Full Papers

Glycogen Synthase Kinase-3 (GSK-3) Inhibitory Activity and Structure–Activity Relationship (SAR) Studies of the Manzamine Alkaloids. Potential for Alzheimer's Disease

Mark Hamann,[†] Diana Alonso,[‡] Ester Martín-Aparicio,[‡] Ana Fuertes,[‡] M. José Pérez-Puerto,[‡] Ana Castro,[‡] Susana Morales,[‡] María Luisa Navarro,[‡] María del Monte-Millán,[‡] Miguel Medina,[‡] Hari Pennaka,[†] Akula Balaiah,[†] Jiangnan Peng,[†] Jennifer Cook,[†] Subagus Wahyuono,[§] and Ana Martínez*,[‡]

Department of Pharmacognosy, Pharmacology, Chemistry, and Biochemistry and National Center for Natural Products Research, School of Pharmacy, University of Mississippi, Mississippi 38677, NeuroPharma, S.A., Avda de la Industria, 52, 28760 Tres Cantos, Madrid, Spain, and Gadjah Mada University, Bulaksumur, Yogyakarta 55281, Indonesia

Received March 3, 2006

Manzamine A and related derivatives isolated from a common Indonesian sponge, *Acanthostrongylophora*, have been identified as a new class of GSK-3β inhibitors. The semisynthesis of new analogues and the first structure–activity relationship studies with GSK-3β are also reported. Moreover, manzamine A proved to be effective in decreasing tau hyperphosphorylation in human neuroblastoma cell lines, a demonstration of its ability to enter cells and interfere with tau pathology. Inhibition studies of manzamine A against a selected panel of five different kinases related to GSK-3β, specifically CDK-1, PKA, CDK-5, MAPK, and GSK-3α, show the specific inhibition of manzamine A on GSK-3β and CDK-5, the two kinases involved in tau pathological hyperphosphorylation. These results suggest that manzamine A constitutes a promising scaffold from which more potent and selective GSK-3 inhibitors could be designed as potential therapeutic agents for Alzheimer's disease.

Glycogen synthase kinase-3 (GSK-3) is a serine–threonine kinase ubiquitously expressed and involved in the regulation of many cell functions.[1] GSK-3 was originally identified as one of the five protein kinases that phosphorylate glycogen synthase,[2] being implicated in type-2 diabetes.[3] GSK-3 is also known to phosphorylate the microtubule-associated protein tau in mammalian cells.[4] This hyperphosphorylation is an early event in neurodegenerative conditions, such as Alzheimer's disease,[5] also involved is a second kinase, called CDK-5.[6] GSK-3 emerged in the 21st century as one of the most attractive therapeutic targets for the development of selective inhibitors as new promising drugs for severe unmet pathologies, such as type-2 diabetes, bipolar disorders, stroke, and Alzheimer's disease, and different tau pathologies, such as Pick's disease, supranuclear palsy, and frontotemporal dementia.[7]

A number of recent publications have emerged describing structural diverse molecules that inhibit GSK-3, such as pyridyloxadiazoles,[8] thiadiazolidindiones,[9] pyrazolopyrimidines[10] and maleimides.[11] Current advances in the search for GSK-3 inhibitors have been recently reviewed.[12–14] Interesting is the prominent role that marine invertebrates have played in the generation of novel GSK-3 inhibitors, including hymenialdisine,[15] meridianines,[16] and indirubines[17] isolated from several diverse sponges, ascidians, and gastropod mollusks, respectively (Figure 1). Indeed, the marine environment represents an enormous resource for the discovery of potential therapeutic agents.[18,19]

In our search for marine compounds active against neurological disorders, we have recently discovered the GSK-3 inhibition of the manzamine alkaloids. Moreover, manzamine A also inhibited CDK-5 and proved to be effective in decreasing tau phosphorylation after treatment in a human neuroblastoma cell line.

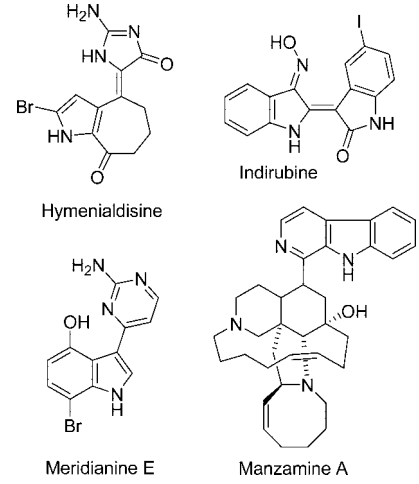


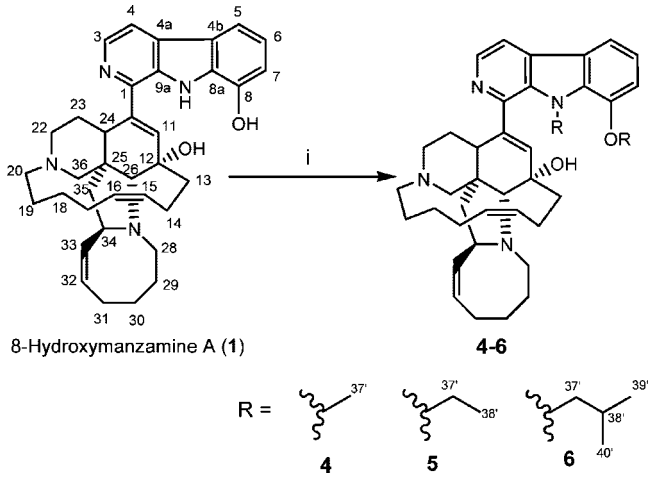
Figure 1. GSK-3 inhibitors isolated from marine organisms.

Manzamines are complex β-carboline alkaloids isolated from Indo-Pacific sponges and characterized as having an intricate and novel polycyclic system. In 1986, Higa and co-workers first reported manzamine A from the Okinawan sponge of the genus *Haliclona*.[20] To date, there are 16 species belonging to 8 families of marine sponges, including *Acanthostrongylophora*, that have been confirmed to yield manzamine-related alkaloids.[21] The occurrence of manzamine alkaloids in a diversity of unrelated sponges has led to the speculation of a possible microbial origin for the biosynthesis of these compounds.[22] Our biological results, the semisynthesis of new manzamine derivatives together with the initial structure–activity relationship (SAR), are described in this paper.

* To whom correspondence should be addressed. Tel: 34-91-806-1130. Fax: 34-91-803-4660. E-mail: amartinez@neuropharma.es.
[†] University of Mississippi.
[‡] NeuroPharma.
[§] Gadjah Mada University.

10.1021/np060092r CCC: $37.00
Published on Web 08/21/2007

Scheme 1[a]



[a] Reagents: (i) R-I, K_2CO_3, Me_2CO, 70 °C.

Chemistry

The previously reported manzamine A, 8-hydroxymanzamine A (**1**),[23] 6-hydroxymanzamine A (**2**),[24] 6-deoxymanzamine X (**9**),[25] manzamine E (**11**), and manzamine F (**12**)[26] were isolated from the Indonesian marine sponge *Acanthostrongylophora* sp. and identified by detailed 1D and 2D nuclear magnetic resonance (NMR) studies and a comparison with the NMR and mass spectrometry (MS) data in the literature.[27–30]

As shown in Scheme 1, refluxing 8-hydroxymanzamine A (**1**) with alkyl iodides in the presence of potassium carbonate in acetone gave the corresponding 9-*N*-alkyl-8-alkoxy manzamine A derivatives (**4**–**6**). The presence of two singlets at 4.10 and 4.02 ppm corresponding to *N*-methyl and *O*-methoxy protons and carbon signals at 36.7 and 55.8 ppm corresponding to *N*-methyl and *O*-methoxy carbons in the distortionless enhancement by polarization transfer (DEPT) spectrum supports the formation of compound **4**.

The methylation at 9-*N* and 8-*O* positions was further confirmed with the help of HMBC correlations. 9-*N*-methyl protons showed ^3J correlations with C-8a (132.1 ppm) and C-9a (133.5 ppm), and 8-*O*-methyl protons showed a ^3J correlation with C-8 (147.9 ppm). Compounds **5** and **6** were prepared by the same methodology. All structures were assigned by ^{1}H, ^{13}C (Table 1), DEPT, and mass spectral data.

