nature chemistry

ARTICLES

PUBLISHED ONLINE: 23 MAY 2011 | DOI: 10.1038/NCHEM.1050

Synthesis of conolidine, a potent non-opioid analgesic for tonic and persistent pain

Michael A. Tarselli[1], Kirsten M. Raehal[2,3], Alex K. Brasher[1], John M. Streicher[2,3], Chad E. Groer[2,3], Michael D. Cameron[2], Laura M. Bohn[2,3]* and Glenn C. Micalizio[1]*

Management of chronic pain continues to represent an area of great unmet biomedical need. Although opioid analgesics are typically embraced as the mainstay of pharmaceutical interventions in this area, they suffer from substantial liabilities that include addiction and tolerance, as well as depression of breathing, nausea and chronic constipation. Because of their suboptimal therapeutic profile, the search for non-opioid analgesics to replace these well-established therapeutics is an important pursuit. Conolidine is a rare C5-nor stemmadenine natural product recently isolated from the stem bark of *Tabernaemontana divaricata* (a tropical flowering plant used in traditional Chinese, Ayurvedic and Thai medicine). Although structurally related alkaloids have been described as opioid analgesics, no therapeutically relevant properties of conolidine have previously been reported. Here, we describe the first *de novo* synthetic pathway to this exceptionally rare C5-nor stemmadenine natural product, the first asymmetric synthesis of any member of this natural product class, and the discovery that (±)-, (+)- and (−)-conolidine are potent and efficacious *non-opioid* analgesics in an *in vivo* model of tonic and persistent pain.

The search for the next generation of therapeutics for the treatment of pain continues to define an active area of scientific pursuit. Among current pharmaceutical interventions, opioid analgesics (i.e. Fig. 1a) represent the most widely embraced[1]. Unfortunately, these agents are clinically problematic as a result of their well-established adverse properties that include addiction, tolerance, depression of breathing, nausea and chronic constipation[2]. Hence the identification of effective non-opioid analgesics to replace these well-established therapeutics is widely held as an important area of investigation. Here, we describe an interdisciplinary study that has led to the discovery that a rare plant-derived natural product, first isolated in 2004[3], possesses potent non-opioid analgesic properties and is effective in alleviating chemically induced, inflammatory and acute tonic pain. Overall, efforts have defined the first *de novo* synthetic pathway to an exceptionally rare C5-nor stemmadenine natural product, and the first asymmetric synthesis of any natural product in this class. These advances have fuelled the discovery that (±)-, (+)- and (−)-conolidine are potent non-opioid analgesics *in vivo*.

Tabernaemonta divaricata is a flowering tropical plant that has been used historically in traditional Chinese, Ayurvedic and Thai medicines, with applications spanning treatment of fever, pain, scabies and dysentery[4]. The search for the medicinally relevant components of this plant has resulted in the isolation of a vast array of indole alkaloids that possess diverse biological profiles[4]. Conolidine (**1**) is an exceedingly rare component of a Malayan *T. divaricata*, isolated in only 0.00014% yield from the stem bark of this small flowering plant (Fig. 1b)[3]. Although no biological or medicinal properties of conolidine have been described, other more abundant alkaloids common to this species have been implicated as opioid analgesics[4,5].

The molecular structure of **1** defines it as a member of the C5-nor stemmadenine family of natural products, other members of which have represented significant challenges to modern asymmetric synthesis. In fact, no asymmetric synthesis of any member of this natural product class has been described, and no robust source of conolidine has been identified to fuel medicinal evaluation. The lack of a source of **1**, the scarcity of chemical approaches suitable for the synthesis of the C5-nor stemmadenine skeleton and the compelling drug-like molecular structure of conolidine motivated our initial chemical investigations aimed at securing ample quantities of this rare alkaloid by synthetic means.

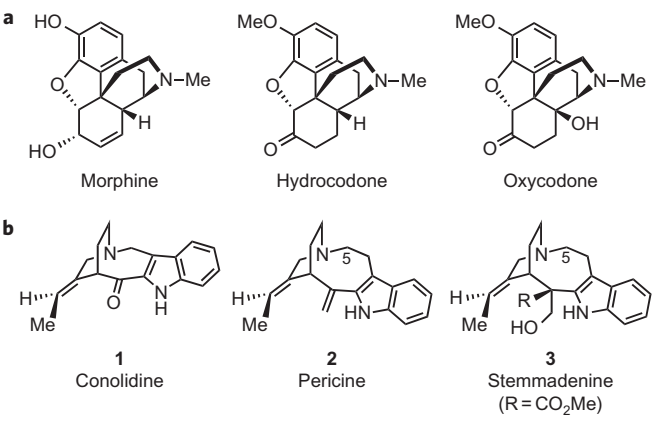

Figure 1 | Opioid analgesics and stemmadenine-based alkaloids. **a**, Common opioid analgesics morphine, hydrocodone and oxycodone. Although extremely effective, well-known side effects compromise the therapeutic profile of these agents. **b**, Conolidine, pericine and stemmadenine are rare alkaloids derived from plants used in traditional Chinese, Ayurvedic and Thai medicine. Conolidine itself can be isolated in just 0.00014% yield from the stem bark of *T. divaricata*. The low natural abundance, established utility of *T. divaricata* in traditional medicine and synthetic challenge posed by the structure of conolidine served to motivate our pursuit of a programme to accomplish the first synthetic pathway to conolidine and evaluate its biological properties.

