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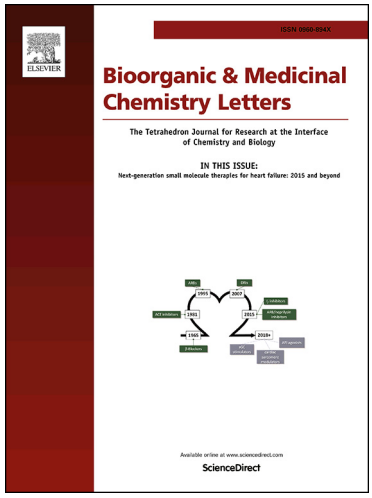

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Discovery of conolidine derivative DS39201083 as a potent novel analgesic without mu opioid agonist activity

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ABSTRACT

We discovered a novel compound, 5-methyl-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3H)-one sulfuric acid salt (**DS39201083**), which was formed by derivatization of a natural product, conolidine. **DS39201083** had a unique bicyclic skeleton and was a more potent analgesic than conolidine, as revealed in the acetic acid-induced writhing test and formalin test in ddY mice. The compound showed no agonist activity at the mu opioid receptor.

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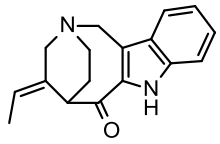
Conolidine (**1**, Figure 1) is a natural indole alkaloid that was first isolated in 2004 by Kam et al.[1] from Tabernaemontana divaricata, along with a number of other monoterpenoid indole alkaloids having opioid analgesic properties. However, research on the biological activity of **1** has not been promoted because it is an extremely rare compound that has been isolated in only 0.00014% yield from the stem bark of the plant. In 2011, Micalizio and coworkers reported the first successful asymmetric total synthesis of conolidine, which involved the formation of a C-ring via the Mannich reaction of a piperidine-indole compound and formaldehyde. They also revealed that the synthetic conolidine showed powerful non-opioid analgesic effects in mouse models.[2,3] Following this work, several other groups have reported the efficient synthesis of this compound.[4,5,6,7] In addition, the research to elucidate the mechanism of action of **1** has also been reported recently.[8] Although the mechanism of action of **1** is not yet completely clear, we are interested in its unique analgesic properties that may render it a promising alternative to opioids which impart severe opioid-related adverse effects[9,10] such as constipation, nausea, vomiting, pruritus, somnolence, cognitive impairment, respiratory depression, tolerance, physical dependence, and addiction. In this letter, we describe the synthesis and *in vivo* structure activity relationship (SAR) study of a series of novel conolidine derivatives.



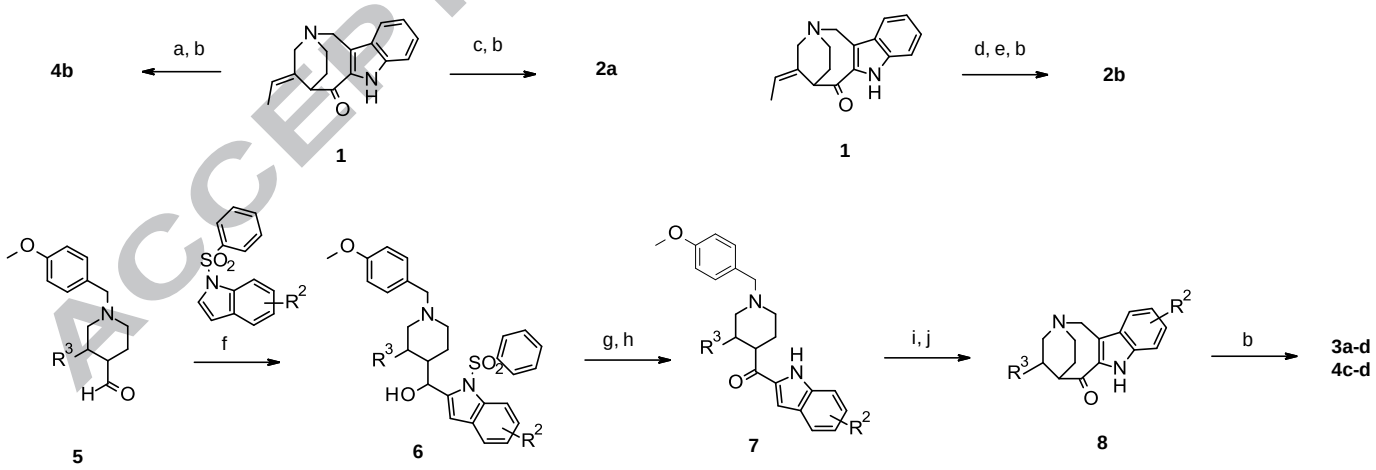
Conolidine (**1**)

Figure 1. Structure of conolidine.