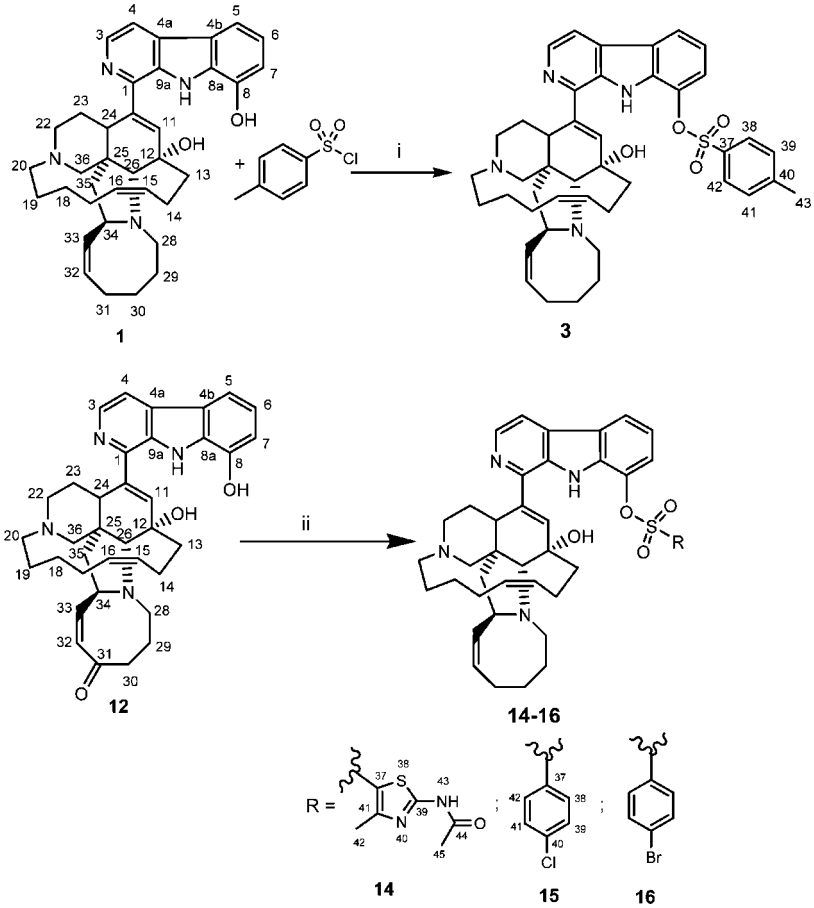
The compounds as shown in Scheme 2 were prepared by the reaction of 8-hydroxymanzamine A (**1**) or manzamine F (**12**) with aryl sulfonyl chlorides in the presence of triethyl amine in dichloromethane (DCM) at 0–25 °C to yield the corresponding

Table 1. ^{13}C and ^{1}H NMR Data of Compounds **4**–**6**[a]

	4		**5**		**6**	
number	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)
1	145.3, *C*		145.7, *C*		145.5, *C*	
3	138.3, *C*H	8.45, d (4.8)	138.4, *C*H	8.44, d (4.8)	138.3, *C*H	8.42, d (4.8)
4	113.6, *C*H	7.81, d (4.8)	113.4, *C*H	7.80, d (5.2)	113.2, *C*H	7.79, d (4.8)
4a	130.9, *C*		130.0, *C*		129.8, *C*	
4b	123.6, *C*		124.8, *C*		122.6, *C*	
5	109.4, *C*H	7.71, d (7.6)	109.9, *C*H	7.70, d (7.2)	110.4, *C*H	7.69, d (7.6)
6	120.4, *C*H	7.19, t (7.6)	120.6, *C*H	7.20, t (7.6)	121.0, *C*H	7.19, t (7.6)
7	112.8, *C*H	7.02, d (7.6)	112.9, *C*H	7.01, d (7.6)	112.8, *C*H	7.0, d (8.0)
8	147.9, *C*		147.3, *C*		147.6, *C*	
8a	132.1, *C*		132.1, *C*		132.2, *C*	
9a	133.5, *C*		132.2, *C*		133.4, *C*	
10	139.4, *C*		138.8, *C*		139.4, *C*	
11	136.4, *C*H	5.87 s	135.2, *C*H	5.88 s	136.1, *C*H	6.04 s
12	70.3, *C*		70.2, *C*		70.5, *C*	
13	40.5, *C*H$_2$	1.45 m, 1.95 m	40.0, *C*H$_2$	1.47 m, 1.93 m	40.3, *C*H$_2$	1.45 m, 1.93 m
14	21.8, *C*H$_2$	2.11 m, 2.23 m	21.7, *C*H$_2$	2.12 m, 2.21 m	21.8, *C*H$_2$	2.11 m, 2.24 m
15	130.2, *C*H	5.34 t, 10	130.2, *C*H	5.33 t, 12	130.1, *C*H	5.32 t 12
16	134.2, *C*H	5.69 m	134.2, *C*H	5.71 m	134.0, *C*H	5.69 m
17	25.9, *C*H$_2$	2.87 m, 3.08 m	26.0, *C*H$_2$	2.88 m, 3.08 m	25.8, *C*H$_2$	2.88 m, 3.08 m
18	28.1, *C*H$_2$	1.56 m, 1.84 m	28.1, *C*H$_2$	1.56 m, 1.83 m	27.9, *C*H$_2$	1.57 m, 1.84 m
19	25.8, *C*H$_2$	1.74 m, 1.95 m	25.8, *C*H$_2$	1.76 m, 1.96 m	25.8, *C*H$_2$	1.77 m, 1.96 m
20	50.7, *C*H$_2$	2.82 m, 3.09 m	50.7, *C*H$_2$	2.80 m, 3.11 m	50.6, *C*H$_2$	2.86 m, 3.08 m
22	49.7, *C*H$_2$	1.88 m, 2.84 m	49.8, *C*H$_2$	1.88 m, 2.84 m	49.7, *C*H$_2$	1.88 m, 2.85
23	31.7, *C*H$_2$	1.77 m, 1.99 m	31.7, *C*H$_2$	1.76 m, 1.97 m	31.5, *C*H$_2$	1.76 m, 1.99 m
24	40.7, *C*H	2.66 m	40.8, *C*H	2.65 m	40.7, *C*H	2.66 m
25	47.3, *C*		47.3, *C*		47.1, *C*	
26	75.3, *C*H	3.53 s	75.3, *C*H	3.55 s	75.2, *C*H	3.53 m
28	53.4, *C*H$_2$	2.64 m, 4.21 m	53.5, *C*H$_2$	2.63 m, 4.20 m	53.4, *C*H$_2$	2.64 m, 4.16 m
29	26.9, *C*H$_2$	1.56 m, 1.75 m	27.0, *C*H$_2$	1.56 m, 1.75 m	27.1, *C*H$_2$	1.56 m, 1.76 m
30	25.8, *C*H$_2$	1.32 m, 1.80 m	25.7, *C*H$_2$	1.32 m, 1.80 m	25.8, *C*H$_2$	1.32 m, 1.80
31	31.5, *C*H$_2$	1.80 m, 1.45 m	31.5, *C*H$_2$	1.80 m, 1.45 m	31.7, *C*H$_2$t	1.81 m, 1.45 m
32	139.6, *C*H	5.95 m	139.0	5.96 m	139.6	5.92 m
33	128.8, *C*H	5.58 m	128.9	5.52 m	128.7	5.55 m
34	54.8, *C*H	2.25 m, 2.82 m	54.9, *C*H	2.28 m, 2.85 m	54.6 d	2.27 m, 3.08 m
35	44.1, *C*H$_2$	1.44 m, 2.15 m	41.1, *C*H$_2$	1.44 m, 2.15 m	44.3	1.46 m, 2.16
36	68.7, *C*H$_2$	1.93 m, 2.38 m	68.7, *C*H$_2$	1.92 m, 2.37 m	68.3, *C*H$_2$	1.92 m, 2.38 m
37	55.8, *C*H$_3$	4.02 s	64.17, *C*H$_2$	4.20 s	68.1, *C*H$_2$	3.97 d
37′	36.7, *C*H$_3$	4.10 s	44.1, *C*H$_2$	4.21 s	53.5, *C*H$_2$	3.95 d
38			14.9, *C*H$_3$	1.38 t	21.8, *C*H	2.13 m
38′			14.7, *C*H$_3$	1.45 t	19.7, *C*H	2.02 m
39, 40					19.6, *C*H$_3$	1.05 d
39′, 40′					19.4, *C*H$_3$	1.04 d

[a] In $CDCl_3$, 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Solvent residue signals were use as the internal standard. Carbon multiplicities were determined by DEPT experiments.

Scheme 2[a]



[a] Reagents: (i) TEA, DCM, 0–30 °C; (ii) R-SO_2Cl, TEA, DCM, 0–30 °C.