[1]Department of Chemistry, The Scripps Research Institute, Jupiter, Florida, USA, [2]Department of Molecular Therapeutics, The Scripps Research Institute, Jupiter, Florida, USA, [3]Department of Neuroscience, The Scripps Research Institute, Jupiter, Florida, USA. *e-mail: lbohn@scripps.edu; micalizio@scripps.edu

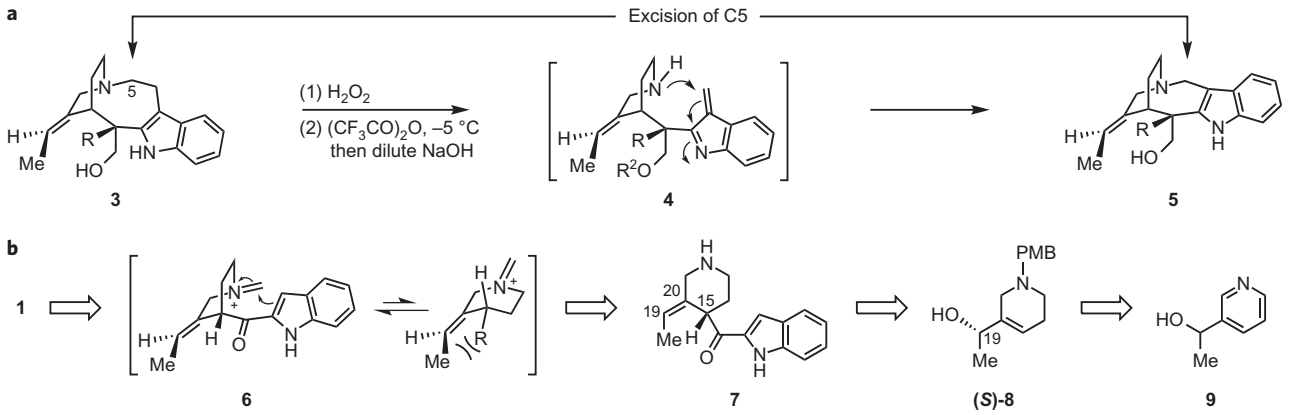

Figure 2 | Development of a synthesis strategy for conolidine inspired by the biosynthetic proposal for the conversion of stemmadenine to vallesamine. **a**, Biomimetic semisynthesis of vallesamine (**5**) from stemmadenine (**3**). **b**, Retrosynthesis of conolidine (**1**) that features a cyclization process supported by the minimization of A-1,3 strain (**7** → **1**) — a strategy inspired by the conformational constraints likely operative in the cyclization of **4** (**a**).