Our objective was to identify orally bioavailable conolidine derivatives that show more potent analgesic efficacy in the *in vivo* animal pain model. Therefore, we designed and synthesized several varieties of conolidine derivatives (**2a–4d**) that were synthesized from **(±)-1** or aldehyde **5** that can be synthesized by the method already reported using the process illustrated in **Scheme 1**. All the derivatives were sulfate salts, and the compounds, except for **4a**,[11] were either racemic (**2a–3d**, **4c–d**) or diastereoisomeric **4b**.

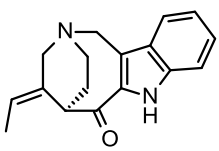
We initiated the SAR study by evaluating the synthesized derivatives in the acetic acid writhing test, which measures the reduction (% inhibition) in the acetic acid induced-writhing behavior upon oral administration of the compounds (30 mg/kg) in Slc:ddY mice for efficient derivatization. The effect of the substituted conolidine derivatives on the various parameters of the SAR study are summarized in **Table 1**.

Introduction of the alkyl group at the R^1 position (**2a–b**) reduced the *in vivo* activities of the compounds as compared with that of **(−)-1**. Moreover, the substituent of the aromatic moiety in the core structure was investigated. For instance, introduction of a methoxy group on the phenyl ring in compounds (**3a–d)** resulted in weaker activities as compared with that of **(−)-1**. We speculated that the analgesic activities are associated with poor or moderate metabolic stabilities against mice cytochromes. Next, we synthesized several derivatives (**4a–d)** focusing on the exo-olefin of conolidine. Among them, the unsubstituted compound **4a** showed drastically improved metabolic stability (97%) and more potent *in vivo* activity (73% inhibition at 30 mg/kg).

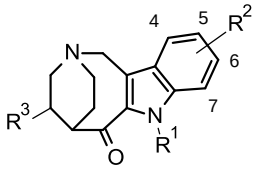


Scheme 1. Reagents and conditions: (a) Pd-C, EtOH, 97%; (b) H_2SO_4 aq., EtOH, 68-98%; (c) MeI, NaH, DMF, 56%; (d) $BrCH_2CH_2OAc$, NaH, DMF, 58%; (e) NaOH, THF/MeOH, 85%; (f) *n*-BuLi, benzenesulfonyl group -protected indole, THF, 72-94%; (g) Cs_2CO_3/MeOH, THF, 82-92%; (h) MnO_2, CH_2Cl_2, 68-89%; (i) ACECl, 1,2-dichloroethane, then MeOH; (j) paraformaldehyde, TFA, 1,2-dichloroethane, 25-38% from **7**.

Table 1. *In vivo* SAR of substituted conolidine derivatives **2a–4d**.



