from the three-carbon bridge are responsible for their reduced AChE inhibitory activity relative to that of huperzine A. On the other hand, comparison of the electrostatic fields of these compounds employing the semiempirical molecular orbital method, AM1, reveals differences in the electrostatic fields of huperzine A and analogues **4a** and **4b** in the region of the three-carbon bridge (isopotential maps and steric and electrostatic field–fit energies available in supplementary material). This result indicates that the reduced activities of **4a** and **4b** relative to that of huperzine A probably stem from the change in their electrostatic fields, and consequently reveals the function (i.e., to present the required electrostatic field to AChE) of the three-carbon bridge of huperzine A.

Again, as shown in Table I, the poor AChE activity of compound **5** can be attributed to the loss of the electrostatic contribution of the double bond together with the possible loss of hydrophobic binding due to the methyl group.

Taken together the foregoing physical properties and biological data of these newly synthesized compounds emphasize the importance of huperzine A's three-carbon bridge in presenting the required electrostatic field to the acetylcholinesterase enzyme. It is therefore unlikely that a potent AChE inhibitor can be found through extensive simplification of the huperzine A structure without considering the electrostatic field contributed by the unsaturated three-carbon bridge. Using some of the findings reported herein, progress on the design of more efficacious huperzine A analogues will be reported in due course.[14]

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Supplementary Material Available: Spectral data for compounds **3**, **4a**, **4b**, and **5**, full X-ray data report on compound **11b**, and complete details of the molecular modeling studies (34 pages); a listing of observed and calculated structure factors for all data (11 pages). Ordering information is given on any current masthead page.

† Neurochemistry Research, Mayo Clinic Jacksonville.
‡ Neuropharmacology Research, Mayo Clinic Jacksonville.
§ University of Pittsburgh.
∥ Merck Sharp & Dohme Research Laboratories.

Alan P. Kozikowski,*,† Chris P. Miller,§ Fumio Yamada† Yuan-Ping Pang,† Jacqueline H. Miller‡ Michael McKinney,‡ Richard G. Ball∥

*Neurochemistry Research and Neuropharmacology Research
Mayo Clinic Jacksonville
4500 San Pablo Road
Jacksonville, Florida 32224*

*Department of Chemistry
University of Pittsburgh
Pittsburgh, Pennsylvania 15260*

*Merck Sharp & Dohme Research Laboratories
P. O. Box 2000
Rahway, New Jersey 07065*

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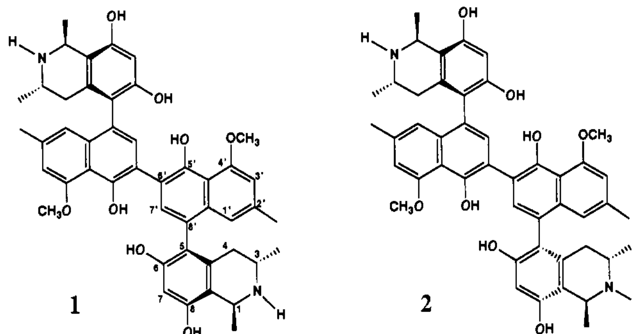
Novel Alkaloids from the Tropical Plant *Ancistrocladus abbreviatus* Inhibit Cell Killing by HIV-1 and HIV-2

The epidemic of acquired immune deficiency syndrome (AIDS) continues to present an urgent requirement for new drug development candidates with antiviral activity toward the human immunodeficiency virus (HIV). The U.S. National Cancer Institute has undertaken a major new initiative to discover novel anti-HIV agents from natural sources.[1]

In the present study, the NCI primary in vitro screen[1,2] initially disclosed anti-HIV-cytopathic activity in the organic extracts of the aerial parts of the tropical liana *Ancistrocladus abbreviatus* (Ancistrocladaceae), collected in Cameroon in March 1987. Bioassay-guided fractionation of those extracts provided the novel atropisomeric pair of anti-HIV-cytopathic alkaloids, michellamines A (1) and B (2).



The antiviral compounds were obtained in three steps. The crude extract was subjected to an acid–base partitioning scheme; the anti-HIV-cytopathic activity was concentrated in the basic fraction. This material was further separated by centrifugal partition chromatography ($CHCl_3$–CH_3OH–0.5% HBr/H_2O, 5:5:3, descending mode) and then by HPLC on an amino-bonded phase column [$CHCl_3$–0.075% $(NH_4)_2CO_3$/MeOH, 43:7] to give the two active compounds, 1 and 2.