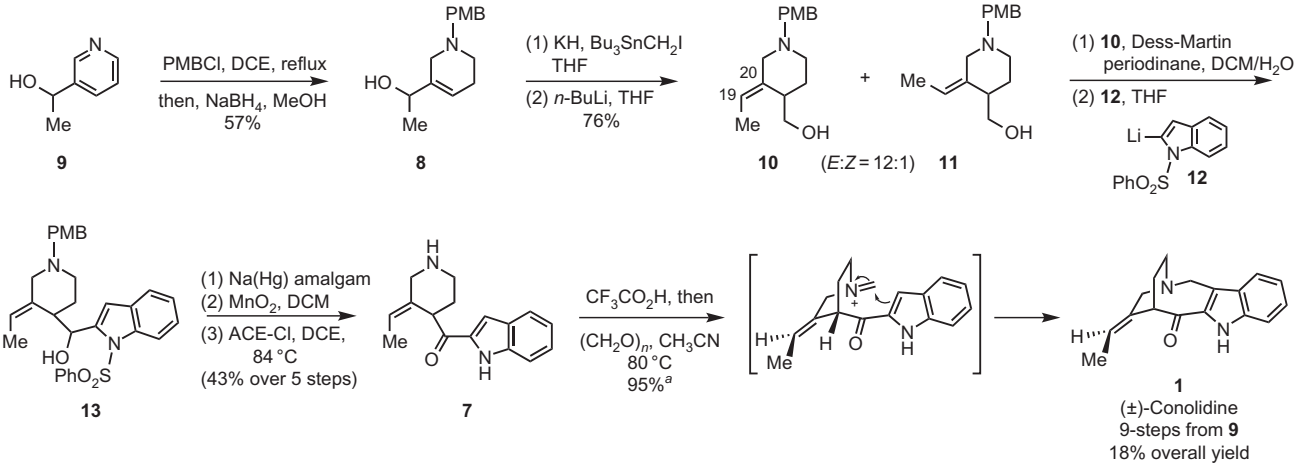

Figure 3 | Execution of a synthesis pathway to conolidine (1). Synthesis of (±)-conolidine (**1**) is accomplished in just nine steps and 18% overall yield from the commercially available pyridine **9**. A Still's allyl stannylmethyl ether-based [2,3]-Wittig rearrangement is used as a key stereoselective transformation in this pathway that culminates in the acid-mediated conversion of **7** to conolidine **1**. [a]Yield reported is for the preparation of the $\frac{1}{2}$ H_2SO_4 salt (the compound used in subsequent *in vivo* experiments); spectral data reported in the Supplementary Information are of the free base of **1**. PMB, *p*-methoxybenzyl; DCE, 1,2-dichloroethane; DCM, dichloromethane; ACE-Cl, α-chloroethyl chloroformate.

Results and discussion

At the outset of our studies, two anticipated challenges guided the conception of a chemical pathway to this class of natural products: (1) formation of a strained 1-azabicyclo[4.2.2]-decane and (2) stereochemical control in the generation of the exocyclic trisubstituted alkene.

Our retrosynthetic planning began with an examination of the biosynthetic pathway for conversion of stemmadenine (**3**) to vallesamine (**5**), a proposal later realized *in vitro* by Scott and colleagues (Fig. 2a)[6,7]. Here, excision of the C5 carbon occurs by a sequence of (1) *N*-oxidation, (2) fragmentation, (3) hydrolysis and (4) cyclization (through **4**)[8].

The ease with which cyclization takes place in this system is probably influenced by a conformational preference imparted by the stereodefined C19–C20 (*E*)-ethylidene unit, thereby providing a bias to support cyclization through **4** (Fig. 2a). With this as a guiding principle, we speculated that conolidine (**1**) could derive from iminium ion **6** through a related ring closure by means of a conformation that would be similarly stabilized by minimization of allylic strain (Fig. 2b)[9]. While providing a conformational bias to facilitate the establishment of the 1-azabicyclo[4.2.2]-core, the stereochemistry of this alkene was expected to impart kinetic stability to the potentially unstable β,γ-unsaturated ketone **7** (ref. 9). The preparation of substrate **7** was then deemed possible from allylic alcohol **8**, and ultimately from the readily available pyridine **9**.

As depicted in Fig. 3a, efforts commenced with commercially available pyridine **9**. Initial *N*-alkylation, followed by hydride reduction, provided **8** in 57% yield. With precedent firmly established for stereoselective Claisen rearrangement of related substrates providing the undesired alkene isomer[11,12], an unusual stereoselective transformation of **8** was required to establish the desired alkene geometry at C19–C20. Although our initial experiments aimed at accomplishing such a stereoselective transformation focused on the application of reductive cross-coupling chemistry between **8** and a suitably functionalized alkyne[13], these studies encountered unanticipated failure as a result of low levels of reactivity associated with the allylic alcohol component in this class of metallacycle-mediated convergent coupling reaction. We sought to identify a stereoselective transformation suitable for the conversion of allylic alcohol **8** to a functionalized (*E*)-exo-ethylidine-containing product.

With this goal in mind, we turned our attention to Still's allyl stannylmethyl ether-based [2,3]-Wittig rearrangement[14]. Whereas few reports describe the application of this reaction to the establishment of stereodefined exo-alkylidene cycloalkanes[15–17], the unique

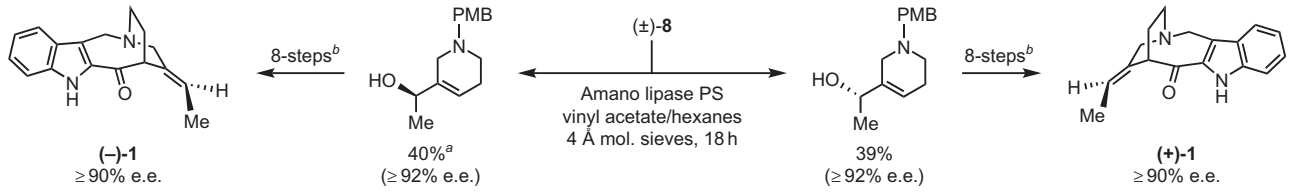

Figure 4 | Asymmetric synthesis of (+)- and (−)-conolidine. Resolution of racemic **8**, by lipase-catalysed acylation, defines a robust means to access optically active samples of either antipode of conolidine (**1**). [a]After hydrolysis. [b]Apart from the conditions for oxidation of an intermediate primary alcohol, this eight-step sequence is identical to that described in Fig. 3.

stereochemical course of this transformation in acyclic settings suggested its potential utility in solving the problem at hand.