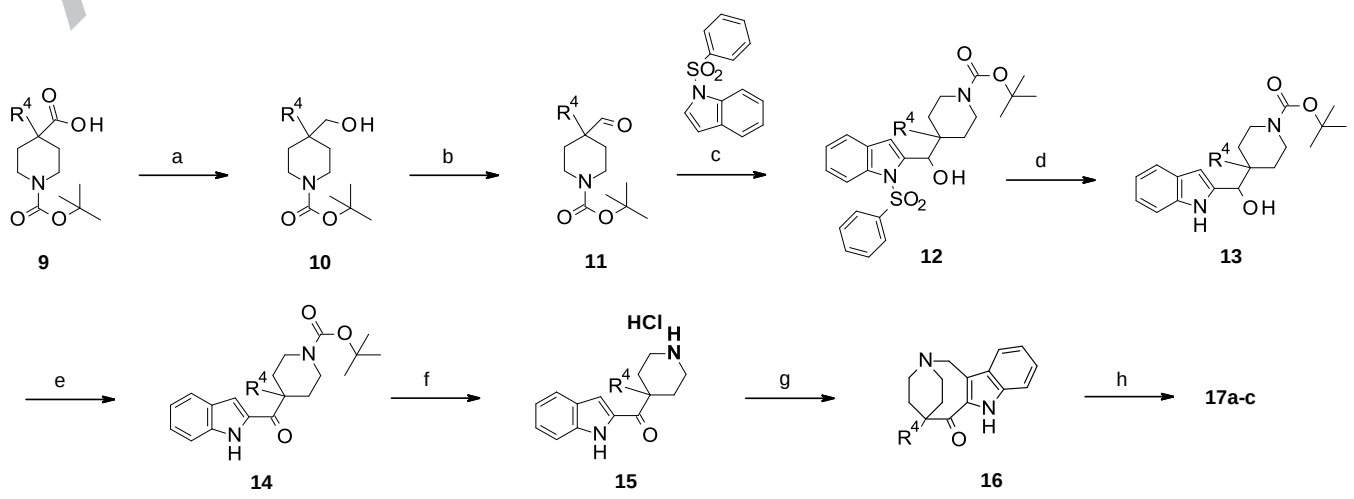
(-)-1 Conolidine



Compound	R^1	R^2	R^3	Acetic acid writhing test (% inhibition at 30 mg/kg)	Log D [a]	Metabolic stability (%)[b]
(±)-1	H	H		49	N.T.[c]	N.T.[c]
(−)-1	H	H		49	2.8	78
2a	Me	H		30	3.5	40
2b	CH_2CH_2OH	H		27	2.7	38
3a	H	4-OMe		42	3.4	22
3b	H	5-OMe		3	3.1	45
3c	H	6-OMe		31	3.1	54
3d	H	7-OMe		34	3.3	N.T.[c]
4a	H	H	H	73	1.8	97
4b	H	H		50	N.T.[c]	N.T.[c]
4c [d]	H	H		18	3.3	56
4d	H	H		0	N.T.[c]	N.T.[c]

[a] The distribution coefficients (log D) were measured between 1-octanol and phosphate buffered saline (pH 7.4).
[b] Percentage of the tested compound remaining after 0.5 h of incubation with mouse liver microsome (0.1 mg/mL).
[c] Not tested, [d] Ervaticine[5,12]

These results encouraged us to synthesize further derivatives on the basis of **4a**. Therefore we decided to investigate the influence of an alkyl group at R^4 position in detail. The synthetic route of compounds (**17a-c**) is shown in **Scheme 2**.[13] Initially, the readily available **9** was reduced by $LiAlH_4$ to produce alcohol **10**, which was subsequently converted to the corresponding aldehyde **11** by Parikh–Doering oxidation using SO_3/pyridine.[14] Addition of the benzenesulfonyl group protected-indole to **11** furnished the corresponding alcohol **12** in a moderate yield. The reaction showed excellent selectivity, and almost no 3-position adduct was produced. Removal of the benzenesulfonyl group under mild conditions using Cs_2CO_3/MeOH gave the desired product **13**. Continuous oxidation of the secondary alcohol with MnO_2 and subsequent removal of the Boc group produced piperidine hydrochloride **15**. Intramolecular Mannich reaction of **15** with formaldehyde furnished the desired bicyclic compound **16**. Finally, conversion of the bicyclic compound **16** to its salt provided the desired compound (**17a-c)**.



9 **10** **11** **12** **13** **14** **15** **16**

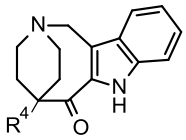
Scheme 2. Reagents and conditions: (a) $LiAlH_4$, THF, 72-90%; (b) SO_3/pyridine, DMSO, $(i\text{-Pr})_2EtN$, CH_2Cl_2; (c) *n*-BuLi, benzenesulfonyl group -protected indole, THF, 60-89% from **11**; (d) Cs_2CO_3/MeOH, THF, 69-85%; (e) MnO_2, CH_2Cl_2; (f) HCl, EtOAc; (g) paraformaldehyde, TFA, 1,2-dichloroethane, 29-40% from **13**; (h) H_2SO_4 aq., EtOH, 68%.

The results of derivatives (**17a–c**) are summarized in **Table 2**. We found that **17a** bearing a methyl group showed the strongest analgesic activity (90% inhibition at 30 mg/kg). In contrast, the introduction of larger substituents such as ethyl **17b** and *sec*-butyl **17c** attenuated the *in vivo* activity presumably because of the low metabolic stability imparted by the increased lipophilicity. The pharmacokinetic (PK) parameters of the compounds were also measured in mice. The most active compound, **17a**, showed the best PK profile (AUC = 3.21 μM, C_{max} = 1.25 μM, $T_{1/2}$ = 2.33 h). Interestingly, these data suggest that the PK profiles and the analgesic activity tend to be almost positively correlated.

