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Discovery of a novel bicyclic compound, DS54360155, as an orally potent analgesic without mu-opioid receptor agonist activity

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ABSTRACT

We synthesized derivatives of a natural alkaloid, conolidine, and evaluated these derivatives in the acetic acid-induced writhing test and formalin test in ddY mice after oral administration. As a result, we identified (5*S*)-6-methyl-1,3,4,5,6,8-hexahydro-7H-2,5-methano[1,5]diazonino[7,8-b]indol-7-one sulfate salt, **15a** (**DS54360155**), with a unique and original bicyclic skeleton, as an analgesic more potent than conolidine. Moreover, **15a** did not exhibit mu-opioid receptor agonist activity.

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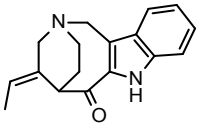
C ver, few studies have evaluated the biological activity of **1**. This is because **1** can only be isolated in small amounts from the bark of the plant. In 2011, Micalizio *et al.* reported the first successful asymmetric total synthesis of **1**. They also reported that synthetic **1** exhibited potent non-opioid analgesic effects in mouse models.[2,3] Since these studiesy, several other groups have reported efficient synthesis[4-6] of this compound and elucidated its mechanism of action.[7] Although the mechanism of action of **1** is not completely understood, we are interested in its unique analgesic properties that may render it a promising alternative to opioids, which have severe opioid-related adverse effects,[8,9] such as constipation, nausea, vomiting, pruritus, somnolence, cognitive impairment, respiratory depression, tolerance, physical dependence, and addiction. In this study, we synthesized a series of novel derivatives of **1** and evaluated their structure-activity relationships (SARs) *in vivo*. As a result, we obtained a potent analgesic **15a** (**DS54360155**) without mu-opioid receptor agonist activity.



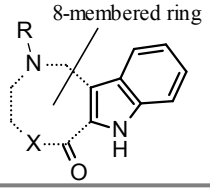
Conolidine (**1**)

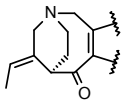
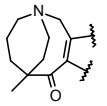
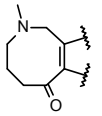
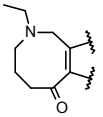
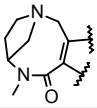
Figure 1. Structure of conolidine (**1**)

This study aimed to identify the derivatives of **1** that are orally bioavailable and exhibit safe and potent analgesic effects in an animal pain model. In this regard, to determine the *in vivo* SARs, we evaluated the effects of the synthesized derivatives on mouse performance during an acetic acid-induced writhing test. The test was used to measure the reduction (% inhibition) in acetic acid-induced writhing behavior in ddY mice, which are outbred mice maintained in closed colonies, in response to the oral administration of the compounds (30 mg/kg). Furthermore, our preliminary screening demonstrated that (−)-**1** was a potent inhibitor of the human ether-a-go-go-related gene (hERG) potassium channel (63% inhibition at 10 μM). The hERG encodes a potassium ion channel responsible for myocardial repolarization. The inhibition of the hERG channel may cause arrhythmia due to QT prolongation. Therefore, overall, we aimed to identify derivatives of **1** that exhibited strong *in vivo* analgesic activity as well as weak inhibition of the hERG channel.