As illustrated in Fig. 3, allylic alcohol **8** was converted to its corresponding tributylstannylmethyl ether, and subsequently exposed to *n*-BuLi. The products from the [2,3]-Wittig rearrangement were generated in 76% yield (over two steps), favouring the formation of the desired isomer **10** (**10**:**11** = 12:1). Oxidation to the corresponding β,γ-unsaturated aldehyde with Dess–Martin periodinane, and nucleophilic addition of the organolithium reagent **12**, then gave a mixture of stereoisomeric alcohols **13** that was subsequently advanced to the cyclization substrate **7** by a simple three-step sequence.

Conversion of amino ketone **7** to its corresponding trifluoroacetate salt, followed by treatment with paraformaldehyde in acetonitrile at 80 °C, delivered (±)-conolidine **1** in 95% yield. The success of this process validated our proposed strategy to access the strained azabicyclo[4.2.2] core of the C5-nor stemmadenine family of natural products, and delivered the first synthetic sample of conolidine by a synthesis pathway that proceeds in just nine steps and 18% overall yield from a readily available pyridine.

Building on the successful synthesis of racemic **1**, we explored the potential of this route to accomplish the first asymmetric synthesis of a C5-nor stemmadenine alkaloid. As illustrated in Fig. 4, resolution of (±)-**8** with a commercially available lipase provided optically enriched substrates (≥92% e.e.) that were advanced to (+)- or (−)-conolidine (≥90% e.e.) by a simple eight-step sequence. Here, the only preparative modification required was a substitute for the Dess–Martin oxidation used to generate the β,γ-unsaturated aldehyde en route to enantio-enriched carbinol **13**. Whereas Dess–Martin oxidation of optically active samples of **10** resulted in inconsistent partial racemization, a modified Parikh–Doering oxidation ensured a reproducible pathway to enantio-defined samples of either enantiomer of conolidine.

With a concise asymmetric chemical pathway established, and the first synthetic samples of (+)-, (−)- and (±)-conolidine in hand, hundreds of milligrams of synthetic material was prepared and our attention shifted to exploring the potential analgesic properties of these substances *in vivo*.

Because related members of this natural product class have been postulated to have opioid properties[4,5], we assessed the efficacy of conolidine in several nociceptive tests in C57BL/6J mice. Unlike opioids, (+)- and (−)-conolidine sulfate (**1**) do not produce antinociception in the hot plate (51 °C) or in the warm water tail immersion assay (49 °C) following systemic injection of 10 mg kg^{-1}, intraperitoneally (i.p.) (Supplementary Fig. S1). However, in a visceral pain model, the acetic acid abdominal constriction assay[18], conolidine (10, 20 mg kg^{-1}) proved to be efficacious in preventing the acetic acid-induced writhing response (Fig. 5a). Therefore, conolidine differs from morphine in that it does not promote antinociception to acute thermal stimulation; however, like morphine, and a host of known analgesics, it does suppress the acetic acid-induced writhing response[19,20].

A pain model designed to assess both acute tonic and persistent pain responses was also used to ascertain conolidine's analgesic efficacy. The injection of a dilute formalin solution into a rodent's paw pad produces a biphasic pain response that is demonstrated by the animals attending to and licking the injected paw (nocifensive behaviours)[21]. Responses to the initial phase (Phase 1) occur within the first 10 min of formalin injection and reflect the direct chemical action at nociceptive primary afferent nerve fibres[22]. Following a brief intermediary phase (10–20 min after formalin injection) in which no pain response occurs, a second pain response (Phase 2) becomes evident (20–40 min after formalin injection) during which the animal once again attends to the injected paw. This second phase models a persistent pain state that is believed to be due in part to injury-provoked sensitization of central nervous system neurons and to inflammatory factors[18,23,24]. The application of local anaesthetics, such as lidocaine, suppresses the first phase of the response, whereas non-steroidal anti-inflammatory drugs are effective in blocking the second phase. Few drugs, such as the opioid narcotics, are effective in suppressing both phases of the pain response[18].

Using a well-characterized mouse line in this particular assay, the sulfate salts of (±)-, (+)- and (−)-conolidine were administered (10 mg kg^{-1}, i.p.) 15 min before formalin (5%, 25 μl, intraplantar) challenge[20]. Compared with vehicle, all three forms of conolidine sulfate reduce the display of nocifensive behaviours in both Phase 1 ($P < 0.001$) and Phase 2 ($P < 0.001$, two-way analysis of variance (ANOVA)) (Fig. 5b). Further, the unnatural antipode (−)-conolidine is effective in a dose-dependent manner (Fig. 5c), displaying potency similar to that shown for morphine sulfate (see Fig. 5d and Table 1)[20].

To investigate the duration of drug action, (−)-conolidine sulfate was administered at increasing time intervals before formalin challenge. Whereas one-way ANOVA reveals no time effect for either phase ($P > 0.05$), subsequent Student's *t*-test analysis reveals that although conolidine significantly attenuates the pain response compared with vehicle at 15 and 30 min before injection in Phase 1, and at 15 min before that in Phase 2, it was less efficacious when administered 1 h before formalin challenge in Phase 1 or 30–60 min before that in Phase 2 (Fig. 5e).