Furthermore, we evaluated some of the selected representative compounds (**(−)-1, 4a**, **17a**) in detail by the acetic acid-induced writhing test, in order to calculate the ED_{50} value (**Table 3**). As expected, **17a** showed excellent dose-dependent activity (ED_{50} = 7.8 mg/kg; 23%, 52%, and 94% inhibition at 3, 10, and 30 mg/kg, respectively) four times higher than that of **(−)-1** (ED_{50} = 32 mg/kg). Consequently, **(−)-1** and **17a** were evaluated in the mice formalin test. In this test, 3.5% formalin (20 μL) was injected into the hind paw pad of Slc:ddY mice (n = 8) 30 min after the compound was administered (30 mg/kg, p.o.). Reduction in the sum of the time spent in paw licking and the biting responses were measured for the first 10 min (the initial phase) following the formalin injection. In general, among the representative clinically used systemic analgesics such as pregabalin, gabapentin, duloxetine, and celecoxib, and opioids such as morphine, only opioids are known to be effective in inhibiting the initial phase.[15,16,17] If a compound without mu opioid agonist activity can inhibit the initial phase, it is a potential analgesic that will also mimic the analgesic effect of opioids without mimicking their side effects.

Compound **17a** was found to have more potent analgesic efficacy (92% inhibition) as compared with **(−)-1** (43% inhibition). Furthermore, we investigated the binding affinity and functional activity of these representative compounds toward the mu opioid receptor (MOR). We confirmed that the analgesic efficacies of these compounds are not mediated by the mu opioid activity of the MOR binding test and MOR cAMP assay. Thus, the orally active **17a**, **DS39201083**, was selected as a promising tool compound.

Table 2. *In vivo* SAR at R^4 and pharmacokinetic (PK) parameters of conolidine derivatives **4a** and **17a–c**.



Compound	R^4	Acetic acid writhing test (% inhibition at 30 mg/kg)	AUC (μM) [a]	C_{max} (μM) [a]	$T_{1/2}$ (h) [a]	Log D	Metabolic stability (%) [b]
4a	H	73	2.26	1.16	1.17	1.8	89
17a	Me	90	3.21	1.25	2.33	2.5	92
17b	Et	48	1.02	0.71	1.28	3.0	43
17c	*sec*-Bu	51	0.14	0.23	1.60	3.7	0

[a] Average of three values dosed at 10 mg/kg orally (p.o.) in ddY mice (0.5 w/v% methylcellulose suspension).

[b] Percentage of the tested compound remaining after 0.5 h of incubation with mouse liver microsome (0.5 mg/mL).

Table 3. *In vivo* analgesic evaluation and MOR assay of **(−)-1**, **4a** and **17a**.

Compound	Acetic acid writhing test ED_{50} (mg/kg) [a]	Formalin test, initial phase1 (% inhibition at 30 mg/kg)	MOR binding test IC_{50} (μM) [b]	MOR cAMP assay EC_{50} (μM) [c]	MOR cAMP assay E_{max} (%) [d]
(−)-1	32	43	70	>100	13
4a	11	N.T. [e]	>100	>100	13
17a	7.8	92	71	>100	12

[a] The ED_{50} value was calculated from the dose response curve of analgesic activity at 10, 30 and 100mpk.

[b] Binding affinities (IC_{50}) were obtained by the competitive displacement of radiolabeled [3H] diprenorphine. Morphine, with an IC_{50} 0.41 μM, was used as a positive control.

[c] cAMP assays were carried out using human MOR-expressed CHO cells. DAMGO, with an EC_{50} 0.088μM, was used as a positive control.

[d] E_{max} was calculated as the response (in %) obtained with DAMGO.

[e] Not tested

In conclusion, we identified a novel compound, 5-methyl-1,4,5,7-tetrahydro-2,5-ethanoazocino[4,3-b]indol-6(3H)-one sulfuric acid salt **17a** [18], derived from a natural product conolidine. Compound, **17a (DS39201083)**, has a unique and non-chiral bicyclic

structure and shows more potent analgesic efficacy upon its oral administration in mice, as revealed by the acetic acid-induced writhing test and formalin test; furthermore, it shows no agonist activity for the MOR. Although the mechanism of action is still unknown, we consider **DS39201083** to be a promising tool compound. Studies on further derivatization of this series of compounds and target identification to understand the mechanism of action are ongoing, and the results will be reported in due course.

