

Alkaloids from frog skin: the discovery of epibatidine and the potential for developing novel non-opioid analgesics

John W. Daly,^a H. Martin Garraffo,^a Thomas F. Spande,^a Michael W. Decker,^b James P. Sullivan^b and Michael Williams^b

^a Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892

^b Neurological and Urological Diseases Research, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064-6125

Received (in Cambridge, UK) 12th October 1999

Published on the Web 22nd February 2000

1 Introduction

Research on the nature, structure and biological activity of the toxins present in the skin of poison-dart frogs of South America began in the Laboratory of Chemistry at the National Institutes of Health in the mid-1960s. The presence of toxins in the skin of such frogs had been discovered long ago by Indians of Western Colombia, who to this day use skin secretions from three Colombian species of dendrobatid frogs (genus *Phyllobates*) to poison the grooved tips of blow darts used in hunting small game and birds. Initial field work on a poison-dart frog of the Río San Juan drainage, and preparation of extracts was first conducted by F. Märki in 1962 and then by Daly in 1964 and 1966. The toxic principles were isolated and proved on structural analysis to be steroidal alkaloids, which were named batrachotoxins.¹ These were then shown to be specific and potent activators of sodium channels.² Both the natural alkaloids and a radioactive analog have proven to be invaluable research tools for the study of sodium channels and their interaction with local anesthetics, anticonvulsants, antiarrhythmics and other drugs.³ The structure of batrachotoxin and other alkaloids, subsequently isolated from frog skin, are shown in Fig. 1.

These initial studies on the batrachotoxin alkaloids from the poison-dart frogs of Western Colombia might never have been extended to some sixty species of poison-frogs of the neotropical family Dendrobatidae, had not Charles W. Myers, a herpetologist working on the reptiles and amphibians of Panama, contacted Daly and proposed a collaboration on the toxicity of an extremely variable dendrobatid frog (genus *Dendrobates*) of the Bocas Archipelago of Panama. The initial hypothesis, namely that the more brightly colored populations would contain higher levels of toxic alkaloids, proved incorrect. However, the analyses revealed not the steroidal batrachotoxins, but instead a variety of simpler bicyclic alkaloids, including the relatively toxic pumiliotoxins and relatively nontoxic decahydroquinolines.⁴ The pumiliotoxins and related alkaloids later were shown to be potent myotonic/cardiotoxic agents⁵ with modulatory effects on sodium channels.⁶ The initial field work by Myers and Daly led to a thirty year friendship and collaboration with the aim of analyzing the distribution, nature, structure and biological activity of alkaloids in frog skin.

A major field trip by Myers and Daly in the early 1970s led to the isolation and structural determination of relatively nontoxic bicyclic histrionicotoxins,⁷ later established as high-affinity noncompetitive blockers of nicotinic acetylcholine receptor-channels (nAChRs).³ Over the next three decades more than 500 alkaloids of at least two dozen structural classes were discovered, most of which have, as yet, not been found elsewhere in nature.^{8,9} This is remarkable, since the dendrobatid