Taken together, these data demonstrate that, like opioid analgesics, conolidine is effective in suppressing responses to both chemically induced and inflammation-derived pain[20]. Further, pharmacokinetic measures demonstrate that conolidine is present in the brain and plasma at relatively high concentrations during the period of its analgesic efficacy and remains present up to 4 h after injection (Fig. 5f)[25].

To further demonstrate that conolidine differs from opioid analgesics, pharmacological studies were performed. On the basis of β-arrestin translocation assays, radioligand binding displacement and G-protein coupling assays, conolidine has no affinity for or efficacy at the μ-opioid receptor, the primary target of morphine (Supplementary Fig. S2 and Table S1). Moreover, radioligand displacement and β-arrestin translocation studies also demonstrate a lack of affinity or efficacy for κ- and δ-opioid receptors (Supplementary Fig. S2a and Table S1).

In an attempt to determine the biological target of conolidine, we subjected (±)-conolidine sulfate to screening by the Psychoactive

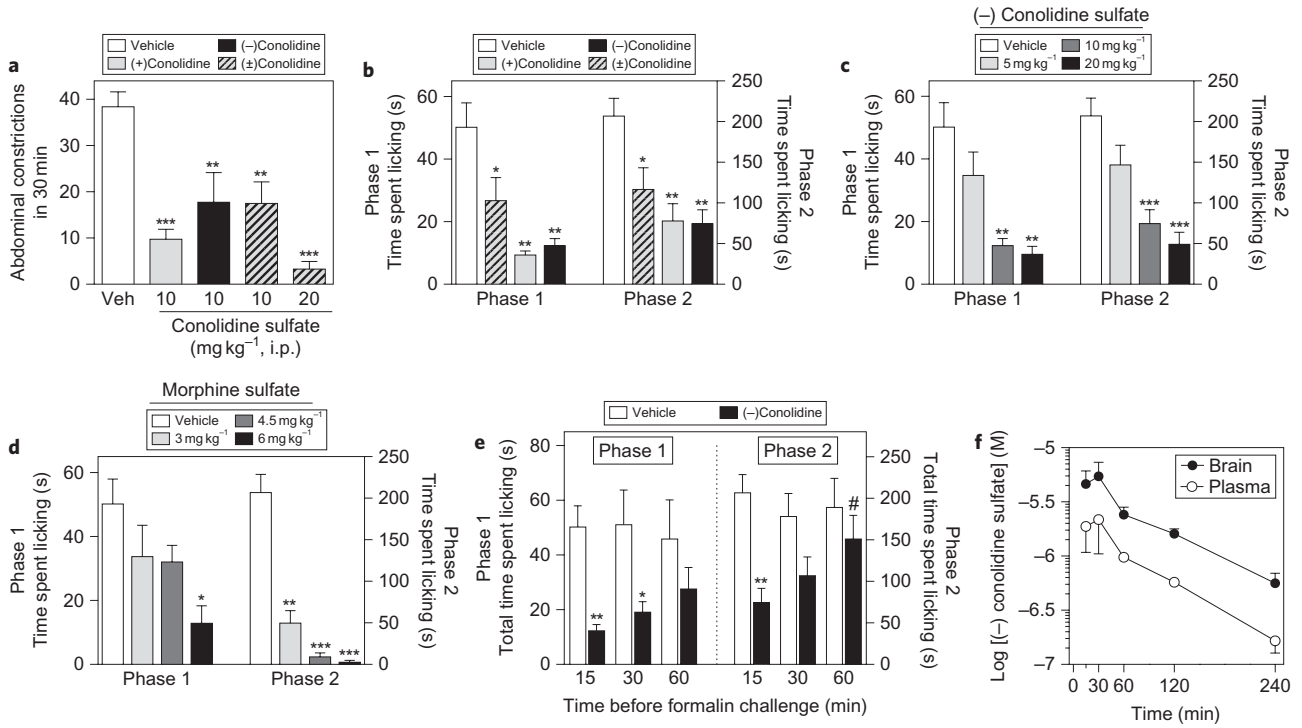

Figure 5 | Conolidine is antinociceptive in visceral, tonic and persistent pain models and is present at micromolar levels in the brain after systemic injection. Male adult (12–16 weeks old) C57BL/6J mice from Jackson Labs were treated with conolidine sulfate or vehicle (10% dimethyl sulfoxide in water, 10 μl g^{-1}, i.p.) at the times indicated. **a**, Conolidine sulfate (10 or 20 mg kg^{-1}, i.p. of the indicated enantiomer) was injected 15 min before acetic acid (0.75% in H_2O)[19]. Abdominal constrictions were recorded during the 30 min immediately following acetic acid injection. Drug effect versus vehicle: $^{**}P < 0.01$, $^{***}P < 0.001$, Student's *t*-test, $n = 4$–8 mice per treatment. **b**, Formalin (25 μl, 5% in saline) was injected into the hind-paw pad (intraplantar) 15 min after conolidine sulfate (10 mg kg^{-1}, i.p.); paw licking was recorded for 40 min following formalin injection. Phase 1 refers to the sum of time spent licking in the first 10 min of response and Phase 2 represents the 20–40-min response period. Drug effect versus vehicle: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, Student's *t*-test, $n = 6$–12 mice per treatment. **c**, Dose effect of (−)-conolidine sulfate in each phase of the formalin test: drug effect by one-way analysis of variance (ANOVA): $P = 0.0004$ for Phase 1 and $P < 0.0001$ for Phase 2; Bonferroni post-hoc analysis: vehicle vs. drug within each phase: $^{**}P < 0.01$; $^{***}P < 0.001$, $n = 7$–12 mice per treatment. **d**, Dose effect of morphine sulfate in 10% dimethyl sulfoxide (i.p.) compared to vehicle. Drug effect by one-way ANOVA: $P = 0.03$ for Phase 1 and $P < 0.0001$ for Phase 2; Bonferronni post-hoc analysis: vehicle vs. drug within each phase: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, $n = 4$–12 mice per treatment. **e**, Time effect of (−)-conolidine sulfate suppression of both phases of the formalin response. Conolidine (10 mg kg^{-1}, i.p.) or vehicle was administered at the times indicated before formalin challenge. Conolidine vs. vehicle: $^{*}P < 0.05$; $^{**}P < 0.01$; 60 vs. 15 min: $^{\#}P < 0.05$, Student's *t*-test, $n = 6$–12 mice per treatment. **f**, Pharmacokinetic profile of (−)-conolidine sulfate in the plasma and brain after a 10 mg kg^{-1} i.p. injection in male C57BL/6J mice at the times indicated[20]. In all cases, the mean ± standard error of the mean is given; statistical analyses were performed with GraphPad Prism 5.0 (GraphPad Software, San Diego, CA).