Reference and notes

1. Kam, T. S.; Pang, H. S.; Choo, Y. M.; Komiyama, K. *Chem. Biodivers*. **2004**, *1*, 646.
2. Tarselli, M. A.; Raehal, K. M.; Brasher, A. K.; Streicher, J. M.; Groer, C. E.; Cameron, M. D.; Bohn, L. M.; Micalizio, G. C. *Nat. Chem*. **2011**, *3*, 449.
3. Bohn, L. M.; Micalizio, G. C. WO2012/088402 (patent).
4. Takanashi, N.; Suzuki, K.; Kitajima, M.; Takayama, H. *Tetrahedron Lett.* **2016**, *57*, 375.
5. Naoe, N.; Yoshida, Y.; Oishi, S.; Fujii, N.; Ohno, H. *J. Org. Chem.* **2016**, *81*, 5690.
6. Huang, Y.; Yang, Y.; Song, H.; Liu, Y1.; Wang, Q. *Sci. Rep*. **2015**, 5, 13516.
7. Sugimoto, K.; Matsuya, Y. *Tetrahedron Lett.* **2017**, *58*, 4420.
8. Mendis G. D. C.; Berecki G.; Morrisroe E.; Pachernegg S.; Li M.; Varney M.; Osborne P. B.; Reid C.A.; Halgamuge S.; Petrou S. *Sci. Rep.* **2019**, 9, 121.
9. Crofford, L. J. *Nat. Rev. Rheum*. **2010**, *6*, 191.
10. Labianca, R.; Sarzi-Puttini, P.; Zuccaro, S. M.; Cherubino, P.; Vellucci, R.; Fornasari, D. *Clin. Drug. Investig.* **2012**, *32*, 53.
11. Scopes, D. I. C.; Allen, M. S.; Hignett, G. J.; Wilson, N. D. V.; Harris, M.; Joule, J. A. *J. Chem. Soc. Perkin Trans. 1*. **1977**, *21*, 2376.
12. Rahman, A.; Muzaffar, A. *Heterocycles*, **1985**, *23*, 2975.
13. See the supporting information for details of the experimental procedure of **17a**.
14. Parikh, J.P.; Doering, W. E. *J. Am. Chem. Soc.* **1967**, *89*, 5505.
15. Field, M. J.; Oles, R. J.; Lewis, A.S.; McCleary, S.; Hughes, J.; Singh, L. *Br. J. Pharmacol.* **1997**, *121*, 1513.
16. Sun Y. H.; Dong Y. L.; Wang Y. T.; Zhao G. L.; Lu G. J.; Yang J.; Wu S. X.; Gu Z. X; Wang W. *PLoS ONE*. **2013,** *8(10)*.
17. Le Bars D.; Gozariu M.; Cadden S. W. *Pharmacol. Rev.* **2001**, *53*, 597.
18. The data for **17a**. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.58 (1H, br s), 7.65 (1H, d, *J* = 8.2 Hz), 7.48 (1H, d, *J* = 8.2 Hz), 7.34 (1H, t, *J* = 7.6 Hz), 7.12 (1H, t, *J* = 7.4 Hz), 4.72 (2H, s), 3.42–3.37 (2H, m), 3.22–3.19 (2H, m), 2.11–2.04 (2H, m), 1.88–1.86 (2H, m), 1.28 (3H, s). MS (ESI) *m/z*: 255 $(M+H)^+$. Anal. Calcd for $C_{16}H_{18}N_2O$. H_2SO_4. $1/3C_2H_5OH$: C, 54.43; H, 6.03; N, 7.62; S, 8.72. Found: C, 54.16; H, 5.80; N, 7.89; S, 8.73.

Graphical Abstract

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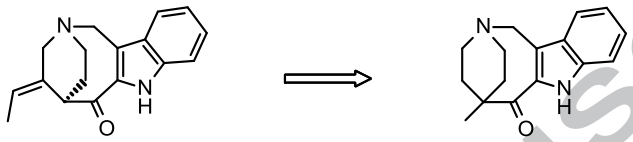

(-)-Conolidine DS39201083