Table 2. ^{13}C and ^{1}H NMR Data of Compound **3**[a]

	3			**3**	
number	^{13}C (mult)	^{1}H (mult, *J* in Hz)	number	^{13}C (mult)	^{1}H (mult, *J* in Hz)
1	144.38, *C*		20	50.8, CH_2	2.83 m, 3.05 m
3	134.86, *C*H	8.46, d (4.8)	22	49.73, CH_2	1.88 m, 2.83 m
4	114.65, *C*H	7.97, d (4.8)	23	31.6, CH_2	1.79 m, 1.98 m
4a	129.82, *C*		24	40.96, *C*H	2.67 m
4b	124.99, *C*		25	47.10, *C*	
5	111.33, *C*H	7.78, d (7.6)	26	75.09, *C*H	3.54 s
6	120.40, *C*H	7.17, t (7.8)	28	53.6, CH_2	2.67 m, 4.25 m
7	113.18, *C*H	7.27, d (8.0)	29	26.97, CH_2	1.57 m, 1.76 m
8	145.81, *C*		30	26.067, CH_2	1.33 m, 1.81 m
8a	132.11, *C*		31	31.64, CH_2	1.81 m, 1.49 m
9a	133.73, *C*		32	133.73, *C*H	5.97 m
10	139.26, *C*		33	128.45, *C*H	5.59 m
11	134.60, *C*H	6.26 s	34	54..92, *C*H	2.27 m, 2.84 m
12	70.04, *C*		35	44.69, CH_2	1.46 m, 2.15 m
13	40.22, CH_2	1.86 m, 2.05 m	36	69.62, CH_2	1.92 m, 2.38 m
14	21.82, CH_2	2.12 m, 2.24 m	37	138.10, *C*	
15	129.82, *C*H	5.30, t (9.6)	38, 42	128.76, *C*H	7.81, d (8.4)
16	133.73, *C*H	5.75 m	39, 41	130.10, *C*H	7.34, d (8.0)
17	25.8, CH_2	2.86 m, 3.04 m	40	139.15, *C*	
18	28.17, CH_2	1.56 m, 1.83	43	21.69, CH_3	2.38 s
19	25.70, CH_2	1.76 m, 1.95 m			

[a] In $CDCl_3$, 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Solvent residue signals were use as the internal standard. Carbon multiplicities were determined by DEPT experiments.

sulfonyl manzamines. The presence of one singlet at 2.38 ppm for methyl protons (C_{43}–H) and two doublets at 7.81 (J = 8.4) and 7.34 (J = 8.0) for phenyl protons (C_{38}–H and C_{42}–H) and (C_{39}–H and C_{41}–H) (Table 2) indicated the formation of compound **3**. Two additional signals corresponding to C_{38} and C_{42} and C_{39} and C_{41} carbons at 128.7 and 130.1 in its DEPT spectrum also support its structure. The rest of the compounds **14–16** were also prepared by using the same procedure, and the structures were confirmed by ^{1}H, ^{13}C (Table 3), and mass spectral data.

The 9-N-alkylation of the β-carboline moiety in manzamine A was completed by reacting manzamine A with alkyl iodides catalyzed by NaH in *N*,*N*-dimethylformamide (DMF) to yield

Table 3. ^{13}C and ^{1}H NMR Data of Compounds **14–16**[a]

	14		**15**		**16**	
number	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)
1	143.1, *C*		142.8, *C*		142.8, *C*	
3	139.3, *C*H	8.48, d (4.8)	139.10, *C*H	8.48, d (4.8)	139.1, *C*H	8.45, d (4.8)
4	113.7, *C*H	8.03, d (7.2)	113.5, *C*H	8.01, d (7.6)	113.6, *C*H	7.99, d (7.6)
4a	130.1, *C*		130.1, *C*		130.2, *C*	
4b	123.2, *C*		123.3, *C*		123.1, *C*	
5	112.2, *C*H	7.83, d (5.2)	112.4, *C*H	7. 76, d (5.2)	112.0, *C*H	7. 77, d (5.2)
6	120.5, *C*H	7.19, t (7.8)	120.4, *C*H	7.19, t (8.0)	120.4, *C*H	7.18, t (7.8)
7	113.2, *C*H	7.28, d (7.6)	113.1, *C*H	7.28, d (6.8)	113.1, *C*H	7.27, d (7.6)
8	143.4, *C*		143.3, *C*		143.5, *C*	
8a	132.5, *C*		132.4, *C*		132.6, *C*	
9a	133.4, *C*		133.3, *C*		133.8, *C*	
10	141.4, *C*		141.2, *C*		141.0, *C*	
11	137.7, *C*H	6.49 s	137.3, *C*H	6.41 s	139.0, *C*H	6.49 s
12	69.4, *C*		69.1, *C*		69.6, *C*	
13	39.8, *C*H_2	1.87 m, 2.05 m	40.3, *C*H_2	1.88 m, 2.03 m	41.2, *C*H_2	1.89 m, 2.04 m
14	21.7, *C*H_2	2.10 m, 2.25 m	21.5,*C*H_2	2.12 m, 2.26 m	21.6, *C*H_2	2.11 m, 2.27 m
15	128.7, *C*H	5.81 dd	128.9, *C*H	5.69 dd	128.4, *C*H	5.72 dd
16	132.2, *C*H	5.56 dddd	132.3, *C*H	5.55 dddd	132.3, *C*H	5.55 dddd
17	25.4, *C*H_2	1.65 m, 2.45 m	25.6, *C*H_2	1.62 m, 2.44 m	25.7, *C*H_2	1.67 m, 2.46 m
18	26.6, *C*H_2	1.38 m, 1.41 m	26.7, *C*H_2	1.37 m, 1.41 m	26.7,*C*H_2	1.39 m, 1.43 m
19	24.7, *C*H_2	1.42 m, 1.72 m	24.4, *C*H_2	1.41 m, 1.73 m	24.8, *C*H_2	1.41 m, 1.74 m
20	52.8, *C*H_2	2.38 m, 2.58 m	52.9, *C*H_2	2.39 m, 2.57 m	52.8, *C*H_2	2.39 m, 2.58 m
22	49.5, *C*H_2	1.90 m, 2.80 m	49.6, *C*H_2	1.90 m, 2.80 m	49.6, *C*H_2	1.91 m, 2.82 m
23	33.1, *C*H_2	1.56 m, 2.25 m	33.6, *C*H_2	1.56 m, 2.26 m	33.5, *C*H_2	1.56 m, 2.26 m
24	42.1, *C*H	3.28 m	42.1, *C*H	3.19 m	42.2, *C*H	3.20 m
25	46.4, *C*		46.7, *C*		46.9, *C*	
26	81.3, *C*H	3.78 s	81.7, *C*H	3.68 s	80.9, *C*H	3.76 s
28	53.0, *C*H_2	2.77 m, 3.40 m	53.0, *C*H_2	2.77 m, 3.38 m	53.0, *C*H_2	2.78 m, 3.37 m
29	32.6, *C*H_2t	1.90 m, 2.07 m	32.7, *C*H_2	1.90 m, 2.06 m	33.0, *C*H_2	1.90 m, 2.08 m
30	44.9, *C*H_2	2.29 m, 2.57 m	45.0, *C*H_2	2.28 m, 2.57 m	44.5, *C*H_2	2.28 m, 2.58 m
31	214.6, *C*		215.2, *C*		215.6, *C*	
32	38.9, *C*H_2	2.40 m, 2.54 m	38.8, *C*H_2	2.39 m, 2.63 m	38.9, *C*H_2	2.39 m, 2.65 m
33	25.4, *C*H_2	1.71 m, 2.11 m	25.1, *C*H_2	1.70 m, 2.10 m	25.3, *C*H_2	1.70 m, 2.10 m
34	63.9, *C*H	2.98 m	63.7, *C*H	2.99 m	63.7, *C*H	2.97 m
35	49.9, *C*H_2	1.52 m, 1.62 m	48.7, *C*H_2	1.51 m, 1.62 m	48.6, *C*H_2	1.52 m, 1.63 m
36	68.4, *C*H_2	2.28 m, 2.45 m	68.9, *C*H_2	2.28 m, 2.45 m	68.6, v	2.30 m, 2.46 m
37	107.5, *C*		140.1		140.2	
38			129.89, *C*H	7.95, d (8.8)	130.1, *C*H	7.86, d (8.8)
39	163.2, *C*		130.14, *C*H	7.55, d (8.4)	132.9, *C*H	7. 71, d (8.8)
40			137.90, *C*		134.8, *C*	
41	145.0, *C*		130.14, *C*H	7.95, d (8.8)	132.9, *C*H	7.34, d (8.0)
42		2.35 s	129.89, *C*H	8.02, d (7.6)	130.1, *C*H	7.81, d (8.4)
44	169.1, *C*					
45	16.6, q	2.12 s				

[a] In $CDCl_3$, 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Solvent residue signals were use as the internal standard. Carbon multiplicities were determined by DEPT experiments.

compounds **7** and **8** (Scheme 4). The methylation at the 9-*N* position was confirmed using the ^{13}C and ^{15}N heteronuclear multiple-bond correlation (HMBC) experiments and the 9-*N*-methyl protons (δ 3.87) showed ^{3}J correlations with the C-8a (δ 142.9) and C-9a (δ 134.8) carbons and the ^{2}J correlation with the 9-*N* (δ 108.7) nitrogen of the β-carboline moiety (Table 4).