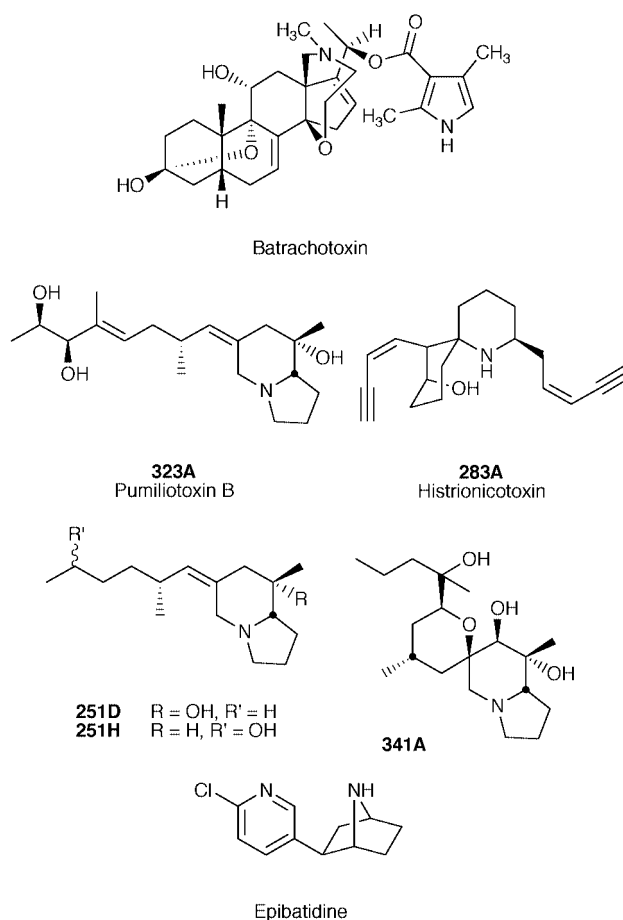


Fig. 1 Structures of epibatidine and other alkaloids discovered in skin extracts from poison frogs (family Dendrobatidae). Batrachotoxin from Colombian *Phyllobates aurotaenia*,¹ pumiliotoxin B from Panamanian *Dendrobates pumilio*,⁴ histrionicotoxin from Colombian *Dendrobates histrionicus*,⁷ and epibatidine and alkaloids **251D**, **251H** and **341A** from Ecuadorian *Epipedobates tricolor*.^{12–14,16}

frogs apparently do not synthesize any of their skin alkaloids, but instead sequester them unchanged into skin glands from dietary sources¹⁰ to be used as secreted chemical deterrents to predators. The search over the past five years for the dietary sources of the batrachotoxins, pumiliotoxins and histrionicotoxins has been frustrating, but some six classes of relatively simple decahydroquinolines, piperidines, pyrrolidines and “izi-dines” of dendrobatid frog skin have been found in ants, while certain of the tricyclic and spiropyrrolizidine alkaloids occur in beetles and millipedes, respectively.^{10,11}

2 Epibatidine

To date, the most clinically relevant discovery among the frog skin alkaloids was made by Daly and Myers after an exploratory field trip to Western Ecuador in 1974. This led to the isolation of a trace frog skin alkaloid with an analgesic potency that was two hundred-fold greater than morphine. The initial trip by Myers and Daly in 1974 provided skin extracts for analysis of alkaloids from a few specimens of a dendrobatid frog, now known as *Epipedobates tricolor*, from two sites in the Río Jubones drainage of Southwestern Ecuador near the Peruvian border. Fortunately, the *in vivo* effects of the alkaloid fraction from these small samples were assessed by subcutaneous injection in mice, whereupon a marked Straub-tail reaction was noted, which is typical for opioid-class alkaloids. It appeared that a trace, previously undetected alkaloid was responsible and that it might prove to be a very potent opioid of novel structure. Clearly, further extracts would be required for isolation and structure elucidation. A subsequent collecting trip by Myers and Daly in 1976 was both disappointing and successful. The frogs from one site, a lowland forested cacao plantation, had inexplicably disappeared. And skin extracts of the same frog collected in large numbers from nearby banana plantations, proved on analysis to contain no alkaloids. Some twenty years later, it is obvious that the dietary sources of the Straub-tail alkaloid and other alkaloids were present in the forested cacao plantation, but not in the banana plantation. Fortunately, the frogs (Fig. 2) from a second collection site, a highland roadside



Fig. 2 Ecuadorian poison frog (*Epipedobates tricolor*). Specimen from near Santa Isabel, Azuay, Ecuador (Photo courtesy of C. W. Myers).

seepage area, were very abundant and skin extracts were obtained from 750 frogs. A total of 60 mg of a complex mixture of alkaloids were isolated. Chromatography yielded 21 mg of the major alkaloid pumiliotoxin **251D**, the structure of which, after crystallization and X-ray analysis,¹² provided the necessary insights to the structures of the myotonic/cardiotoxic pumiliotoxins discovered in the mid-1960s in a Panamanian dendrobatid frog.⁴ Other alkaloids isolated were a deoxy-pumiliotoxin **251H**,¹³ a unique cyclic ether-containing pumiliotoxin **341A**¹⁴ and, of course, the Straub-tail alkaloid. The latter was characterized as a novel chlorine-containing alkaloid with an empirical formula of $C_{11}H_{13}N_2Cl$. On the basis of apparent molecular ions at 208 and 210, it was initially referred to as alkaloid **208/210**. Mass spectral analysis indicated the presence of an aliphatic moiety containing one nitrogen ($C_4H_7N^+$) and a highly unsaturated moiety containing the other nitrogen and the chlorine ($C_7H_7NCl^+$). Less than 500 μ g of relatively pure material were obtained and the compound was shown to be 200-fold more potent than morphine as an analgesic in mice.