We hypothesized that **1** derivatives, which exhibit lower lipophilicity (measured as a distribution coefficient; LogD), may reduce the hERG channel inhibition. However, the introduction of polar groups, such as the hydroxyl or carboxyl group, to the indole group of **1** resulted in the attenuation of analgesic activity *in vivo*. In addition, the conversion of indole moiety was synthetically difficult. Hence, we fixed the indole moiety and transformed the bicyclic skeleton. Although the conversion of the bicyclic ring was also not easy, we tenaciously attempted the synthesis of various derivatives. Finally, we could stably synthesize several 8-membered ring derivatives. The data of the synthesized conolidine derivatives (**2**–**5**) are summarized in **Table 1**. All the derivatives were sulfate salts, and compounds **2** and **5** were racemic forms. Compound **2** was a novel compound converted to the [4.3.2] bicyclic skeleton from the [4.2.2] bicyclic skeleton of **1.** Compounds **3** and **4** were simplified to a monocyclic 8-membered ring from a sterically bulky bicyclic structure to reduce the LogD value. Compound **5** was an original compound that had an amide bond in the [5.2.1] bicyclic skeleton. Among the synthesized derivatives, compound **5** had potent analgesic activity (83% inhibition at 30 mg/kg), whereas compounds **2**, **3**, and **4** showed weaker activity than **1**. From the results of compounds **1**, **2**, and **5**, we considered that the difference in bicyclic 8-membered ring structure greatly affected the analgesic activity *in vivo*. In addition, we observed a general correlation between the LogD value of the synthesized derivatives and hERG inhibition. As a result, compound **5**, which had the lowest LogD (0.7), exhibited the weakest hERG channel inhibition (26% inhibition at 10 μM). Bicyclic compounds **2** and **5** exhibited a higher metabolic stability than compounds **3** and **4**. We speculated that the bicyclic skeleton had a stable conformation by fixing the ring and hindering access of metabolic enzymes such as cytochrome P450 to substrates. The low metabolic stability of **(−)-1** may be due to the high LogD.

Table 1. *In vivo* structure activity relationship (SAR) of conolidine derivatives **2–5**