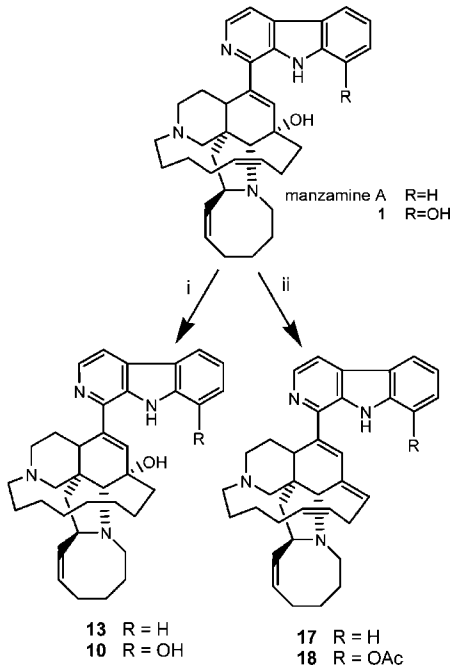
Reduction of manzamine A in absolute ethanol in the presence of excess Pd/carbon at room temperature for 4 h under hydrogen gas afforded 15,16,32,33-tetrahydromanzamine A (**13**) (Scheme 3), as a major product (68.3%). The same reaction with 8-hydroxy-manzamine A (**1**) afforded compound **10** (51.7%) (Table 5).

Compound **17** was obtained from the reaction of manzamine A with acetic anhydride in the presence of dry K_2CO_3 in dry acetone under reflux for 9 h (Scheme 3). The same reaction procedure was applied to prepare compound **18** starting with compound **1**. The high-resolution Fourier transform mass spectrometry (HRFTMS) data indicated the addition of 24 mass units in compound **18** and the loss of 18 mass units in compound **17**. The presence of one additional olefinic proton signal at δ 5.67 in the ^{1}H NMR spectrum and replacement of the C-12 signal at δ 69.8 with a quaternary olefinic carbon signal at δ 142.6 and an additional carbon signal at δ 115.8 in the ^{13}C and DEPT spectra indicate the presence of an additional double bond at the C-12 carbon. The correlation spectroscopy (COSY) spectrum of **17** and **18** helped to establish the double bond between C-12 and C-13 because the H-13 proton showed a correlation with H_2-14 at δ 2.14, and it was further confirmed by HMBC studies. The ^{1}H NMR of **18** showed a methyl singlet at δ 2.02, and the ^{13}C showed the presence of a methyl carbon at δ 17.8 and a carbonyl carbon at δ 167.8, indicating the presence of an acetyl group (Table 6). Thus, compounds **17** and **18** were shown to be 12,13-dehydromanzamine A and 8-*O*-acetyl-12,13-dehydromanzamine A, respectively.

Biological Results and SAR Discussion

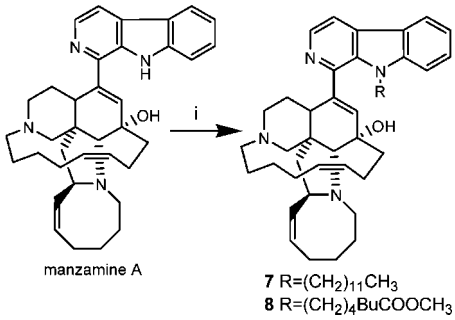
As a continued effort to identify new GSK-3 inhibitors from marine sources, we found that manzamine A inhibits human GSK-3β *in vitro* (70% at 25 μM), with an IC_{50} value of 10.2 μM. To investigate the mechanism of action for manzamine A on GSK-3β, kinetic experiments were performed. A double-reciprocal Lineweaver–Burk plot of enzyme kinetics is shown in Figure 2. The intercept of the plot in the vertical axis (1/*V*) rises when the manzamine A concentration increases, whereas the intercept in the horizontal axis (1/*S*) does not change, meaning that, while the enzyme maximal rate (V_{max}) decreases in the presence of the inhibitor, Michael's constant (K_m) remains unaltered. These results

Scheme 3[a]



[a] Reagents: (i) Pd/C-H_2, EtOH, 4 h, room temperature; (ii) K_2CO_3, Ac_2O, Me_2CO, 9 h, reflux.

Scheme 4[a]



[a] Reagents: (i) DMF, NaH, R-I, 0 °C, 2–3 h.

strongly suggest that manzamine A acts as a noncompetitive inhibitor of ATP binding, because increasing the ATP concentration does not interfere with inhibition (Figure 2).

To identify the pharmacophore responsible for this new GSK-3 inhibitor, we tested the potential GSK-3 inhibition of carboline and ircinal A, which are considered the chemical precursors of manzamine A. Both moieties are inactive with regard to binding to GSK-3 (Figure 3), indicating that the entire manzamine molecule is required for this activity.

Next, we investigated the SAR on this new chemical class by evaluating several manzamine analogues isolated from different sponges or prepared through semisynthetic modification. In all of the cases where the percentage of GSK-3 inhibition at 25 μM is greater than 50%, the IC_{50} values were determined. This determination was not possible for compounds **4** and **9** because of the lack of compound. IC_{50} values were in the micromolar range for all of the tested manzamine derivatives. In several cases, we obtained compounds more potent than the lead compound manzamine A.

First, we examined the effect of some substituents on the carboline moiety (Table 7). Replacement of hydrogen at the carboline 6 or 8 position with OH, OMe, OEt, or OTs groups is well-tolerated by the enzyme, and compounds **1–5** are equipotent

Table 4. ^{13}C and ^{1}H NMR Data of Compounds **7** and **8**[a]

	7		**8**	
number	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)
1	142.8, *C*		144.8, *C*	
3	138.1, *CH*	8.40, d (5.2)	138.6, *CH*	8.42, d (5.2)
4	113.8, *CH*	7.78, d (5.2)	113.6, *CH*	7.81, d (5.2)
4a	130.0, *C*		129.6, *C*	
4b	123.2, *C*		121.8, *C*	
5	120.5, *CH*	7.51, d (7.8)	122.0, *CH*	7.69, d (7.8)
6	120.7, *CH*	7.36, t (8.0)	120.0, *CH*	7.34, t (8.0)
7	127.4, *CH*	7.56, d (7.4)	128.3, *CH*	7.53, d (7.4)
8	113.4, *C*	7.59, d (8.0)	112.0, *CH*	7.57, d (8.0)
8a	142.9, *C*		142.3, *C*	
9a	134.8, *C*		134.1, *C*	
10	139.5, *C*		144.2, *C*	
11	136.5, *CH*	5.78, s	137.3, *C*	5.83, s
12	70.1, *C*		68.7, *C*	
13	37.6, CH_2	1.59, m 2.23, m	41.2, CH_2	1.42, m 2.13, m
14	20.3, CH_2	2.42, m 2.79, m	21.3, CH_2	2.30, m 2.61, m
15	128.1, *CH*	5.54, br s	128.3, *CH*	5. 59, br s
16	128.4, *CH*	5.30, br s	132.7, *CH*	5.34, br s
17	27.1, CH_2	1.55, m 1.81, m	25.8, CH_2	1.57, m 1.77, m
18	29.6, CH_2	1.30, m 1.56, m	26.9, CH_2	1.37, m 1.62, m
19	28.0, CH_2	1.42, m 1.50, m	25.6, CH_2	1.48, m 1.51, m
20	58.9, CH_2	2.31, m 2.82, m	53.1, CH_2	2.38, m 2.73, m
22	52.3, CH_2	2.07, m 2.94, m	49.9, CH_2	2.01, m 2.99, m
23	32.7, CH_2	2.45, m 2.71, m	33.8, CH_2	2.30, m 2.62, m
24	44.1, *CH*	2.29, m	42.0, *CH*	2.14, m
25	37.9, *C*		46.0, *C*	
26	75.0, *CH*	3.76, s	75.2, *CH*	3.78, s
28	53.6, CH_2	2.79, m 3.19, m	52.7, CH_2	2.68, m 3.15, m
29	26.7, CH_2	1.70, m 1.79, m	25.9, CH_2	1.82, m 1.95, m
30	31.7, CH_2	1.23, m 1.70, m	28.5, CH_2	1.35, m 1.67, m
31	24.6, CH_2	1.58, m 1.79, m	25.4, CH_2	1.51, m 1.66, m
32	129.2, *CH*	5.90, br s	28.9, *CH*	5.92, br s
33	130.5, *CH*	5.66, br s	29.9, *CH*	5.63, br s
34	63.8, *CH*	3.05, m	63.6, *CH*	3.11, m
35	51.8, CH_2	2.30, m 2.43, m	49.3, CH_2	2.25, m 2.56, m
36	69.5, CH_2	2.64, d (11.3) 2.21, d (11.3)	69.2, CH_2	2.62, d (11.3) 2.30, d (11.3)
37	33.6, CH_3	3.87, s	44.8, CH_2	3.85, t (6.1)
38			29.7, CH_2	1.68, m
39			35.8, CH_2	1.57, m
40			174.8, *C*	2.31, m
42			48.2, CH_3	3.92, s

[a] In $CDCl_3$, 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Solvent residue signals were use as the internal standard. Carbon multiplicities were determined by DEPT experiments.

to manzamine A, with the hydroxy derivatives **1** and **3** a little more active. However, substitution of nitrogen 9 of the carboline heterocycle yields noticeable changes in GSK-3β inhibition. Thus, small groups, such as Me or Et, are well-tolerated by the enzyme (compounds **4** and **5**), while the larger ones, such as *i*-But, $(CH_2)_{11}CH_3$, or *t*-BuOCOMe, led to inactive derivatives (compounds **6–8**).

We have also examined the effect of some changes in the aliphatic heterocyclic system (Table 8). In this case, conformational restriction of this polycyclic part elicits a significant effect on GSK-3 inhibition. Thus, the absence of a double bond between positions 15 and 16 results in inactive derivatives (compounds **10** and **13**), while the absence of Δ^{32} unsaturation yields slight activity changes. While compound **9** with an epoxy function in the cyclooctane ring maintains the same GSK-3 inhibition of manzamine A, derivatives **11** and **12** with a carbonyl group in position 31 are less potent than manzamine A and compound **1**, respectively. However, if the hydroxyl group of derivative **12** is substituted with different bulky moieties (compounds **14–16**), an increase in GSK-3 inhibition is observed. This fact is in agreement with data obtained for compounds **1** and **3–5** and should point to the possibility of a pocket in the GSK-3 enzyme, where the phenyl ring of the carboline heterocycle should be allocated.

Moreover, if conformational restriction is increased in the aliphatic part of manzamines (Table 9), the inhibition of the enzyme is slightly increased (compounds **17** and **18**).

Table 5. ^{13}C and ^{1}H NMR Data of Compounds **10** and **13**[a]

	10		**13**	
position	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)
1	142.9, *C*		144.8, *C*	
3	138.2, *CH*	8.40, d (5.2)	138.6, *CH*	8.64, d (5.0)
4	114.0, *CH*	7.78, d (5.2)	113.6, *CH*	7.83, d (5.1)
4a	130.2, *C*		129.6, *C*	
4b	123.4, *C*		121.8, *C*	
5	112.6, *CH*	7.56, d (7.7)	122.0, *CH*	8.09, d (7.7)
6	121.0, *CH*	7.03, dd (7.7, 7.5)	120.0, *CH*	7.26, dd (7.6, 7.5)
7	112.4, *CH*	6.96, d (7.5)	128.3, *CH*	7.53, dd (7.6, 7.5)
8	144.0, *C*		112.0, *CH*	7.58, d (7.5)
8a	130.8, *C*		141.8, *C*	
9a	133.6, *C*		133.5, *C*	
10	140.7, *C*		144.2, *C*	
11	137.7, *CH*	6.78, s	137.3, *CH*	6.61, s
12	69.2, *C*		68.7, *C*	
13	37.9, CH_2	2.07, m	41.2, CH_2	2.02, m 1.83, m
14	20.6, CH_2	1.60, m	21.3, CH_2	2.30, m 2.21, m
15	23.8, CH_2	1.42, m	24.1, CH_2	1.53, m
16	25.4, CH_2	1.73, m	25.3, CH_2	1.68, m
17	27.3, CH_2	1.58, m	25.8, CH_2	2.57, m 1.57, m
18	25.7, CH_2	1.55, m	26.9, CH_2	1.70, m
19	26.3, CH_2	1.64, m, 1.43, m	25.6, CH_2	1.72, m
20	59.1, CH_2	2.45, dd (12.0, 5.3) 2.32, m	53.1, CH_2	2.68, m 2.43, m
22	52.5, CH_2	2.97, m 2.34, m	49.9, CH_2	2.80, m 1.99, m
23	33.2, CH_2	2.05, m 1.74, m	33.8, CH_2	2.30, m
24	41.2, *CH*	3.36, m	42.0, *CH*	3.14, m
25	46.8, *C*		46.0, *C*	
26	81.4, *CH*	3.76, s	81.3, *CH*	3.78, s
28	45.8, CH_2	3.44, m 2.97, m	52.7, CH_2	3.38, m 2.95, m
29	29.9, CH_2	1.70, m	25.9, CH_2	1.80, m 1.65, m
30	25.9, CH_2	2.05, m 1.75, m	28.5, CH_2[b]	1.55, m
31	28.7, CH_2 [b]	1.58, m	25.4, CH_2[b]	1.77, m
32	25.2, CH_2[b]	1.75, m	28.9, CH_2[b]	2.15, m 1.55, m
33	30.8, CH_2	1.72, m	29.9, CH_2	1.65, m
34	64.3, *CH*	2.99, m	63.6, *CH*	3.11, m
35	52.0, CH_2	2.40, m	49.3, CH_2	1.85, m 1.56, m
36	69.6, CH_2	2.62, d (11.2) 2.18, d (11.2)	69.2, CH_2	2.62, brd (11.9) 2.30, d (12.0)

[a] In $CDCl_3$, 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Solvent residue signals were use as the internal standard. Carbon multiplicities were determined by DEPT135 experiments. [b] Interchangeable in the same column.

Table 6. ^{13}C and ^{1}H NMR Data of Compounds **17** and **18**[a]

	17		**18**	
number	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)
1	146.5, *C*		146.3, *C*	
3	137.9, *CH*	8.41, d (5.3)	138.5, *CH*	8.38, d (5.6)
4	113.0, *C*	7.81, d (5.3)	113.5, *CH*	7.82, d (5.6)
4a	128.3, *C*		128.9, *C*	
4b	124.2, *C*		124.4, *C*	
5	118.9, *C*	7.96, d (7.5)	119.1, *CH*	7.97, d (7.8)
6	119.5, *C*	7.14, dd (7.6, 7.5)	119.5, *CH*	7.23, dd (7.8, 7.6)
7	120.2, *C*	7.30, d (7.6)	120.2, *CH*	7.29, d (7.6)
8	112.6, *C*	7.68, d (7.6)	136.7, *C*	
8a	133.3, *C*		133.2, *C*	
9a	134.6, *C*		133.9, *C*	
10	144.0, *C*		144.0, *C*	
11	135.0, *C*	6.60, s	135.0, *CH*	6.50, s
12	138.7, *C*		138.5, *C*	
13	131.1, *CH*	6.45, dd (8.5, 7.0)	131.1, *CH*	6.43, dd (8.5, 7.0)
14	21.6, CH_2	2.12, m	21.5, CH_2	2.21, m 1.80, m
15	130.6, *CH*	5.43, m	127.4, *CH*	5.56, m
16	131.2, *CH*	5.35, m	133.1, *CH*	5.50, m
17	23.7, CH_2	1.89, m 1.73, m	25.6, CH_2	1.65, m
18	24.7, CH_2	1.38, m	27.0, CH_2	1.42, m 1.30, m
19	27.7, CH_2	1.92, m	26.7, CH_2	1.40, m 1.32, m
20	59.6, CH_2	2.62, m 2.51, m	53.1, CH_2	2.60, m 2.43, m
22	51.2, CH_2	2.71, m 1.98, m	49.4, CH_2	2.83, m 1.95, m
23	28.0, CH_2	2.73, m 2.41, m	34.2, CH_2	2.81, m
24	38.7, *CH*	3.96, m	41.7, *CH*	2.44, m
25	52.2, *C*		47.7, *C*	
26	64.3, *CH*	4.88, s	72.3, *CH*	3.50, s
28	45.6, CH_2	3.85, dd (14.7, 4.7) 3.34, dd (14.7, 10.8)	50.5, CH_2	3.14, dd (11.9, 8.7) 2.99, dd (11.8, 4.7)
29	24.8, CH_2	1.38, m	25.7, CH_2	1.76, m
30	28.4, CH_2	1.88, m 1.60, m	27.7, CH_2	2.15, m 2.05, m
31	29.5, CH_2	2.15, m	31.7, CH_2	2.42, m
32	132.6, *CH*	5.87, dd (7.7, 7.6)	132.8, *CH*	5.84, dd (7.7, 7.6)
33	131.3, *CH*	5.24, m	131.1, *CH*	5.21, m
34	161.8, *C*		53.7, *CH*	3.99, dd (8.3, 8.2)
35	44.9, CH_2	2.41, m 1.46, d (12.9)	44.8, CH_2	2.42, m 1.49, d (12.9)
36	62.7, CH_2	2.62, d (10.9) 2.51, d (10.9)	68.6, CH_2	2.76, d (11.5) 2.30, m
8-OAc			169.5, *C* 21.4, CH_3	2.53, s

[a] In $CDCl_3$, 400 or 500 MHz for ^{1}H NMR and 100 or 125 MHz for ^{13}C NMR. Solvent residue signals were use as the internal standard. Carbon multiplicities were determined by DEPT135 experiments.

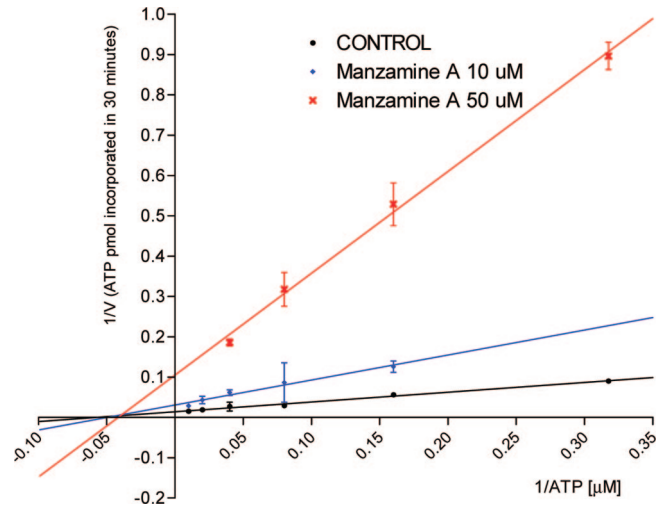


Figure 2. Double-reciprocal Lineweaver–Burk plot of kinetic data from assays of GSK-3β protein kinase activity at different concentrations of manzamine A. The ATP concentration in the reaction mixture varied from 3 to 100 μM. Manzamine A concentrations were 10 and 50 μM. The kinase activity was measured by increasing ATP up to 100 mM, where enzyme saturation could be ensured.

To further assess the potential of manzamine A in the treatment of Alzheimer's disease, we investigated its ability in inhibiting several different kinases and decreasing the hyperphosphorylation of tau protein mediated by GSK-3 in human neuroblastoma cell cultures.

Manzamine A was evaluated in a select kinase panel composed of GSK-3β, GSK-3α, CDK-5, CDK-1, PKA, and MAPK. Manzamine inhibits specifically GSK-3β and CDK-5 with an IC_{50} of 10 and 1.5 μM, respectively, while no inhibition was observed in the others. These two kinases are the key players in the hyperphosphorylation of tau protein in Alzheimer's disease.[31]

Moreover, treatment of the SH-SY5Y cell culture with manzamine A at different concentrations (5, 15, and 50 μM) resulted in a decrease of tau phosphorylation measured with a specific enzyme-linked immunosorbent assay (ELISA) sandwich method at the epitope Ser396, which is phosphorylated specifically by GSK-3. Cell survival was determined in parallel by measuring lactate dehydrogenase (LDH) release. As a positive control, alsterpaullone (Alst) was used in the experiment (Figure 4).

Conclusion

Manzamine A and several related derivatives have been identified as new GSK-3β inhibitors. The first SAR results for the manzamine class against GSK-3β are reported here. Additionally, manzamine A inhibits not only GSK-3 but also CDK-5, the two main kinases implicated in tau hyperphosphorylation *in vivo*. Moreover, manzamine A proved to be effective in decreasing tau hyperphosphorylation on human neuroblastoma cell lines, a demonstration of its ability to enter cells and interfere with tau pathology. These results suggest that manzamine A constitutes a new scaffold from which more potent and selective GSK-3 inhibitors could be designed as

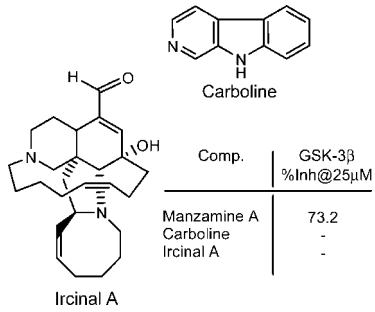
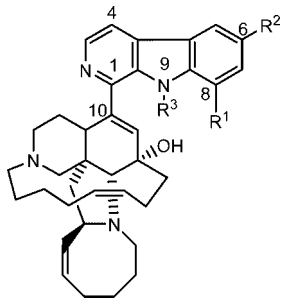


Figure 3. GSK-3 inhibition by manzamine A and its precursors.

Table 7. GSK-3 Inhibition by Manzamine A and Analogues **1**–**8**[a]



compounds	R^1	R^2	R^3	GSK-3β percent inhibition at 25 μM	GSK-3β IC_{50} (μM)
manzamine A	H	H	H	73.2	10.2
1	OH	H	H	86.7	4.8
2	H	OH	H	74.3	16.6
3	OTs	H	H	80.4	
4	OMe	H	Me	72.4	nd
5	OEt	H	Et	78.0	10.4
6	O-*i*-But	H	*i*-But	24.2	nd
7	H	H	$(CH_2)_{11}CH_3$	0	nd
8	H	H	*t*-BuOCOMe	3	nd

[a] nd = not determined.

potential therapeutic agents for the treatment of diseases mediated by GSK-3, such as the Alzheimer's disease, and further validates the marine environment as a rich source for new therapeutic agents for neurodegenerative diseases.

Experimental Section

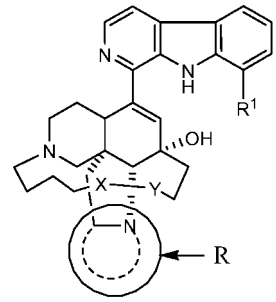
General Experimental Procedures. Optical rotations were measured with a JASCO DIP-310 digital polarimeter. The IR spectra were recorded on ATI Mattson Genesis Series Fourier transform infrared (FTIR) spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$, on NMR spectrometers operating at 400 or 500 MHz for ^1H NMR and 100 or 125 MHz for ^{13}C NMR. Chemical shift (δ) values are expressed in parts per million (ppm) and are referenced to the residual solvent signals of $CDCl_3$ at δ_H/δ_C 7.26/77.0. The high-resolution electrospray ionization mass spectrometry (HRESIMS) spectra were measured using a Bioapex FTESI-MS with electrospray ionization. High-performance liquid chromatography (HPLC) was carried out on a Waters 510 model system and a dual wavelength detector.

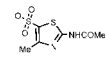
Preparation of 9-*N*-Alkyl-8-alkoxy-manzamine A (4–6). To a solution of 8-hydroxymanzamine-A (0.1 mmol) in 5 mL of dry acetone were added 1.5 equiv of potassium carbonate and alkyl iodide. The solution was refluxed overnight. The reaction was monitored by TLC. After completion of the reaction, water was added and the crude product was extracted with chloroform and dried over Na_2SO_4. The solvent was concentrated under reduced pressure, and the crude product was purified by HPLC.

9-*N*-Methyl-8-methoxy-manzamine A (4). Yield 80%. mp 172−174 °C. ^1H and ^{13}C NMR data: see Table 1. HRESIMS *m/z*: found, 593.3879 (calcd for $C_{38}H_{49}N_4O_2$, $[M + H]^+$, 593.3856).

9-*N*-Ethyl-8-ethoxy-manzamine A (5). Yield 76%. mp 187–189 °C. ^1H and ^{13}C NMR data: see Table 1. HRESIMS *m/z*: found, 621.4151 (calcd for $C_{40}H_{53}N_4O_2$, $[M + H]^+$, 621.4169).

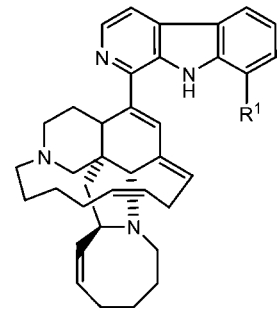
Table 8. GSK-3 Inhibition by Manzamine Analogues **9**–**17**[a]



Compd	R^1	X-Y	R	GSK-3β %Inhibition @25μM	GSK-3β IC_{50} (μM)
9	H	CH=CH		88.4	n.d.
10	OH	$(CH_2)_2$		29	n.d.
11	H	CH=CH		53.6	25
12	OH	CH=CH		30.0	n.d.
13	H	$(CH_2)_2$		0	n.d.
14		CH=CH		78.8	8.5
15		CH=CH		76.7	7.2
16		CH=CH		63.0	23

[a] nd = not determined.

Table 9. GSK-3 Inhibition by Dehydromanzamine derivatives **17** and **18**[a]



compounds	R^1	GSK-3β percent inhibition at 25 μM	GSK-3β IC_{50} (μM)
17	H	71.2	5.4
18	OCOMe	79.1	4.8

[a] nd = not determined.

9-*N*-Isobutyl-8-isobutyloxy-manzamine A (6). Yield 75%. mp 200–201 °C. ^1H and ^{13}C NMR data: see Table 1. HRESIMS *m/z*: found, 677.4779 (calcd for $C_{44}H_{61}N_4O_2$, $[M + H]^+$, 677.4795).

Synthesis of 8-Arylsulfonyl-manzamines (3 and 14–16). To a solution of manzamine F (**4**) or 8-hydroxymanzamine A (0.1 mmol) in dry DCM (5 mL), Et_3N (0.15 mmol) was added at 0 °C. After 5

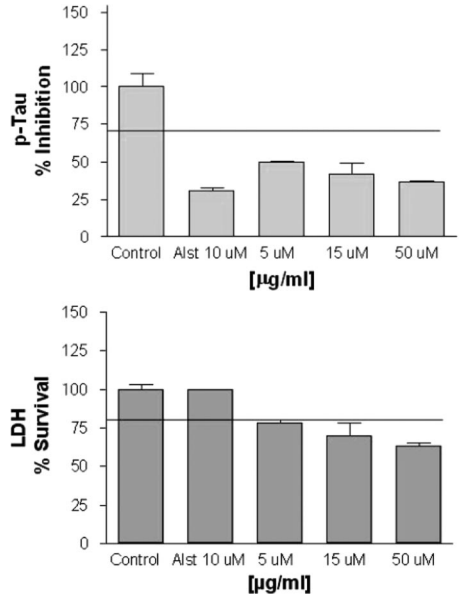


Figure 4. Inhibition of tau phosphorylation by manzamine A.

min of stirring at 0 °C, RSO_2Cl (0.15 mmol) was added and stirring was continued for 24 h at room temperature. The reaction mixture was diluted with DCM and washed with water and brine. The obtained crude product after solvent evaporation was purified by HPLC.