Even more exciting was the fact that the opioid receptor antagonist naloxone did not block either the Straub-tail response or the hot plate analgesia.

In the late 1970s, the sensitivity and power of NMR spectrometers was not sufficient to provide definitive structural data. Further amounts of the alkaloid, later to be named epibatidine, were needed. However, on subsequent field trips in 1979 and 1982, skin extracts from frogs at and near the original highland site proved to have only trace amounts of the Straub-tail alkaloid, while frogs raised in captivity were alkaloid-free reinforcing a developing hypothesis that a dietary source was the origin of the alkaloid. It appeared that whatever the dietary source of epibatidine, it was neither abundant nor widely distributed.

By 1990, the sensitivity and power of NMR spectroscopy had advanced remarkably and it was decided that the structure of epibatidine, now contained in a small, irreplaceable sample, was potentially solvable. Vapor-phase FTIR spectral analysis indicated the presence of a chloropyridine moiety. Rather than attempt a further HPLC purification of pooled fractions containing epibatidine (a secondary amine) and some pumiliotoxins (tertiary amines), it was decided by Garraffo, Spande and Daly to convert epibatidine to a weakly basic *N*-acetyl derivative followed by acid extraction of the highly basic pumiliotoxins from *N*-acetylepibatidine. Garraffo demonstrated a quantitative conversion and complete purification of *N*-acetylepibatidine on a few micrograms. Nearly all the pooled sample of epibatidine was then acetylated and partitioned and NMR analysis provided the structure of epibatidine, which was reported in 1992.¹⁵

In addition to Daly's laboratory, several others embarked at this point on the synthesis of this remarkably potent analgesic. In May of 1993, E. J. Corey of Harvard University informed Daly that his group had synthesized both enantiomers of epibatidine and three analogs in which the chloro substituent on the pyridyl ring was replaced by hydrogen, methyl or iodo substituents. These compounds were offered for biological evaluation and Barbara Badio in Daly's lab confirmed the suspicion that the target of activity was the nicotinic acetylcholine receptor (nAChR), based both on affinity in binding assays and functional agonist assays.¹⁶ Ray Baker of Merck also contacted Daly in May of 1993 and provided additional (+)- and (–)-epibatidine. In return, Daly supplied Baker's group with natural *N*-acetylepibatidine, so that they could determine the absolute configuration of the natural compound. The previously reported potent analgesic activity of natural epibatidine¹⁵ was confirmed for both synthetic enantiomers and shown to be blocked by mecamylamine, an nAChR antagonist.¹⁶ Epibatidine had marked analgesic activity at a dose of $0.01 \mu\text{mol kg}^{-1}$, but at only slightly higher doses it was quite toxic.

Despite recent statements in the media, there was no tradition in Ecuadorian folklore that the skin of *Epipedobates tricolor* had analgesic or other medicinal properties and, in fact, the frog was considered locally as an unimportant part of the fauna. At the time of collection (1975–1976), the scientific collection of amphibians was not regulated by any Ecuadorian agency; the samples collected by Daly and Myers were legally exported from Ecuador with appropriate US Fish and Wildlife import documentation. In the 1980s, such permit-issuing agencies were established in Ecuador and, in 1984, the Convention on International Trade in Endangered Species (CITES) placed restrictions on the trade in brightly colored frogs of the dendrobatid family including *Epipedobates tricolor*. These restrictions have greatly hindered further research on alkaloids from such brightly colored frogs.¹⁷

Since the report of the structure of epibatidine in 1992¹⁵ many syntheses in addition to those of Corey¹⁸ and Baker¹⁹ have been forthcoming, the majority involving either an intramolecular nucleophilic cyclization or a Diels–Alder condensation to form the 7-azabicyclo[2.2.1]heptane ring system.^{20–23} An extensive

set of citations to syntheses is also available in a general review on alkaloids from amphibian skin.⁹

In vitro and *in vivo* studies on the activity of epibatidine and its analogs at various nAChR subtypes were facilitated by the early commercial availability of racemic epibatidine and both enantiomers. This compound has now become a ligand of choice for any investigation of nAChRs. [³H]Epibatidine was developed as a high-affinity radioligand for the study of nAChRs by Kenneth Kellar at Georgetown University.^{24,25} An [¹²⁵I] analog of epibatidine has been used for autoradiography,²⁶ while ¹⁸F and ¹³C analogs have proven useful for *in vivo* positron emission tomography (PET).^{27–29}

In animals, tolerance to the analgesic effects of epibatidine was minimal for the unnatural (+)-enantiomer and modest for the natural (–)-enantiomer,³⁰ and hence epibatidine had the potential for long-term treatment of chronic neuropathic, arthritic or cancer-elicited pain. However, a major obstacle to the clinical utility of epibatidine as an analgesic was its very limited therapeutic index, the result of its lack of marked selectivity towards the multiple nAChR subtypes.^{31,32} Indeed the separation between efficacy and seizures in mice is less than five. Thus, while the proof of principle had been established for nAChR ligands being potent analgesics, further research efforts were focused on developing safer synthetic compounds that retained the analgesic potency and lack of tolerance development observed with epibatidine, while minimizing the toxicity.^{33,34}

3 Nicotinic analgesics

An ongoing research program, initiated at Abbott Laboratories in 1990, was targeted towards developing selective $\alpha 4 \beta 2$ neuronal nAChR agonists for the treatment of Alzheimer's disease and had identified ABT-418,^{35,36} which was in Phase II clinical trials for Alzheimer's disease in the 1994 time frame. This compound was subsequently discontinued due to a failure of the compound to differentiate from placebo. ABT-418 was however, shown to have cognition-enhancing activity in an acute trial in Alzheimer's disease patients³⁷ and in a pilot trial for attention-deficit hyperactivity disorder.³⁸ A natural extension of the activities of the Abbott group, led by Stephen Arneric, was to examine the spectrum of other beneficial activities known to be associated with (–)-nicotine including the weak analgesic effects of the alkaloid first reported in 1932.³⁹ In 1993, evaluation of a number of compounds from the Abbott chemical library in acute analgesia models led to the identification of the pyridyl ether A-87048, which was found to have analgesic activity at a dose of 6.2 $\mu\text{mol kg}^{-1}$, providing an impetus for the group at Abbott to pursue the analgesic potential for novel nAChR ligands,⁴⁰ an initiative subsequently reinforced by the report on the discovery of the nicotinic mechanism of action of the potent analgesic epibatidine.¹⁶ Structures of nicotine and several synthetic nicotinic agonists are shown in Fig. 3.

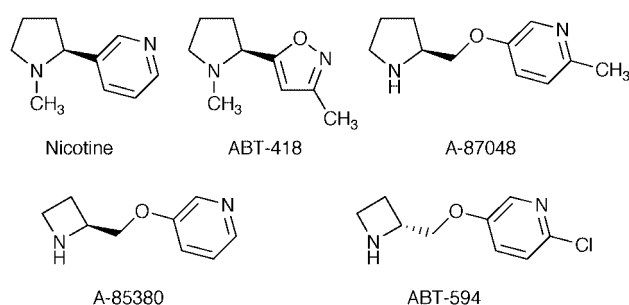


Fig. 3 Nicotine and synthetic agonists for nicotinic acetylcholine receptor-gated channels.

In addition to commercially available racemic epibatidine and its enantiomers, 500 other compounds resulting from

synthetic chemistry efforts at Abbott led by Mark Holladay were evaluated for analgesic activity⁴⁰ with the goal of identifying compounds with analgesic efficacy and reduced activity at autonomic and neuromuscular nAChRs. For example, the azetidine-2-pyridyl ether analog A-85380, a very potent and selective agonist for $\alpha 4 \beta 2$ nAChR binding sites in brain, was a potent analgesic but retained some of the same toxicities as the frog alkaloid.^{41,42} Subsequent medicinal chemistry around the azetidine pharmacophore led to ABT-594^{43–45} a compound that had reduced interactions with autonomic and neuromuscular nAChRs (Table 1) and was eventually selected

Table 1 Pharmacological comparison of (±)-epibatidine and ABT-594. A potency ratio of 1 indicates equal potency, while higher numbers indicate a 6–3700-fold greater potency for epibatidine. Data from references 32, 45 and 49

Measure	Potency Ratio (ABT-594 versus epibatidine)
Binding^a	
$\alpha 4 \beta 2$ (³ H-Cytisine)	
Rat (whole brain)	1
Human (K177 cells)	1
$\alpha 7$ (¹²⁵ I- α -Bungarotoxin)	
Rat (whole brain)	60
Human (K28 cells)	60
$\alpha 1 \beta 1 \delta \gamma$ (¹²⁵ I- α -Bungarotoxin)	
Torpedo	3700
In Vitro Function	
Human $\alpha 4 \beta 2$ (K177) ^b	8
Human $\alpha 3 \beta x$ ganglionic-like (IMR32 cells) ^b	49
Human $\alpha 7$ (<i>Xenopus</i> oocytes) ^c	43
Effects in Mice (i.p. dosing)	
Antinociception (hot-plate) ^d	6
Acute lethality ^e	30

^a ABT-594 K_i /Epibatidine K_i . ^b ABT-594 EC_{50} /Epibatidine EC_{50} ; determined by ⁸⁶Rb⁺ efflux assay. ^c ABT-594 EC_{50} /Epibatidine EC_{50} ; determined electrophysiologically. ^d ABT-594 Maximal Effective Dose/Epibatidine Maximal Effective Dose. ^e ABT-594 Approximate Lethal Dose/Epibatidine Approximate Lethal Dose.

as a potential clinical lead. *In vitro* pharmacological profiling subsequent to the identification of its favorable analgesic profile *in vivo*, showed it to be selective for the cloned human $\alpha 4 \beta 2$ nAChR with reduced affinities for $\alpha 3 \beta 4$ and neuromuscular nAChRs (Table 1). The compound was also without significant activity at 73 other neurotransmitter receptor and uptake sites. The analgesic activity of ABT-594 in acute (Hargreaves hot box), chemical (formalin test) and neuropathic (Chung) pain models was equivalent in efficacy to morphine but the compound was 30–100-fold more potent than morphine.^{43–45} The effects of ABT-594, like those of epibatidine, were antagonized by nAChR antagonists (e.g. mecamylamine, chlorisondamine), but not by an opioid antagonist, naltrexone. Unlike nicotine and epibatidine, ABT-594 had an improved therapeutic index in regard to its potential cardiovascular liabilities. Unlike morphine, ABT-594 had no effect on respiration or gastrointestinal motility and, in a 5-day twice a day dosing regimen in the Chung neuropathic pain model, showed no evidence of tolerance.⁴⁴ In the same model, the analgesic effects of morphine decreased with time.

While key clinical data are not yet available on the efficacy of ABT-594, this compound and epibatidine have renewed interest in the study of the role of nAChRs in pain perception. This led to the examination of antisense oligonucleotides to the $\alpha 4$ subunit demonstrating that the absence of this subunit attenuated nicotinic-induced analgesia.⁴⁶ Other studies by Jean-Pierre Changeux and Imad Damaj,⁴⁷ using knockout mice lacking both the $\alpha 4$ subunit and $\beta 2$ subunit of the nAChR, showed that both subunits were critical to the modulation of pain perception. However, these findings do not conclusively point to the $\alpha 4 \beta 2$ subunit combination as being the only nAChR involved in

analgesia, since other nAChR agonists with potent and selective activity at $\alpha 4\beta 2$ receptors (e.g. ABT-418) lack robust analgesic effects.

Despite the excitement generated by the discovery of epibatidine and its role in rekindling active interest in pain mechanisms involving nAChRs and a host of publications, there is still much to be learnt especially in regard to the anatomical site(s) of action of both epibatidine and ABT-594. An ^{18}F analog of A-85380 has been recently described as a new tool for PET scanning of nAChRs⁴⁸ which, together with ^{18}F and ^{13}C analogs of epibatidine,^{27–29} is sure to aid in these efforts.

4 Current status

The first report on ABT-594,⁴³ published in *Science* in January of 1998, had wide media coverage. Daly and Williams were contacted by US TV networks and Garraffo appeared on CNN. The discovery of ABT-594 was also covered in the *Financial Times* and the *New York Times*. During these heady times, at least two US network TV crews requested permission from Abbott to shoot video footage of the frogs and were disappointed to find that not only did Abbott not have a photogenic colony of *Epipedobates tricolor*, but that no one at Abbott had actually seen the frog beyond photographs in various publications. Furthermore, Daly's group at the NIH had no frogs and had not actually worked with the frogs since early breeding efforts in 1980.

A challenge for both Daly and the Abbott group was to ensure that the chronology of the finding of epibatidine and the development of ABT-594 were correctly attributed. Numerous reports in both the US and international press mistakenly indicated that Abbott was developing epibatidine rather than ABT-594 as an analgesic. In fact, there have been no efforts to develop epibatidine for clinical use. ABT-594 was conceived at Abbott Laboratories by Holladay's group and is the subject of international patents on composition of matter for this novel nAChR agonist.

The discovery of epibatidine is noteworthy from the perspective of the continued importance of natural product research and its transition to applied biomedical research. Without the long-standing interest of Daly and Myers in frog alkaloids and their potential pharmacological activities, it is highly unlikely that epibatidine and its potent analgesic properties and the subsequent determination of its mechanism of action would have ever occurred. While there was considerable interest in both the academic and industrial communities in efforts to synthesize epibatidine as documented above, it was not until the mechanism of action of epibatidine was determined by Badio and Daly¹⁶ that the value of this novel frog alkaloid was fully appreciated. Questions remain to be answered as to the specific nAChR constructs involved in mediating the analgesic actions of epibatidine as well as nicotine and ABT-594. The dietary source of epibatidine remains to be discovered. In addition, over 100 trace alkaloids whose structures remain undefined, have been detected in frog skin extracts,⁹ presenting yet more challenges for the future.

References

- 1 T. Tokuyama, J. Daly and B. Witkop, *J. Am. Chem. Soc.*, 1969, **91**, 3931.
- 2 E. X. Albuquerque, J. W. Daly and B. Witkop, *Science*, 1971, **172**, 995.
- 3 J. W. Daly and T. F. Spande, in *Alkaloids: Chemical and Biological Perspectives*, ed. S. W. Pelletier, John Wiley and Sons, New York, 1986, **4**, pp. 1–274.
- 4 J. W. Daly and C. W. Myers, *Science*, 1967, **196**, 970.

- 5 J. W. Daly, E. McNeal, F. Gusovsky, F. Ito and L. E. Overman, *J. Med. Chem.*, 1988, **31**, 477.
- 6 F. Gusovsky, W. L. Padgett, C. R. Creveling and J. W. Daly, *Mol. Pharmacol.*, 1992, **42**, 1104.
- 7 J. W. Daly, I. Karle, C. W. Myers, T. Tokuyama, J. A. Waters and B. Witkop, *Proc. Natl. Acad. Sci. U.S.A.*, 1971, **68**, 1870.
- 8 J. W. Daly, *J. Nat. Prod.*, 1998, **61**, 162.
- 9 J. W. Daly, H. M. Garraffo and T. F. Spande, in *Alkaloids: Chemical and Biological Perspectives*, ed. S. W. Pelletier, Elsevier Science Ltd., Oxford, 1999, **13**, pp. 1–161.
- 10 J. W. Daly, H. M. Garraffo and C. W. Myers, *Pharmaceut. News*, 1997, **4**, 9.
- 11 T. H. Jones, J. S. T. Gorman, R. R. Snelling, J. H. C. Delabie, M. S. Blum, H. M. Garraffo, P. Jain, J. W. Daly and T. F. Spande, *J. Chem. Ecol.*, 1999, **25**, 1179.
- 12 J. W. Daly, T. Tokuyama, T. Fujiwara, R. J. Highet and I. Karle, *J. Am. Chem. Soc.*, 1980, **102**, 830.
- 13 P. Jain, H. M. Garraffo, T. F. Spande, H. J. C. Yeh and J. W. Daly, *J. Nat. Prod.*, 1995, **58**, 100.
- 14 P. Jain, T. F. Spande, H. M. Garraffo and J. W. Daly, *Heterocycles*, 1999, **50**, 903.
- 15 T. F. Spande, H. M. Garraffo, M. W. Edwards, H. J. C. Yeh, L. Pannell and J. W. Daly, *J. Am. Chem. Soc.*, 1992, **114**, 3475.
- 16 B. Badio and J. W. Daly, *Mol. Pharmacol.*, 1994, **45**, 563.
- 17 C. W. Myers and J. W. Daly, *Science*, 1993, **262**, 1193.
- 18 E. J. Corey, T. P. Loh, S. AchyuthaRao, D. C. Daley and S. Sarshar, *J. Org. Chem.*, 1993, **58**, 5600.
- 19 S. R. Fletcher, R. Baker, M. S. Chambers, S. C. Hobbs and P. J. Mitchell, *J. Chem. Soc., Chem. Commun.*, 1993, **15**, 1216.
- 20 C. A. Broka, *Med. Chem. Res.*, 1994, **4**, 449.
- 21 C. Szantay, Z. Kardos-Balogh and C. Szantay Jr., in *The Alkaloids*, ed. G. A. Cordell, Academic Press, San Diego, 1995, **46**, pp. 95–125.
- 22 Z. Chen and M. L. Trudell, *Chem. Rev.*, 1996, **96**, 1179.
- 23 C. Kibayashi and S. Aoyagi, in *Studies in Natural Product Chemistry*, ed. Atta-ur-Rahman, Elsevier, Amsterdam, 1997, **19**, 66.
- 24 R. A. Houghtling, M. I. Davila-Garcia and K. J. Kellar, *Mol. Pharmacol.*, 1995, **48**, 280.
- 25 D. C. Perry and K. J. Kellar, *J. Pharmacol. Exp. Therap.*, 1995, **275**, 1030.
- 26 M. I. Davila-Garcia, J. L. Musachio, D. C. Perry, Y. Xiao, A. Horti, E. D. London, R. F. Dannals and K. J. Kellar, *J. Pharmacol. Exp. Therap.*, 1997, **282**, 445.
- 27 F. Liang, H. A. Navarro, P. Abraham, P. Kotian, Y.-S. Ding, J. Fowler, N. Volkow, M. J. Kuhar and F. I. Carroll, *J. Med. Chem.*, 1997, **40**, 2293.
- 28 L. Dolci, F. Dolle, H. Valette, F. Vaufrey, C. Fuseau, M. Bottlaender and C. Crouzel, *Bioorg. Med. Chem.*, 1999, **7**, 467.
- 29 J. T. Pratt, J. E. Spang, G. Westera, A. Buck and P. A. Schubiger, *Nucl. Med. Biol.*, 1999, **26**, 165.
- 30 M. I. Damaj and B. R. Martin, *Eur. J. Pharmacol.*, 1996, **300**, 51.
- 31 J. P. Sullivan and A. W. Bannon, *CNS Drug Rev.*, 1996, **2**, 21.
- 32 J. P. Sullivan, M. W. Decker, J. D. Brioni, D. L. Donnelly-Roberts, D. A. Anderson, A. W. Bannon, C.-H. Kang, P. Adams, M. Piattoni-Kaplan, M. J. Buckley, M. Gopalakrishnan, M. Williams and S. P. Arneric, *J. Pharmacol. Exp. Ther.*, 1994, **271**, 624.
- 33 M. W. Holladay, M. J. Dart and J. K. Lynch, *J. Med. Chem.*, 1997, **40**, 4169.
- 34 J. R. Trayner, *Brit. J. Anaesth.*, 1998, **81**, 69.
- 35 S. P. Arneric, J. P. Sullivan, C. Briggs, D. A. Anderson, E. D. Cadman, P. Adams, D. L. Donnelly-Roberts, D. M. Hughes, D. S. Garvey, J. Wasicak and M. Williams, *J. Pharmacol. Exp. Ther.*, 1994, **270**, 310.
- 36 M. W. Decker, J. D. Brioni, J. P. Sullivan, M. J. Buckley, R. J. Radek, J. L. Raskiewicz, C.-H. Kang, D. J. B. Kim, W. J. Giardina, M. Williams, and S. P. Arneric, *J. Pharmacol. Exp. Ther.*, 1994, **270**, 319.
- 37 A. Potter, J. Corwin, J. Lang, M. Piasecki, R. Lenox and P. A. Newhouse, *Psychopharmacology*, 1999, **142**, 334.
- 38 T. E. Wilens, J. Biederman, T. J. Spencer, J. Bostic, J. Prince, M. C. Monuteaux, J. Soriano, C. Fine, A. Abrams, M. Rater and D. Polissner, *Am. J. Psychiatry*, 1999, in press.
- 39 L. Davis, L. J. Pollock and T. T. Stone, *Surg. Gynecol. Obstet.*, 1932, **55**, 418.
- 40 M. W. Holladay and M. W. Decker, *Adv. Med. Chem.*, 1999, in press.
- 41 J. P. Sullivan, D. Donnelly-Roberts, C. A. Briggs, D. J. Anderson, M. Gopalakrishnan, M. Piattoni-Kaplan, J. E. Campbell, D. G. McKenna, E. Molinari, A. M. Hettinger, D. S. Garvey, J. T. Wasicak, M. W. Holladay, M. Williams, and S. P. Arneric, *Neuropharmacology*, 1996, **35**, 725.
- 42 P. Curzon, A. L. Nikkel, A. W. Bannon, S. P. Arneric and M. W. Decker, *J. Pharmacol. Exp. Ther.*, 1998, **287**, 847.