Drug Screening Program (PDSP) sponsored by the National Institute of Mental Health (Bethesda, Maryland). Conolidine was able to displace more than 50% of radioligand binding to the serotonin-3 receptor ion channel, the norepinephrine transporter as well as the α2B adrenergic, α2C adrenergic and histamine-2 receptors, with low affinity ($K_i > 1$ μM, Supplementary Table S2). Functional assays, however, show marginal efficacy and potency for conolidine as an agonist or antagonist at these potential protein targets (Supplementary Table S2), suggesting that these candidates may not represent the primary mechanism of action for this compound.

There may be some significance in the fact that conolidine does not produce antinociception in the hot plate and tail flick assays, yet is efficacious in other pain tests because it may give some clues as to its mechanism of action. For example, if conolidine were acting at M2/M4 muscarinic receptors[26], α2C receptors[27] and opioid receptors or by blocking serotonin or norepinephrine transporters[28], some degree of hot plate and/or tail flick antinociception would be expected. Interestingly, other analgesics, such as gabapentin, do not suppress responses in the acute thermal antinociception tests; moreover, gabapentin, which also lacks a definitive mechanism of action, is primarily effective against the second phase of the formalin test[29–32] when administered systemically.

To gain further insight into conolidine's pharmacological profile *in vivo*, we performed open-field activity monitoring in C57BL/6J mice. Although conolidine (10 mg kg^{-1}, i.p.) readily enters the brain, it does not alter locomotor activity—specifically horizontal activity, total distance travelled, vertical rearing or stereotypic movements—in C57BL/6J mice (Supplementary Fig. S3). This is in direct contrast to morphine, which is classically known to stimulate locomotor activity in mice[33]. Although it is clear that conolidine is not an opioid analgesic, its mechanism of action remains to be determined. Regardless, its lack of adverse effects in the open-field test is encouraging because this may be indicative of fewer side effects, such as sedation, or elevations of dopamine—a characteristic of all addictive substances[34].

Conolidine's analgesic efficacy is promising, and the enantioconvergence in analgesic efficacy is interesting (Fig. 5a,b). If both antipodes of **1** operate by a similar pharmacological mechanism, then the local symmetry about the azabicyclo[4.2.2]-decane indicates a tolerance for ethylidene substitution at either site of the system. Future studies will explore the impact that diverse substitution at these sites has on analgesic properties of unnatural C5-nor stemmadenines.

Overall, we describe the first total synthesis of the rare C5-nor stemmadenine alkaloid, conolidine, and the first asymmetric

Table 1 | Relative drug potencies in both phases of the formalin test (i.p.; 95% confidence interval) from data presented in Fig. 5c,d.