8-*O*-(4-Toluenesulfonyl)manzamine A (3). Yield 78%. mp 167–169 °C. ^{1}H and ^{13}C NMR data: see Table 2. HRESIMS *m/z*: found, 719.3617 (calcd for $C_{43}H_{51}N_4O_4S$, $[M + H]^+$, 719.3631).

8-*O*-(2-Acetamido-4-methylthiazole-5-sulfonyl)manzamine F (14). Yield 79.5%. mp >205 °C (dec). ^{1}H and ^{13}C NMR data: see Table 3. HRESIMS *m/z*: found, 799.3325 (calcd for $C_{42}H_{51}N_6O_6S_2$, $[M + H]^+$, 799.3311).

8-*O*-(4-Chlorobenzenesulfonyl)manzamine F (15). Yield 81%. mp >198 °C (dec). ^{1}H and ^{13}C NMR data: see Table 3. HRESIMS *m/z*: found, 755.3023 (calcd for $C_{42}H_{48}ClN_4O_5S$, $[M + H]^+$, 755.3034).

8-*O*-(4-Bromobenzenesulfonyl)manzamine F (16). Yield 82%. mp 209 °C (dec). ^{1}H and ^{13}C NMR data: see Table 3. HRESIMS *m/z*: found, 799.2536 (calcd for $C_{42}H_{48}BrN_4O_5S$, $[M + H]^{+\cdot}$ 799.2529).

Preparation of Compounds 7 and 8. Manzamine A (0.1 mmol) was dissolved in 5 mL of dry DMF and stirred for 15 min in a Dewar flask at 0 °C. To this solution, 95% NaH (0.12 mmol) was added with care and stirred for an additional 5 min; alkyl iodide (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 allowed to stir for 15 min. The mixture was extracted with Et_2O (2 × 50 mL), and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude product was chromatographed on a Phenomenex Luna C8, 5 μm, 250 × 10 mm column using a water and acetonitrile gradient with the flow rate of 8 mL/min to obtain pure compounds **7** and **8**.

9-*N*-Dodecylmanzamine A (7). Yield 81%. mp 163–164 °C. $[\alpha]_D^{25}$ +33 (*c* 0.1, $CHCl_3$). UV λ_{max} (log ε): (MeOH) 236 (3.43), 237 (3.91), 276 (2.18) nm. IR ν_{max} (film): 3659 (NH), 3014, 1747, 1718, 1654, 1558, 1406, 1337, 1024 cm^{-1}. ^{1}H and ^{13}C NMR data: see Table 4. HRESIMS *m/z*: found, 717.5478 (calcd for $C_{48}H_{69}N_4O$ $[M + H]^+$ 717.5471).

9-(4-Methylcarboxybutyl)-manzamine A (8). Yield 76%. mp 154–155 °C. $[\alpha]_D^{25}$ +32 (*c* 0.1, $CHCl_3$). UV λ_{max} (log ε): (MeOH) 237 (3.71), 251 (3.36), 285 (2.01) nm. IR ν_{max} (film): 3650 (NH), 3024–2815, 1725, 1701, 1685, 1569, 1476, 1427, 1236, 1019 cm^{-1}. ^{1}H and ^{13}C NMR data: see Table 4. HRESIMS *m/z*: found, 663.4291 (calcd for $C_{42}H_{55}N_4O$ $[M + H]^+$ 663.4274).

Preparation of Compounds 10 and 13. Manzamine A (0.1 mmol) was dissolved in 5 mL of ethanol, and 5 mg of palladium charcoal was added 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 a hexane/ethyl acetate gradient yielding **13** as a major compound (68.3%). A similar reaction procedure has been applied to 8-hydroxymanzamine A yielding **10** as a major compound (51.7%).

Tetrahydro-8-hydroxymanzamine A (10). Yield 51.7%. mp > 173 °C (dec). $[\alpha]_D^{25}$ +23 (*c* 0.1, $CHCl_3$). UV λ_{max} (log ε): (MeOH) 254 (3.23), 271 (3.86), 349 (2.71) nm. IR ν_{max} (film): 3661 (NH), 3037, 1728, 1614, 1438, 1271, 1053 cm^{-1}. ^{1}H and ^{13}C NMR data: see Table 5. HRESIMS *m/z*: found, 569.3961 (calcd for $C_{36}H_{49}N_4O_2$ $[M + H]^+$ 569.3856).

Tetrahydromanzamine A (13). Yield 68.3%. mp 171–172 °C. $[\alpha]_D^{25}$ +28 (*c* 0.1, $CHCl_3$). UV λ_{max} (log ε): (MeOH) 241 (3.62), 249 (3.93), 279 (2.46) nm. IR ν_{max} (film): 3653 (NH), 3042, 1716, 1663, 1526, 1451, 1418, 1273, 1052 cm^{-1}. ^{1}H and ^{13}C NMR data: see Table 5. HRESIMS *m/z*: found, 553.3922 (calcd for $C_{36}H_{49}N_4O$ $[M + H]^+$ 553.3906).

Preparation of Compounds 17 and 18. Manzamine A (0.1 mmol) was dissolved in 15 mL of dry acetone, and 1.2 mmol of anhydrous K_2CO_3 and 11 μL of anhydrous acetic anhydride were added and 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 ethylacetate was added and washed with an equal amount of brine solution. The organic layer was dehydrated on Na_2SO_4 and then removed *in vacuo*. The mixture was chromatographed on a silica gel column using a hexane/ethylacetate gradient yielding compound **17** (49.8%). A similar reaction procedure has been applied to 8-hydroxymanzamine A yielding compound **18** (46.4%).

12,13-Dehydromanzamine A (17). Yield 49.8%. mp >161 °C (dec). $[\alpha]_D^{25}$ +34 (*c* 0.1, $CHCl_3$). UV λ_{max} (log ε): (MeOH) 241 (3.29), 258 (3.91), 283 (3.14), 357 (2.38) nm. IR ν_{max} ($CHCl_3$): 3668 (NH), 3035, 1715, 1611, 1546, 1442, 1275, 1036 cm^{-1}. ^{1}H and ^{13}C NMR data: see Table 6. HRESIMS *m/z*: found, 531.3495 (calcd for $C_{36}H_{47}N_4$ $[M + H]^+$ 531.3488).

12,13-Dehydro-8-*O*-acetylmanzamine A (18). Yield 46.4%. mp >173 °C (dec). $[\alpha]_D^{25}$ +37 (*c* 0.1, $CHCl_3$). UV λ_{max} (log ε): (MeOH) 241 (3.29), 258 (3.91), 283 (3.14), 357 (2.38) nm. IR ν_{max} ($CHCl_3$): 3668 (NH), 3035, 1721, 1624, 1553, 1474, 1235, 1012 cm^{-1}. ^{1}H and ^{13}C NMR data: see Table 6. HRESIMS *m/z*: found, 589.3548 (calcd for $C_{38}H_{49}N_4O_2$ $[M + H]^+$ 589.3543).

Biological Section.

GSK-3β Inhibition. Recombinant human glycogen synthase kinase 3β (Upstate, catalog number 14-306) was assayed in 11 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) at pH 7.4, 0.2 mM ethylenediaminetetraacetic acid (EDTA), 1.25 mM ethylene glycol bis(2-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 26.25 mM $MgCl_2$, and 0.25 mM sodium orthovanadate in the presence of 62.5 μM phosphoglycogen synthase peptide-2 (GS-2) (TOCRIS, catalog number 1352), 0.5 μCi γ-[^{33}P]ATP and unlabeled ATP (Sigma, A-9187) at a final concentration of 12.5 μM. The final assay volume was 20 μL. After incubation for 30 min at 30 °C, 15 μL aliquots were spotted onto P81 phosphocellulose papers. Filters were washed 4 times for at least 10 min each and counted with 1.5 mL of scintillation cocktail in a scintillation counter (Perkin-Elmer, Microbeta 1450). IC_{50} values were calculated, analyzing inhibition curves by nonlinear regression using GraphPad Prism. Kinetic experiments were performed, varying both ATP levels (ranging from 3 to 100 μM) and two inhibitor concentrations. The kinase activity was measured, increasing ATP up to 100 mM, where enzyme saturation could be ensured.

GSK-3α Inhibition. The protocol is the same as the one used for GSK-3β, but the recombinant GSK-3α was purchased from Upstate (catalog number 14-492) and the final GS2 concentration was 20 μM. The reaction was incubated for 12 min, with a 26 μM ATP concentration.

CDK-5 Inhibition. The CDK-5 inhibition method was the same as the one described for GSK-3β, and only the source of the enzyme and substrate are different. Human recombinant cdk5-p35 was purchased from MBL, MA, and the histone H1 as the substrate was from Calbiochem.

CDK-1 Inhibition. Recombinant CDK-1 (Upstate, catalog number 14-450) was used for the assay in ADBI buffer. The substrate was histone H1 (Upstate, catalog number 14-155) and was used at 3.12 mM. The final ATP concentration was 22 μM. The reaction was carried out at 30 °C for 15 min.

PKA Inhibition. Recombinant PKA, catalytic subunit (catalog number 14-440) was assayed in ADBI buffer in the presence of PKA Kemptide (catalog number 20-199) and 35 μM of unlabeled ATP. The

rest of the protocol was the same as the one described for the other kinases. The reaction was incubated for 20 min.

MAPK Inhibition. Recombinant MAP kinase (ERK1, Upstate, catalog number 14-439) was assayed in 20 mM MOPS at pH 7.2, 25 mM β-glycerol phosphate, 5 mM EGTA, and 1 mM sodium orthovanadate (ADBI buffer), in the presence of 10 μg of map kinase substrate [myelinic basic protein (MBP); Upstate, catalog number 13-104]. The final ATP concentration was 20 μM. The final volume of the assay was 20 μL, and the rest was the same as for GSK-3β. The incubation time was 15 min.

Inhibition of Tau Phosphorylation. Human neuroblastoma SHSY5Y cells were seeded in 96-well plates (30 000 cells well^{-1}) in MEM/F-12 Ham (minimum essential medium/nutrient mixture F-12 Ham) supplemented with 10% fetal calf serum, 2 mM L-glutamine, and penicillin (100 units/mL)/streptomycin (100 μg/mL). After 1 day, cells were treated with samples for 18 h at 37 °C. After treatment, cultures were washed with phosphate-buffered saline and lysed for 30 min at 4 °C in 120 μL of extraction buffer [10 mM Tris-HCl at pH 7.4, 100 mM NaCl, 1 mM EDTA, 2 mM Na_3VO_4, 1% Triton X-100, 10% glycerol, 0.1% sodium dodecyl sulfate (SDS), 0.5% sodium deoxycholate, 1 mM phenylmethylsulphonyl fluoride (PMSF), and a protease inhibitor cocktail (Roche, catalog number 1 697 498)].

The quantitative determination of phosphorylated human tau was made by taking 40 μL of the cell lysate and using a phosporylation-specific antibody against tau [pS396] in a sandwich ELISA (Biosource, catalog number KHB7031). Tau phosphorylation was estimated by measuring the absorbance at 450 nm in a microtiter plate reader (Cultek, Anthos 2010).

In addition to tau phosphorylation assays, quantification of cell death and cell lysis was made by measuring LDH release (Roche, catalog number 1 644 793). For the quantitative determination of cell survival, 40 μL of the cell lysate was incubated with an equal volume of reaction mixture at room temperature for 20–30 min. The measure of absorbance was made in a microtiter plate reader with 490–492 nm filter (Cultek, Anthos 2010).

References and Notes

(1) Cohen, P.; Frame, S. *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 769–776.
(2) Woodgett, J. R.; Cohen, P. *Biochim. Biophys. Acta* **1984**, *788*, 339–347.
(3) Nikoulina, S. E.; Ciaraldi, T. P.; Mudailar, S.; Mohideen, P.; Carter, L.; Henry, R. R. *Diabetes* **2000**, *49*, 263–271.
(4) Lovestone, S.; Reynolds, C. H.; Latimer, D.; Davis, D. R.; Anderton, B. H.; Gallo, J. M.; Hanger, D.; Mulot, S.; Marquardt, B. *Curr. Biol.* **1994**, *4*, 1077–1086.
(5) Imahori, K.; Uchida, T. *J. Biochem.* **1997**, *121*, 179–188.
(6) Takashima, A.; Murayama, M.; Yasutake, K.; Takahashi, H.; Yokoyama, M.; Ishiguro, K. *Neurosci. Lett.* **2001**, *306*, 37–40.
(7) Martinez, A.; Castro, A.; Dorronsoro, I.; Alonso, M. *Med. Res. Rev.* **2002**, *22*, 373–384.
(8) Naerum, L.; Norskov-Lauritsen, L.; Olesen, P. H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1525–1528.
(9) Martinez, A.; Alonso, M.; Castro, A.; Perez, C.; Moreno, F. J. *J. Med. Chem.* **2002**, *45*, 1292–1299.
(10) Witherington, J.; Bordas, V.; Garland, S. L.; Hickey, D. M. B.; Ife, R. J.; Liddle, J.; Saunders, M.; Smith, D. G.; Ward, R. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1577–1580.
(11) Coghlan, M. P.; Culbert, A. A.; Cross, D. A. E.; Corcoran, S. L.; Yates, J. D.; Pearce, N. J.; Rausch, O. L.; Murphy, G. J.; Carter, P. S.; Cox, L. R.; Mills, D.; Brown, M. J.; Haigh, D.; Ward, R. W.; Smith, D. G.; Murray, K. J.; Reith, A. D.; Holder, J. *Chem. Biol.* **2000**, *7*, 793–803.
(12) Alonso, M.; Martinez, A. *Curr. Med. Chem.* **2004**, *11*, 755–763.
(13) Meijer, L.; Flajolet, M.; Greengard, P. *Trends Pharmacol. Sci.* **2004**, *2*, 471–480.
(14) Dorronsoro, I.; Castro, A.; Martinez, A. *Expert Opin. Ther. Pat.* **2002**, *12*, 1527.
(15) Meijer, L.; Thunnissen, A. M.; White, A. W.; Garnier, M.; Nikolic, M.; Tsai, L. H.; Walter, J.; Cleverley, K. E.; Salinas, P. C.; Wu, Y. Z.; Biernat, J.; Mandelkow, E. M.; Kim, S. H.; Pettit, G. R. *Chem. Biol.* **2000**, *7*, 51.
(16) Gompel, M.; Leost, M.; De Kier, J. E. B.; Puricelli, L.; Franco, L. H.; Palermo, J.; Meijer, L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1703.
(17) Meijer, L.; Skaltsounis, A. L.; Magiatis, P.; Polychronopulos, P.; Knockaert, M.; Leost, M.; Ryan, X. P.; Vonica, C. A.; Brivanlou, A.; Dajani, R.; Crovace, C.; Tarricone, C.; Musacchio, A.; Roe, S. M.; Pearl, L.; Greengard, P. *Chem. Biol.* **2003**, *10*, 1144.
(18) Frenz, J. L.; Col, A. C.; Kerr, R. G. *Expert Opin. Ther. Pat.* **2004**, *14*, 17.
(19) Alonso, D.; Castro, A.; Martinez, A. *Exp. Opin. Ther. Pat.* **2005**, *15*, 1377–1386.
(20) Sakai, R.; Higa, T.; Jefford, C. W.; Bernardinelli, G. *J. Am. Chem. Soc.* **1986**, *108*, 6404.
(21) Hu, J. F.; Hamann, M. T.; Hill, R. S.; Kelly, M. In *The Alkaloids*; Cordell, G. A., Ed.; Elsevier Science: New York, 2003; Vol. 60, pp 207–285.
(22) Peng, J.; Hu, J.-F.; Kazi, A. B.; Li, Z.; Avery, M.; Peraud, O.; Hill, R.; Franzblau, S. G.; Zhang, F.; Schinazi, R. F.; Wirtz, S. S.; Tharnish, P.; Kelly, M.; Wahyuono, S.; Hamann, M. T. *J. Am. Chem. Soc.* **2003**, *125*, 13382–13386.
(23) Ichiba, T.; Corgiat, J. M.; Scheuer, P. J.; Kelly-Borges, M. *J. Nat. Prod.* **1994**, *57*, 168–170.
(24) Kobayashi, M.; Chen, Y. J.; Aoki, S.; In, Y.; Ishida, T.; Kitagawa, I. *Tetrahedron* **1995**, *51*, 3727–3736.
(25) Edrada, R. A.; Proksch, P.; Wray, V.; Witte, L.; Müeller, W. E. G.; van Soest, R. W. M. *J. Nat. Prod.* **1996**, *59*, 1056–1060.
(26) Ichiba, T.; Sakai, R.; Kohmoto, S.; Saucy, G.; Higa, T. *Tetrahedron Lett.* **1988**, *29*, 3083–3086.
(27) 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.; Hamann, M. T. *J. Nat. Prod.* **2006**, *69*, 1034–1040.
(28) Rao, K. V.; Kasanah, N.; Wahyuono, S.; Tekwani, B. L.; Schinazi, R. F.; Hamann, M. T. *J. Nat. Prod.* **2004**, *67*, 1314–1318.
(29) Yousaf, M.; Hammond, N. L.; Peng, J.; Wahyuono, S.; McIntosh, K. A.; Charman, W. N.; Mayer, A. M. S.; Hamann, M. T. *J. Med. Chem.* **2004**, *47*, 3512–3517.
(30) Rao, K. V.; Santarsiero, B. D.; Mesecar, A. D.; Schinazi, R. F.; Tekwani, B. L.; Hamann, M. T. *J. Nat. Prod.* **2003**, *66*, 823–828.
(31) Flaherty, D. B.; Soria, J. P.; Tomasiewicz, H. G.; Wood, J. G. *J. Neurosci. Res.* **2000**, *62*, 463–472.

NP060092R