- 43 A. W. Bannon, M. W. Decker, M. W. Holladay, P. Curzon, D. L. Donnelly-Roberts, P. Puttfarcken, R. S. Bitner, A. Diaz, A. H. Dickenson, R. D. Posolt, M. Williams and S. P. Arneric, *Science*, 1998, **289**, 77.
- 44 A. W. Bannon, M. W. Decker, D. J. B. Kim, J. E. Campbell and S. P. Arneric, *Brain Res.*, 1998, **801**, 158.
- 45 D. L. Donnelly-Roberts, P. S. Puttfarcken, T. A. Kuntzweiler, C. A. Briggs, D. J. Anderson, J. E. Campbell, M. Piattoni-Kaplan, D. G. McKenna, J. T. Wasicak, M. W. Holladay, M. Williams and S. P. Arneric, *J. Pharmacol. Exp. Ther.*, 1998, **285**, 777.
- 46 R. S. Bitner, D. L. Donnelly-Roberts, A. L. Nikkel, P. Curzon and M. W. Decker, *Soc. Neurosci. Abstr.*, 1998, **24**, 336.
- 47 L. M. Marubio, M. D. M. Arroyo-Jimenez, M. Cordero-Erausquin, C. Lena, N. Le Novère, A. D. K. D'Exaerde, M. Huchet, M. I. Damaj and J. P. Changeux, *Nature*, 1999, **398**, 805.
- 48 F. Dolle, L. Dolci, H. Valette, F. Hinnen, F. Vaufrey, I. Guenther, C. Fuseau, C. Coulon, M. Bottlaender and C. Crouzel, *J. Med. Chem.*, 1999, **42**, 2251.
- 49 M. W. Decker, A. W. Bannon, M. J. Buckley, D. J. B. Kim, M. W. Holladay, K. B. Ryther, N.-H. Lin, J. T. Wasicak, M. Williams and S. P. Arneric, *Eur. J. Pharmacol.*, 1998, **346**, 23.

Review 9/00728H