	Relative potencies (ED_{50}, mg kg^{-1})	
Drug	Phase 1	Phase 2
(−)-Conolidine sulfate	5.6 (4.0–7.8)	6.0 (3.7–9.7)
Morphine sulfate	4.6 (3.3–6.4)	2.4 (1.7–3.2)

synthetic pathway to any C5-nor stemmadenine. With an efficient source of this alkaloid secured by synthetic means (nine steps, 18% overall yield), the production of sufficient quantities of the natural product (and related antipode) was followed by the first evaluation of this alkaloid *in vivo*. These combined studies have resulted in the discovery that conolidine (**1**) is a potent non-opioid analgesic that is effective at alleviating chemically induced, acute and persistent tonic pain. Further studies in neuropathic pain models will be undertaken to determine more widespread therapeutic promise for the treatment of chronic pain. Finally, although determination of the pharmacological mechanism of action associated with the potent analgesic properties of this alkaloid remains an area of intense current investigation, the results of these studies mark the establishment of a chemical foundation suitable for investigating the therapeutic potential of this unique alkaloid as a potent non-opioid analgesic.

Received 16 December 2010; accepted 4 April 2011; published online 23 May 2011

References

1. Melnikova, I. The pain market. *Nat. Rev. Drug Discov.* **9,** 589–590 (2010).
2. Crofford, L. J. Adverse effects of chronic opioid therapy for chronic musculoskeletal pain. *Nat. Rev. Rheum.* **6,** 191–197 (2010).
3. Kam, T.-S., Pand, H.-S., Choo, Y.-M. & Komiyama, K. Biologically active ibogan and vallesamine derivatives from *Tabernaemontana divaricata*. *Chem. Biodivers.* **1,** 646–656 (2004).
4. Pratchayasakul, W., Pongchaidecha, A., Chattipakorn, N. & Chattipakorn, S. Ethnobotany & ethnopharmacology of *Tabernaemontana divaricata*. *Indian J. Med. Res.* **127,** 317–335 (2008).
5. Ingkaninan, K., Ijzerman, A. P., Taesotikul, T. & Verpoorte, R. Isolation of opioid-active compounds from *Tabernaemopntana pachysiphon* leaves. *J. Pharm. Pharmacol.* **51,** 1441 (1999).
6. Scott, A. I., Yeh, C.-L. & Greenslade, D. Laboratory model for the biosynthesis of vallesamine, apparicine, and related alkaloids. *J. Chem. Soc. Chem. Commun.* 947–948 (1978).
7. Lim, D-H., Low, T-Y. & Kam, T-S. Biomimetic oxidative transformations of pericine: partial synthesis of apparicine and valparicine, a new pentacyclic indole alkaloid from *Kopsia*. *Tetrahedron Lett.* **47,** 5037–5039 (2006).
8. Ahond, A., Cavé, A., Kan-Fan, C., Langlois, Y. & Potier, P. The fragmentation of *N,N*-dimethyltryptamine oxide and related compounds: a possible implication in indole alkaloid biosynthesis. *J. Chem. Soc. Chem. Commun.* 517 (1970).
9. Hoffmann, R. W. Allylic 1,3-strain as a controlling factor in stereoselective transformations. *Chem. Rev.* **89,** 1841–1860 (1989).
10. Bennasar, M.-L., Zulaica, E., Solé, D., Roca, T., García-Díaz, D. & Alonso, S. Total synthesis of the bridged indole alkaloid apparicine. *J. Org. Chem.* **74,** 8359–8368 (2009).
11. Amat, M., Dolors Coll, M., Passarella, D. & Bosch, J. An enantioselective synthesis of the *Strychnos* alkaloid (−)-tubifoline. *Tetrahedron: Asymmetry* **7,** 2775–2778 (1996).
12. Amat, M., Dolors Coll, M., Bosch, J., Espinosa, E. & Molins, E., Total syntheses of the *Strychnos* indole alkaloids (−)-tubifoline, (−)-tubifolidine, and (−)-19,20-dihydroakuammicine. *Tetrahedron: Asymmetry* **8,** 935–948 (1997).
13. Kolundzic, F. & Micalizio, G. C. Synthesis of substituted 1,4-dienes by direct alkylation of allylic alcohols. *J. Am. Chem. Soc.* **129,** 15112–15113 (2007).
14. Still, W. C. & Mitra, A. A highly stereoselective synthesis of *Z*-trisubstituted olefins via [2,3]-sigmatropic rearrangement. Preference for a pseudoaxially substituted transition state. *J. Am. Chem. Soc.* **100,** 1927–1928 (1978).
15. Hart, S. A., Trindle, C. O. & Etzkorn, F. A. Solvent-dependent stereoselectivity in a Still–Wittig rearrangement: an experimental and ab initio study. *Org. Lett.* **3,** 1789–1791 (2001).
16. Wang, X. J., Hart, S. A., Xu, B., Mason, M. D., Goodell, F. R. & Etzkorn, F. A. Serine-*cis*-proline and serine-*trans*-proline isosteres: stereoselective synthesis of (*Z*)- and (*E*)-alkene mimics by Still–Wittig and Ireland–Claisen rearrangements. *J. Org. Chem.* **68,** 2343–2349 (2003).
17. Fristad, W. E. & Paquette, L. A. Oxidation chemistry of a *cis*-2-(3-flurylidene)ethanol. *Heterocycles* **31,** 2219–2224 (1990).
18. Le Bars, D., Gozariu, M. & Cadden, S. W. Animal models of nociception. *Pharmacol. Rev.* **53,** 597–652 (2001).
19. Collier, H. O. J., Dinneedn L. C., Johnson, C. A. & Schneider, C. The abdominal constriction response and its suppression by analgesics in the mouse. *Br. J. Pharmac. Chemother.* **32,** 295–310 (1968).
20. Mogil, J. S., Kest, B., Sadowski, B. & Belknap, J. K. Differential genetic mediation of sensitivity to morphine in genetic models of opiate antinociception: influence of nociceptive assay. *J. Pharmacol. Exp. Ther.* **276,** 532–544 (1996).
21. Dubuisson, D. & Dennis, S. G. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain* **4,** 161–174 (1977).
22. McNamara, C. R. *et al.* TRPA1 mediates formalin-induced pain. *Proc. Natl Acad. Sci. USA* **104,** 13525–13530 (2007).
23. Woolf, C. J. Evidence for a central component of post-injury pain hypersensitivity. *Nature* **306,** 686–688 (1983).
24. Tjølsen, A., Berge, O. G., Hunskaar, S., Rosland, J. H. & Hole, K. The formalin test: an evaluation of the method. *Pain* **51,** 5–17 (1992).
25. Li, X., Kamenecka, T. M. & Cameron, M. D. Bioactivation of the epidermal growth factor receptor inhibitor gefitinib: implications for pulmonary and hepatic toxicities. *Chem. Res. Toxicol.* **22,** 1736–1742 (2009).
26. Wess, J. *et al.* Muscarinic receptor subtypes mediating central and peripheral antinociception studied with muscarinic receptor knockout mice: a review. *Life Sci.* **72,** 2047–2054 (2003).
27. Stone, L. S., Fairbanks, C. A. & Wilcox, G. L. Moxonidine, a mixed alpha(2)-adrenergic and imidazoline receptor agonist, identifies a novel adrenergic target for spinal analgesia. *Ann. NY Acad. Sci.* **1009,** 378–385 (2003).
28. Jann, M. W. & Slade, J. H. Antidepressant agents for the treatment of chronic pain and depression. *Pharmacotherapy* **27,** 1571–1587 (2007).
29. Shimoyama, N., Shimoyama, M., Davis, A. M., Inturrisi, C. E. & Elliot, K. J. Spinal gabapentin is antinociceptive in the rat formalin test. *Neurosci. Lett.* **222,** 65–67 (1997).
30. Hunter, J. C. *et al.* The effect of novel anti-epileptic drugs in rat experimental models of acute and chronic pain. *Eur. J. Pharmacol.* **324,** 153–160 (1997).
31. Munro, G. Pharmacological assessment of the rat formalin test utilizing the clinically used analgesic drugs gabapentin, lamotrigine, morphine, duloxetine, tramadol and ibuprofen: influence of low and high formalin concentrations. *Eur. J. Pharmacol.* **605,** 95–102 (2009).
32. Baillie, J. K. & Power, I. The mechanism of action of gabapentin in neuropathic pain. *Curr. Opin. Investig. Drugs* **7,** 33–39 (2006).
33. Goldstein, A. & Sheehan, P. Tolerance to opioid narcotics. I. Tolerance to the "running fit" caused by levorphanol in the mouse. *J. Pharmacol. Exp. Ther.* **169,** 175–184 (1969).
34. Wise, R. A. & Bozarth, M. A. A psychomotor stimulant theory of addiction. *Psychol. Rev.* **94,** 469–492 (1987).

Acknowledgements

We gratefully acknowledge T.-S. Kam (University of Malaya, Kuala Lumpur, Malaysia) for providing authentic spectra of natural (+)-conolidine for comparison with our synthetic samples (see Supplementary Information).

Author contributions

G.C.M. conceived, initiated and directed the project. M.A.T. and A.K.B. conducted all chemical experiments. L.M.B. initiated and directed the *in vivo* and *in vitro* pharmacological evaluation. L.M.B. and M.D.C. directed the pharmacokinetic experiments, and K.M.R. and C.G. conducted all biochemical and *in vivo* experiments. Receptor binding profiles were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract no. HHSN-271-2008-00025-C (NIMH PDSP). The NIMH PDSP is directed by Bryan L. Roth (MD, PhD) at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscol at NIMH, Bethesda, Maryland, USA. G.C.M. and L.M.B. wrote the manuscript.

Additional information

The authors declare no competing financial interests. Supplementary information and chemical compound information accompany this paper at www.nature.com/naturechemistry. Reprints and permission information is available online at http://www.nature.com/reprints/. Correspondence and requests for materials should be addressed to L.M.B. and G.C.M.