Plasma desorption mass spectrometry (^{252}Cf PDMS) demonstrated that the two compounds had identical molecular weights (m/z 756). The molecular formula was established as $C_{46}H_{48}N_2O_8$ by high-resolution, fast-atom-bombardment mass spectrometry. While the family Ancistrocladaceae is well known as a source of naphthalene–tetrahydroisoquinoline alkaloids,[3-5] the mass spectral data and the complex NMR spectra of our isolates suggested that they were heretofore unknown dimeric relatives of the Ancistrocladaceae alkaloids.[6]

The presence of only 23 resonances in the ^{13}C NMR spectrum of 1 indicated that the two naphthalene–isoquinoline components were equivalent. The structure and relative stereochemistry of the tetrahydroisoquinoline subunit could be readily discerned from ^{1}H–^{1}H coupling constant analyses and difference NOE experiments. The

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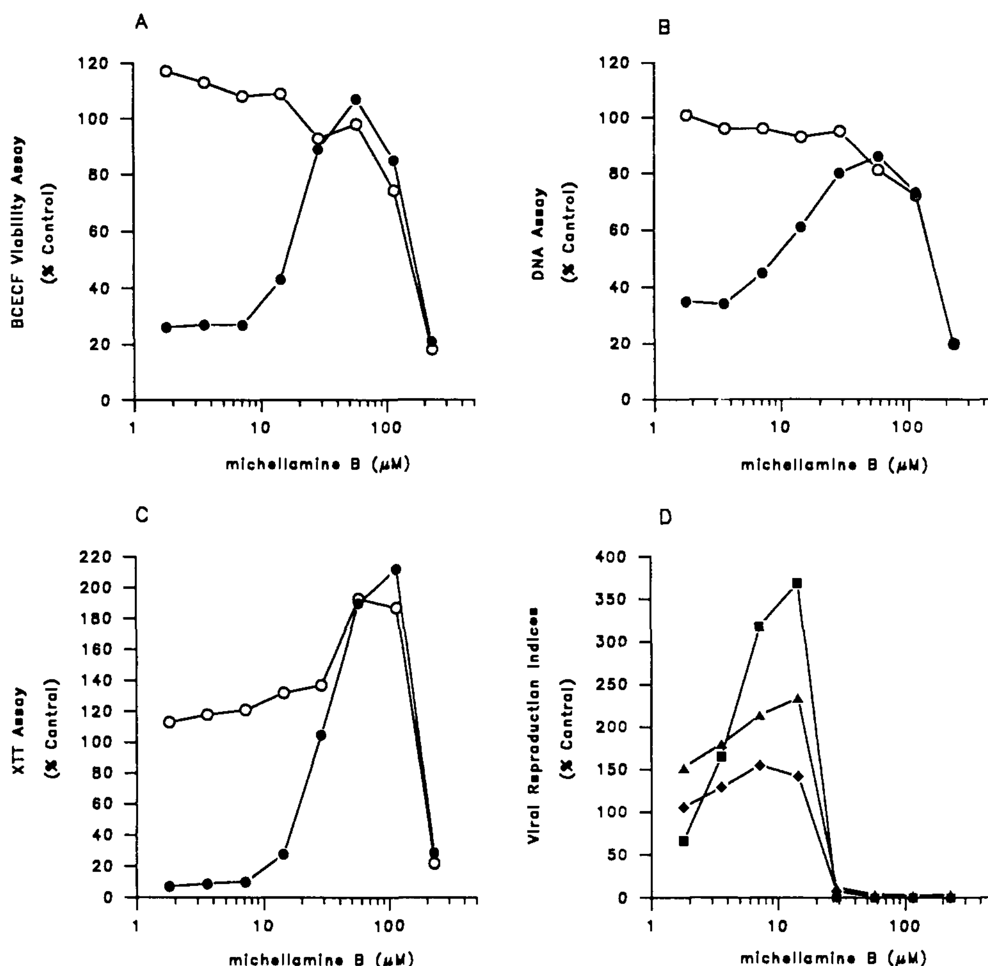

Figure 1. Graphs A, B, and C show the effects of a range of concentrations of 2 upon unifected CEM-SS cells (○) and upon HIV-1 infected CEM-SS (●), as determined after 6 days in culture. Graph A depicts the relative numbers of viable CEM-SS cells as assessed by the BCECF assay; graph B depicts the relative DNA content of the respective cultures; graph C depicts the relative numbers of viable CEM-SS cells, as assessed by the XTT assay. Graph D shows the effects of a range of concentrations of 2 upon indices of infectious virus or viral replication; these indices include viral reverse transcriptase activity (▲), production of viral core protein p24 (♦), and syncytium-forming units (■). In graphs A, B, and C, the data points are represented as the percent of the respective uninfected, non-drug-treated control values. In graph D the data points are represented as the percent of the respective infected, non-drug-treated control values.

H-3 proton served as a linchpin in the analysis. A pseudoaxial position on the ring was evident from its couplings to the H-4 protons (11.8, 4.3 Hz); a moderate to strong NOE response (7%) to the methyl group attached to C-1 established the 1,3 diaxial relationship between the two and therefore the trans relationship between the methyl groups attached to C-1 and C-3.

The composition of one ring in the naphthalene system was established through heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC), and difference NOE (nuclear Overhauser effect) experiments as a pair of meta-disposed protons, with an intervening methyl group and a flanking methoxyl.

(6) The numbering scheme follows the commonly accepted practice for the *Ancistrocladus* alkaloids.[3-5] The absolute stereochemistry has not been determined; that shown is an arbitrary selection based upon literature precedent.[4] Physicochemical and spectral features of 1 were as follows: 0.1% yield from dried plant; $[\alpha]_D$ −10.5°, $[\alpha]_{365}$ 65.7° (*c* 0.38, MeOH); FAB-MS *m/z* 757.342 (MH^+, calcd for $C_{46}H_{49}N_2O_8$ 757.3487); UV λ_{max} (MeOH) 230 nm (log ε = 4.4), 262 (4.1), 287 (3.8), 312 (3.8), 331 (3.8), 344 (3.8); IR ν_{max} (neat) 3380, 1617, 1584 cm^{-1}; ^{13}C NMR (125 MHz, CD_3OD, HBr salt, no. attached H from DEPT experiment) δ 49.5 (C-1, 1), 45.2 (C-3, 1), 33.1 (C-4, 2), 133.1 (C-4a, 0), 120.3 (C-5, 0), 156.9 (C-6, 0), 102.0 (C-7, 1), 155.4 (C-8, 0), 113.0 (C-8a, 0), 119.1 (C-1′, 1), 137.6 (C-2′, 0), 108.0 (C-3′, 1), 158.1 (C-4′, 0), 115.2 (C-4a′, 0), 152.2 (C-5′, 0), 119.0 (C-6′, 0), 134.8 (C-7′, 1), 124.1 (C-8′, 0), 136.6 (C-8a′, 0), 57.1 (OCH_3, 3), 19.4 (C-3 Me, 3), 18.4 (C-1 Me, 3), 22.1 (C-2′ Me, 3); ^1H NMR (500 MHz, CD_3OD, HBr salt) δ 7.30 (H-7′, s), 6.84 (H-3′, s), 6.75 (H-1′, s), 6.40 (H-7, s), 4.64 (H-1, q, *J* = 6.5), 4.10 (OCH_3, s), 3.54 (H-3, ddq, J = 11.8, 4.3, 6.5), 2.69 (H-4e, dd, *J* = 18.6, 4.3), 2.33 (C-2′ Me, s), 2.05 (H-4a, dd, *J* = 18.6, 11.8), 1.57 (C-1 Me, d, *J* = 6.5), 1.16 (C-3 Me, d, 6.5). Physicochemical and spectral features of 2 were as follows: 0.4% yield from dried plant; $[\alpha]_D$ −14.8°, $[\alpha]_{365}$ −23.4° (*c* 0.74, MeOH); FAB-MS *m/z* 757.350 (MH^+, calcd for $C_{46}H_{49}N_2O_8$ 757.3487); UV λ_{max} (MeOH) and ν_{max} identical to those of 1; ^{13}C NMR (125 MHz, CD_3OD, HBr salt, no. attached H from DEPT experiment) δ 49.6/49.3 (C-1, 1), 45.3/45.2 (C-3, 1), 33.9/33.0 (C-4, 2), 133.1/133.0 (C-4a, 0), 120.3/120.2 (C-5, 0), 156.90/156.88 (C-6, 0), 102.1/102.0 (C-7, 1), 155.54/155.51 (C-8, 0), 113.2/113.0 (C-8a, 0), 119.2 (C-1′, 1, 2×), 137.60/137.56 (C-2′, 0), 108.12/108.11 (C-3′, 1), 158.1/158.0 (C-4′, 0), 115.22/115.17 (C-4a′, 0), 152.3/152.2 (C-5′, 0), 119.1/119.0 (C-6′, 0), 136.7/136.5 (C-7′, 1), 124.12/124.10 (C-8′, 0), 135.2/134.7 (C-8a′, 0), 57.05/57.04 (OCH_3, 3), 22.2/22.1 (C-2′ Me, 3), 19.3 (C-3 Me, 3, 2×), 18.42/18.40 (C-1 Me, 3); ^1H NMR (500 MHz, CD_3OD, free base) δ 7.28/7.24 (H-7′, s), 6.84/6.82 (H-3′, s), 6.86/6.77 (H-1′, s), 6.34 (H-7, s, 2×), 4.44/4.26 (H-1, q, *J* = 6.5), 4.09/4.08 (OCH_3, s), 3.27/3.21 (H-3, ddq, *J* = 11, 4.5, 6.5), 2.49 (H-4e, dd, *J* = 17.5, 4.5), 2.31/2.22 (H-2′ Me, s), 2.22 (H-4a, dd, *J* = 17.5, 11), 2.08 (H-4e, dd, *J* = 17.5, 4.5), 1.86 (H-4a, dd, *J* = 17.5, 11), 1.52/1.48 (C-1 Me, d, *J* = 6.5), 1.05/1.01 (C-3 Me, d, *J* = 6.5).

The remaining ring had a single proton, one hydroxyl group, and linkages to two other aryl systems. HMBC and HMQC data suggested a 1,3 relationship of the proton and hydroxyl substituents. The complete substitution of that ring and the relative stereochemistry and conformation of the naphthalene–tetrahydroisoquinoline connection were secured from difference NOE data. Each of the benzylic methylene protons (C-4) of the tetrahydroisoquinoline system exhibited an NOE relationship to different naphthalene protons, H-4e to H-7′ (9%) and H-4a to H-1′ (7%). Thus, the tetrahydroisoquinoline was linked to the naphthalene by a bond from C-5 to C-8′. Assignment of this linkage was supported by long-range (three bond) correlations of the H-4 and H-7′ protons to C-5. The naphthalenes, therefore, had to be connected at C-6′.

In contrast, the ^{13}C NMR spectrum of 2 was comprised of 46 signals. A similar series of NMR experiments provided the same gross structure found for 1 and the same relative configuration in the tetrahydroisoquinoline rings. The differences between the two compounds lay in the relative configuration of the naphthalene–tetrahydroisoquinoline ring connections. In 2, the C-4 methylene signals appeared as four discrete resonances and each produced an NOE enhancement of an aromatic proton signal upon irradiation. In one set, the relationships were the same as those in 1: H-4e and H-7′ (4%), H-4a and H-1′ (4%). The relationships were reversed in the other half of the molecule: H-4e and H-1′ (3%), H-4a and H-7′ (5%). As before, the assignments of the protons in the tetrahydroisoquinoline system were clearly established from coupling constant and NOE data. Variable-temperature NMR experiments have failed to show evidence of interconversion, but either 1 or 2 can be converted to a mixture of the two (1:1) by prolonged treatment with base (equilibrium is reached in approximately 5 days).

For a more definitive demonstration of the anticytopathic activity of the pure michellamines, a battery of interrelated assays was performed concurrently on individual wells from 96-well microtiter plates.[7,8] We estimated cellular viability, in the presence and absence of the michellamines, in uninfected and HIV-infected cells, both by an adaptation of the published XTT method[2] and by means of the fluorescent probe 2′,7′-bis(carboxyethyl)-5-(6)-carboxyfluorescein acetoxymethyl ester (BCECF),[9] a nonfluorescent molecule which enters viable cells where it is hydrolyzed by cellular esterases to a fluorescent marker. Total cellular DNA content was measured with the dye diamidino-2-phenylindole (DAPI),[10] which fluoresces when intercalated at A-T specific sites in chromatin. Viral reverse transcriptase (RT), P24 antigen (p24), and syncytium-forming units (SFU) were assayed as described.[7]

As illustrated in Figure 1A–D, 2 was capable of complete inhibition of the cytopathic effects of HIV-1 upon CEM-SS human lymphoblastoid target cells in vitro (EC_{50} ~20 μM); at higher concentrations, it exerted only a weakly cytotoxic effect on uninfected target cells (IC_{50} ~200 μM). Similar results were obtained with 1: moreover, the water-soluble HBr and HOAc salts of both compounds were equally as effective as the free bases (data not shown). Both alkaloids also inhibited the production of RT, p24, and SFU in HIV-infected CEM-SS cells at the inhibitory effective concentrations, indicating a diminution of viral replication. Interestingly, at subeffective concentrations of either 1 or 2, there was an apparent enhancement of RT, p24, and

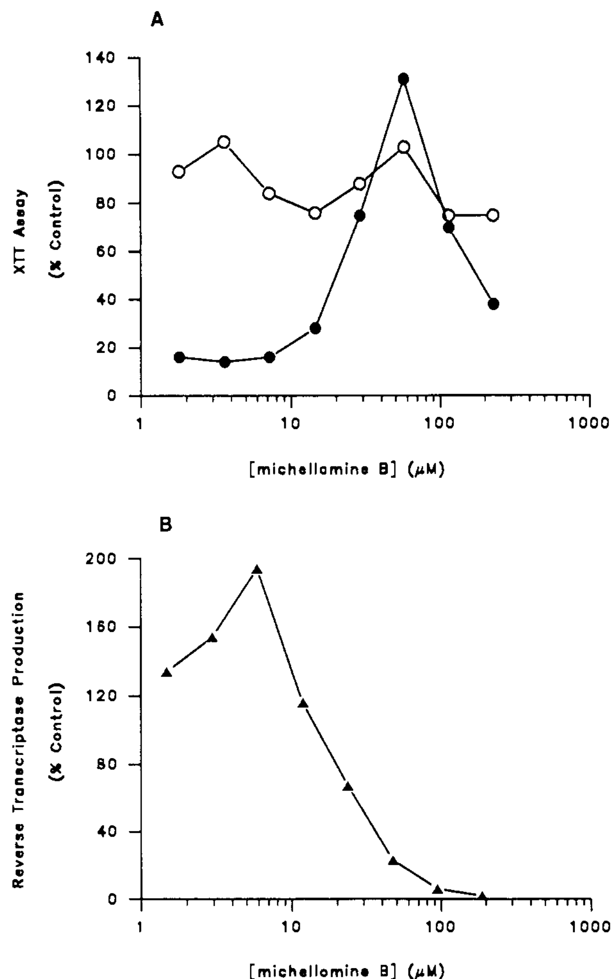

Figure 2. Graph A shows the effect of 2 upon uninfected (○) and HIV-2 infected (●) MT-2 cells assessed after 6 days in culture using the XTT metabolic activity assay. Data points are represented as the percent of the respective uninfected, non-drug-treated control values. Graph B shows the effect of a range of concentrations of 2 upon production of viral reverse transcriptase. Data points are represented as the percent of the respective infected, non-drug-treated control values.

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(8) The human lymphocytic target cell lines (CEM-SS and MT-2) used in the antiviral assays were maintained in RPMI 1640 medium (Gibco, Grand Island, NY) without phenol red and supplemented with 5% fetal bovine serum, 2 mM L-glutamine and 50 μg/mL gentamicin (complete medium). Exponentially growing CEM-SS or MT-2 cells were pelleted and resuspended at a concentration of 2.0 × 10^5 cells/mL in complete medium. For the HIV-1 studies, the Haitian variant of HIV, HTLV-III_{RF} (3.54 × 10^6 SFU/mL), was used. For the HIV-2 studies, the NIH-DZ strain (2.8 × 10^5 SFU/mL) was used. Frozen virus stock solutions were thawed immediately before use and resuspended in complete medium to yield 1.2 × 10^5 SFU/mL. The appropriate amounts of the pure compounds for anti-HIV evaluations were dissolved in 100% dimethyl sulfoxide (DMSO) and then diluted in complete medium to the desired initial concentration (and with final DMSO content not exceeding 1%). Then, all serial drug dilutions, reagent additions and plate-to-plate transfers were carried out as described[7] using an automated Biomek 1000 Workstation (Beckman Instruments, Palo Alto, CA). Uninfected CEM-SS or MT-2 cells were plated at a density of 1 × 10^4 cells in 50 μL of complete medium. Diluted HIV-1 or HIV-2 virus was then added to appropriate wells in a volume of 50 μL to yield a multiplicity of infection of 0.6. Appropriate cell, virus, and drug controls were incorporated in each experiment; the final volume in each microtiter well was 200 μL. Quadruplicate wells were used for virus-infected cells and duplicate wells were used for uninfected cells. Plates were incubated at 37 °C in an atmosphere

SFU; however, the significance of these observations with respect to the potential utility of the michellamines as cytoprotective agents against HIV is unknown.