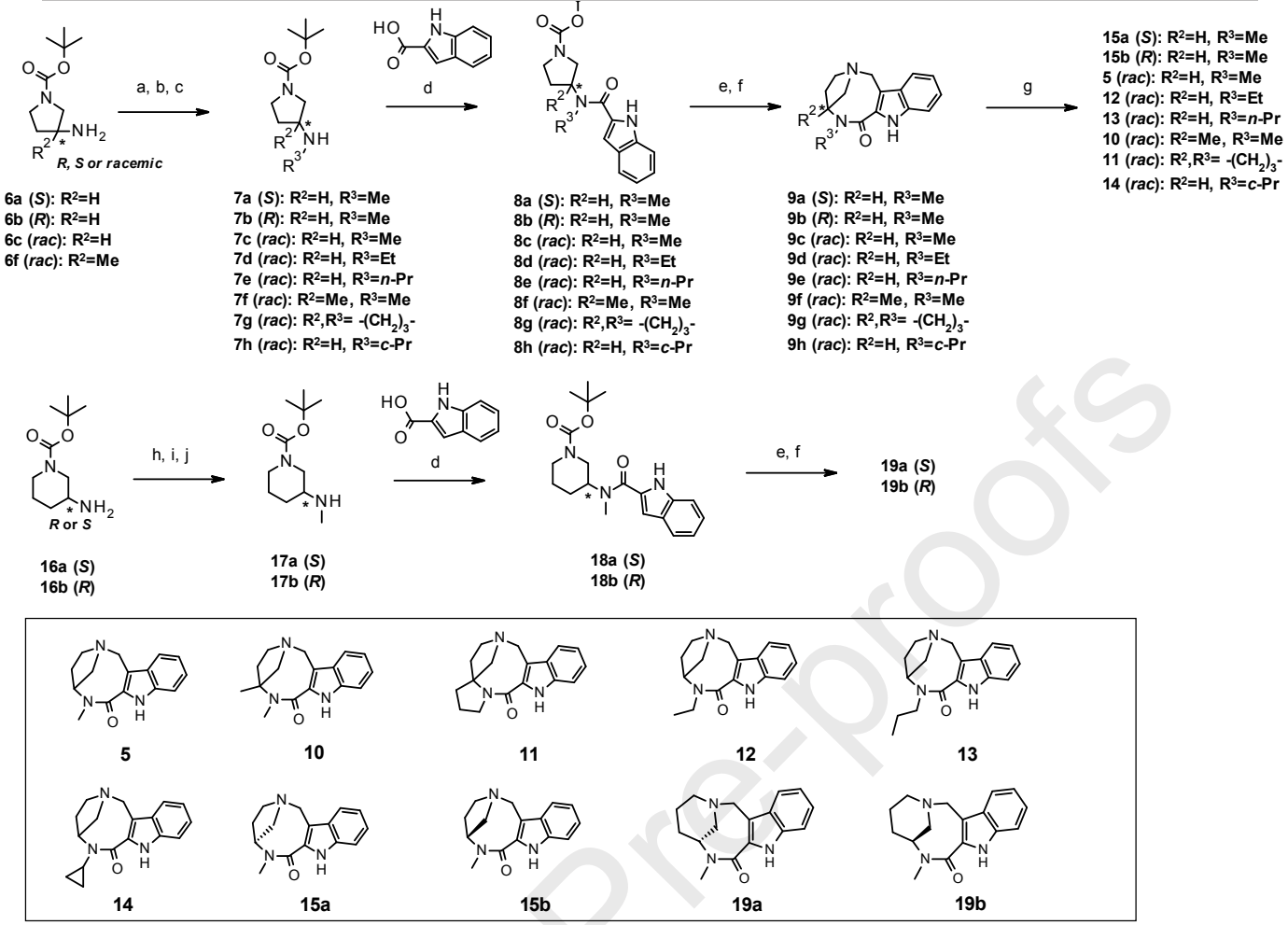


Compound	8-membered ring (R, X)	Salt	Inhibition of acetic acid-induced writhing (% inhibition at 30 mg/kg)	LogD[a]	Metabolic stability (%)[b]	hERG channel inhibition (% inhibition at 10 μM)
(−)-1		H_2SO_4	49	2.8	34	63
2		H_2SO_4	21	1.5	91	88
3		H_2SO_4	31	1.1	53	50
4		H_2SO_4	36	1.3	49	73
5		H_2SO_4	83	0.7	>100	26

[a]The distribution coefficient (LogD) was measured between 1-octanol and phosphate-buffered saline (pH 7.4).

[b]The remaining percentage (%) of the tested compounds after 0.5-h incubation with mouse liver microsome (0.5 mg/mL).

We narrowed down on a [5.2.1] bicyclic skeleton containing an amide bond in the molecule, such as compound **5**, as it was a novel and original structure. Therefore, we decided to perform further derivatization based on the structure of compound **5**. The synthesis of derivatives from the novel bicyclic compound **5** is shown in **Scheme 1**. Compounds **5** and **10–14** were racemic, and compounds **15a** and **15b** were optically active compounds of compound **5,** synthesized to investigate the differential efficacy between the enantiomers. Moreover, the optically active compounds **19a** and **19b** were synthesized to examine the influence of changing the size of the bicyclic ring. Although compounds **5**, **10–14**, **15a**, and **15b** were synthesized as sulfate salts and compounds **19a** and **19b** were synthesized as free forms, we did not observe any difference in pharmacokinetic (PK) profile between the sulfate salts and free forms. We synthesized compounds **10** and **11** to investigate the effects of the R^2 and R^3 substituents and compounds **12–14** to examine the optimal size of the R^3 substituent. The commercially available amines **6a-c**[10], **6f**, **16a**, and **16b**[11] were protected by the 2-nitrobenzenesulfonyl (Ns)[12] or benzyloxycarbonyl (Cbz) group. These protected amines were subjected to alkylation and deprotection to obtain the corresponding amines **7a-f** and **17a, 17b** with a high yield from the starting material. Compounds **11** and **14** were synthesized from commercially available **7g**[13] and **7h**[14] as the starting materials, respectively. Amide formation with amines **7a-h**, **17a**, and **17b**, and indole-2-carboxylic acid using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) yielded compounds **8a-h**, **18a**, and **18b**, which were subjected to Boc group deprotection to form the corresponding amine hydrochloride salt. Subsequently, the intramolecular Mannich reaction of amine hydrochloride salt with formaldehyde resulted in the formation of bicyclic compounds **9a-h**, **19a**, and **19b**. Only compound **9a-h**, which had a pyrrolidine ring in the molecule, was converted to sulfate salt to obtain the corresponding desired compounds (**5**, **10-14**, **15a**, and **15b**).



