.; Ryu, H.; Fitzsimmons, C.; Sullivan, D.; Conant, K. Glial activation and matrix metalloproteinase release in cerebral malaria. *J. Neurovirol*. **2007**, *13*, 2–10.
(57) Medana, I. M.; Hunt, N. H.; Chan-Ling, T. Early activation of microglia in the pathogenesis of fatal murine cerebral malaria. *Glia* **1997**, *19*, 91–103.
(58) Hiestand, P. C.; Petersen, F.; Roggo, S.; Hamann, M. T. Use of manzamines in transplantation and autoimmune diseases. Patent EP7678, **2006**.
(59) Golenser, J.; McQuillan, J.; Hee, L.; Mitchell, A. J.; Hunt, N. H. Conventional and experimental treatment of cerebral malaria. *Int. J. Parasitol*. **2006**, *36*, 583–593.
(60) Akiyama, H.; Barger, S.; Barnum, S.; Bradt, B.; Bauer, J.; Cole, G. M.; Cooper, N. R.; Eikelenboom, P.; Emmerling, M.; Fiebich, B. L.; Finch, C. E.; Frautschy, S.; Griffin, W. S.; Hampel, H.; Hull, M.; Landreth, G.; Lue, L.; Mrak, R.; Mackenzie, I. R.; McGeer, P. L.; O'Banion, M. K.; Pachter, J.; Pasinetti, G.; Plata-Salaman, C.; Rogers, J.; Rydel, R.; Shen, Y.; Streit, W.; Strohmeyer, R.; Tooyoma, I.; Van Muiswinkel, F. L.; Veerhuis, R.; Walker, D.; Webster, S.; Wegrzyniak, B.; Wenk, G.; Wyss-Coray, T. Inflammation and Alzheimer's disease. *Neurobiol. Aging* **2000**, *21*, 383–421.
(61) Block, M. L.; Hong, J. S. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. *Prog. Neurobiol*. **2005**, *76*, 77–98.
(62) Sastre, M.; Klockgether, T.; Heneka, M. T. Contribution of inflammatory processes to Alzheimer's disease: molecular mechanisms. *Int. J. Dev. Neurosci*. **2006**, *24*, 167–176.
(63) Hill, R. T.; Hamann, M. T.; Peraud, O.; Kasanah, N. Manzamine-producing actinomycetes. U.S. Patent US24238, **2004**.
(64) Shilabin, A. G.; Kasanah, N.; Tekwani, B. L.; Hamann, M. T. Kinetic studies and bioactivity of potential manzamine prodrugs. *J. Nat. Prod*. **2008**, *71*, 1218–1221.

(65) Ibrahim, M. A.; Shilabin, A. G.; Prasanna, S.; Jacob, M.; Khan, S. I.; Doerksen, R. J.; Hamann, M. T. 2-*N*-Methyl modifications and SAR studies of manzamine A. *Bioorg. Med. Chem.* **2008**, *16*, 6702–6706.
(66) Makler, M. T.; Ries, J. M.; Williams, J. A.; Bancroft, J. E.; Piper, R. C.; Gibbins, B. L.; Hinrichs, D. J. Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity. *Am. J. Trop. Med. Hyg.* **1993**, *48*, 739–741.
(67) Borenfreund, E.; Babich, H.; Martin-Alguacil, N. Rapid chemosensitivity assay with human normal and tumor cells in vitro. *In Vitro Cell Dev. Biol.* **1990**, *26*, 1030–1034.
(68) NCCLS. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard M27-A2*; National Committee on Clinical Laboratory Standards, 2002; Vol. 22, Issue 15.
(69) NCCLS. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard M24-T2*; National Committee on Clinical Laboratory Standards, 2000, Vol. 20, Issue 26.
(70) NCCLS. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically M7-A5*; National Committee on Clinical Laboratory Standards, 2000; Vol. 20, Issue 2.
(71) Franzblau, S. G.; Witzig, R. S.; McLaughlin, J. C.; Torres, P.; Madico, G.; Hernandez, A.; Degnan, M. T.; Cook, M. B.; Quenzer, V. K.; Ferguson, R. M.; Gilman, R. H. Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue assay. *J. Clin. Microbiol.* **1998**, *36*, 362–366.
(72) Madgula, V. L.; Avula, B.; Reddy, V. L. N.; Khan, I. A.; Khan, S. I. Transport of decursin and decursinol angelate across Caco-2 and MDR-MDCK cell monolayers: in vitro models for intestinal and blood−brain barrier permeability. *Planta Med.* **2007**, *73*, 330–335.