The mechanism of anti-HIV-cytopathic effects of the michellamines is as yet unknown. However, it is notable that **2**, either as the free base or the HBr salt, exhibited the same potency against the cytopathic effects of HIV-2 upon MT-2 target cells in vitro (Figure 2) as it did against HIV-1 upon CEM-SS cells. This is significant because very few of the known anti-HIV-1 agents demonstrate any activity against HIV-2. Michellamine A (**1**) was somewhat less effective than **2** against HIV-2, affording only partial protection at comparable concentrations (data not shown). These compounds represent a novel new active chemotype for investigation in the quest for effective anti-HIV drug candidates.

Chemically, the michellamines are unique in several regards. They are the first dimeric alkaloids of this class to be discovered. None of the known "monomeric" alkaloids have the C-5/C-8′ linkage between the two ring systems.[3-5] Further, they are the most polar compounds in the class, containing more free phenols per monomeric unit than any of the known compounds.[3-5] Extracts of *Ancistrocladus tectorius*, which contain monomeric alkaloids of this series,[5] were inactive in the anti-HIV assay. It cannot yet be ascertained whether this is a reflection of the differences in functionalities or in the linkage of the naphthalene and isoquinoline units, or whether the dimeric unit is required for activity.

containing 5% CO_2 for 6 days. Subsequently, aliquots of cell-free supernatant were removed from each well using the Biomek, and analyzed for reverse transcriptase activity, p24 antigen production, and synthesis of infectious virions as described.[7] Cellular growth or viability then was estimated on the remaining contents of each well using the XTT,[2] BCECF,[9] and DAPI[10] assays as described.[7] To facilitate graphical displays and comparisons of data, the individual experimental assay results (of at least quadruplicate determinations for each) were averaged, and the mean values were used to calculate percentages in reference to the appropriate controls. Standard errors of the mean values used in these calculations typically averaged less than 10% of the respective mean values.

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* To whom correspondence should be addressed at the Laboratory of Drug Discovery Research and Development, National Cancer Institute, Building 1052, Room 121, Frederick, MD 21702-1201.
† On leave from the University of Canterbury, Christchurch, New Zealand.
‡ Laboratory of Drug Discovery Research and Development, National Cancer Institute.
§ Laboratory of Bioorganic Chemistry, National Institute of Diabetes, and Digestive and Kidney Diseases.
‖ Natural Products Branch, National Cancer Institute.

Kirk P. Manfredi,[‡] John W. Blunt[†,‡]
John H. Cardellina, II,[‡] James B. McMahon[‡]
Lewis L. Pannell,[§] Gordon M. Cragg[‖]
Michael R. Boyd*[,‡]

Laboratory of Drug Discovery Research and Development
National Cancer Institute
Frederick, Maryland 21702-1201

Laboratory of Bioorganic Chemistry
National Institute of Diabetes, and
Digestive and Kidney Disease
Bethesda, Maryland 20892

Natural Products Branch
National Cancer Institute
Frederick, Maryland 21702-1201

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Additions and Corrections

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Sumalee Chumpradit, Mei-Pung Kung, Jeffrey J. Billings, and Hank F. Kung*: Synthesis and Resolution of (±)-7-Chloro-8-hydroxy-1-(3′-iodophenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (TISCH): A High Affinity and Selective Iodinated Ligand for CNS D1 Dopamine Receptor.

Page 877. One of the structures in Chart I, IMAB (**1e**) R_1: Cl, R_2: N_3, R_3: I, should be changed to IMAB (**1e**) R^1: I, R_2: N_3, R_3: H.

Ronald H. Erickson,* Roger N. Hiner, Scott W. Feeney, Paul R. Blake, Waclaw J. Rzeszotarski, Rickey P. Hicks, Diane G. Costello, and Mary E. Abreu: 1,3,8-Trisubstituted Xanthines. Effects of Substitution Pattern upon Adenosine Receptor A_1/A_2 Affinity.

Page 1432. Reference 18 is incorrect. The correct reference is: Yoneda, F.; Higuchi, M.; Mori, K.; Senga, K.; Kanamori, Y.; Shimizu, K.; Nishigaki, S. *Chem. Pharm. Bull.* 1978, *26*, 2905–2910.