Scheme 1. Reagents and conditions: (a) 2-Nitrobenzenesulfonyl chloride, Et_3N, THF, 95–100%; (b) R^2 halide, K_2CO_3, DMF, 60 °C, 96–99%; (c) 2-Fluorothiophenol, K_2CO_3, MeCN, 94–100%; (d) EDCI, THF, 88–99%; (e) HCl, 1,4-dioxane/AcOEt; (f) paraformaldehyde, TFA, 1,2-dichloroethane, 4–35% 2 steps from **8a-h**, **18a**, and **18b**; (g) 1 M H_2SO_4 aq., EtOH, 0 °C, 80–99%; (h) carbobenzoxy chloride., Et_3N, CH_2Cl_2, 70-94%; (i) MeI, NaH, THF, 92–97%; (j) Pd/C, H_2, MeOH, 98–100%

The data of the derivatives synthesized in **Scheme 1** are shown in **Table 2**. As expected, the inhibition of the hERG channel tended to be weaker as the LogD value was lower, albeit with some exceptions, as the basicity of the compound also affected the inhibition of the hERG channel. The metabolic stability of most compounds also showed a good value due to the low LogD. Unfortunately, the *in vivo* analgesic activity of compounds **10–14** was attenuated when compared with that of compound **5**. Hence, the introduction of the alkyl substituents to the R^2 and R^3 positions was not permitted (**10** and **11**). Moreover, from the results of **12–14**, the introduction of a larger R^3 substituent (Et, *n*-Pr, *c*-Pr) was found to be ineffective. These compounds generally have good metabolic stability; therefore, the substituents do not appear to affect the physical properties of the compounds. Next, we investigated the differential efficacy between the enantiomers (**15a** and **15b**) of the optically active compounds of compound **5**. We observed that compound **15a** (*S*-form) exhibited considerably strong analgesic activity (**15a**: 95% inhibition at 30 mg/kg) and weak inhibition of the hERG channel (14% inhibition at 10 μM). Furthermore, compound **19a** (*S*-form), which included a piperidine ring in its structure, also showed stronger analgesic activity (**19a**: 77% inhibition at 30 mg/kg) than **19b** (R-form). These results indicate that the target site was recognized by the enantiomer of the compound, although the exact identity of target was unknown. Among these highly active compounds, the optically active compound **15a**, which has a pyrrolidine ring, was the most active. Hence, we identified that a compact 8-membered bicyclic structure, such as a [5.2.1] bicyclic skeleton, was preferred to ensure potent *in vivo* analgesic activity.

Table 2. *In vivo* SAR derivatives **5–19b**

Compound.	Inhibition of acetic acid-induced writhing (% inhibition at 30 mg/kg)	LogD[a]	Metabolic stability (%)[b]	hERG channel inhibition (% inhibition at 10 μM)
5	83	0.7	>100	26
10	37	0.7	93	36
11	47	1.2	82	13
12	43	1.1	91	14
13	44	1.6	72	43
14	55	1.1	92	19
15a	95	0.7	99	14
15b	49	0.7	>100	23
19a	77	1.0	74	38
19b	65	0.9	82	N.T.[c]

[a]The distribution coefficient (LogD) was measured between 1-octanol and phosphate-buffered saline (pH 7.4).

[b]The remaining percentage (%) of the tested compounds after 0.5-h incubation with mouse liver microsome (0.5 mg/mL).

[c]Not tested.

Subsequently, we calculated the ED_{50} value of compounds **(−)-1, 5**, **15a**, **15b**, and **19a**, which exhibited strong analgesic activities (**Table 2)** in the acetic acid writhing test. In addition, we also measured the PK parameters for these compounds (**Table 3**). The ED_{50} value was calculated from the dose-response curve of the analgesic activities at 10, 30, and 100 mg/kg. We observed that compound **15a** exhibited the strongest dose-dependent analgesic effect (ED_{50} = 12 mg/kg; 19%, 23%, and 95% inhibition at 3, 10, and 30 mg/kg, respectively). The results presented in **Table 2** further indicated the high potential of the analgesic efficacy of compound **15a** compared with that of other derivatives. In addition, compound **15a** exhibited a good PK profile (area under the curve = 5.80 μM, C_{max} = 2.15 μM, $T_{1/2}$ = 2.27 h, Cl = 24.9 mL/min/kg, Vd = 1.72 L/kg). Between compounds **15a** and **15b**, compound **15a** had stronger analgesic efficacy despite its lower exposure. This result suggests that compound **15a** has an intrinsically strong analgesic effect.

Consequently, compounds **(−)-1, 15a,** and **15b** were evaluated using a mouse formalin test (**Table 4**). In this test, 3.5% formalin (20 μL) was injected in the hind-paw pad of ddY mice (n = 8) 30 min after the compound was administered (30 mg/kg, p.o.). The reduction in the sum of the time spent in the paw licking and biting responses was measured for the first 10 min (the initial phase) after formalin injection. Generally, among the representative clinically used systemic analgesics such as pregabalin, gabapentin, duloxetine, and celecoxib, and opioids, including morphine, only opioids are known to be effective in inhibiting the initial phase.[15-17] If a compound without mu-opioid receptor (MOR) agonist activity could inhibit the initial phase, it was considered a potential compound that could mimic the analgesic effect of opioids without the opioid-associated side effects. Compound **15a** was observed to have more potent analgesic efficacy (83% inhibition) than **(−)-1** (43% inhibition). Furthermore, we investigated the binding affinity and functional activity of these representative compounds toward the MOR. By using a MOR binding test and MOR cAMP assay, we confirmed that the analgesic efficacy of these compounds is not mediated by mu-opioid receptor activity. This result indicated that **15a** may be a powerful ideal analgesic without side effects. Thus, orally active **15a**, named **DS54360155**, was selected as a promising candidate compound for analgesics.

Table 3. ED_{50} values and pharmacokinetic parameters of (−)-conolidine **1**, **5**, **15a**, **15b**, and **19a** in the acetic acid writhing test

Compound	Analgesic activity against acetic acid-induced writhing ED_{50} (mg/kg)[a]	AUC (μM)[b]	Cmax (μM)[b]	$T_{1/2}$ (h)[b]	Cl (mL/min/kg)[c]	Vd (L/kg)[c]
(−)-1	32	N.T.	N.T.	N.T.	N.T.	N.T.
5	21	3.29	1.46	1.69	N.T.	N.T.
15a	12	5.80	2.15	2.27	24.9	1.72
15b	27	12.2	2.99	2.52	12.9	1.14
19a	23	1.59	0.72	2.18	N.T.	N.T.

[a]The ED_{50} value was calculated from the dose-response curve of the analgesic activities at 10, 30, and 100 mg/kg.

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

[c]Average of three values dosed at 1.0 mg/kg intravenously in the ddY mice (20% HP-b-CyD, solution).

N.T., not tested; AUC, area under the curve

Tab

Compound	Formalin test initial phase (% inhibition at 30 mg/kg)	MOR binding assay IC_{50} (μM)[a]	MOR cAMP assay EC_{50} (μM)[b]	MOR cAMP assay E_{max} (%)[c]
(−)-1	43	70	>100	13
15a	83	>100	>100	19
15b	30	>100	>100	18

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

[b]Cyclic adenine monophosphate (cAMP) assay was performed using human mu-opioid receptor (MOR)-expressing CHO cells. The EC_{50} of DAMGO, the positive control, was 0.075 μM.

[c]E_{max} was calculated as the % of the response obtained with DAMGO.

In conclusion, we identified a novel compound, (5*S*)-6-methyl-1,3,4,5,6,8-hexahydro-7H-2,5-methano[1,5]diazonino[7,8-b]indol-7-one sulfate salt **15a**, derived from the natural product conolidine. Compound **15a (DS54360155)**[18] has a unique and original [5.2.1] bicyclic structure comprising an amide bond and a pyrrolidine ring. The compound exhibited potent analgesic efficacy following oral administration in mice, as revealed by both the acetic acid-induced writhing test and formalin test. Furthermore, the compound exhibited weak hERG channel inhibition and no MOR agonist activity. Although the mechanism of action of **DS54360155** is not completely understood, we consider it a promising candidate compound for application as an analgesic. Currently, studies on further derivatization of this series of compounds and target identification are ongoing to understand the mechanism of action, and the results will be reported in due course.

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Graphical Abstract

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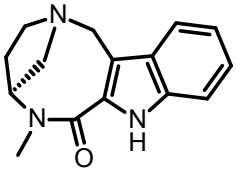

DS54360155

Analgesic activity against acetic acid-induced writhing ED_{50} 12 mg/kg

Formalin test initial phase
83% inhibition at 30 mg/kg

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: