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Design and synthesis of novel α-aminoamides derivatives as Nav1.7 inhibitors for antinociception

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ABSTRACT

Three novel series of α-aminoamides derivatives were designed and synthesized based on ralfinamide, and their Nav1.7 inhibitory activities were evaluated using manual patch clamp electrophysiology. Active compounds inhibited Nav1.7 with half maximal inhibitory concentration (IC_{50}) values ranging from 2.9 μmol/L to 21.4 μmol/L. Among them, the most potent compound **19h** exhibited about 12-fold potency better than ralfinamide. The investigation of their structure-activity relationship gives a strategy to improve the Nav1.7 inhibition of ralfinamide analogues. Compound **19h** was efficacious in antinociception in the mouse spared nerve injury (SNI) model of neuropathic pain without causing sedation in the open field test.

Chronic pain has serious and negative impact on quality of life [1-3]. Currently available analgesics are limited with either greater side-effects or low levels of analgesic efficacy, thus presenting an unmet medical need [4]. Voltage-gated sodium channel Nav1.7 encoded by *SCN9A* gene plays a key role in transmission of pain signaling [5,6]. The loss-of-function mutations in Nav1.7 lead to congenital insensitivity to pain (CIP) [7,8]. Conversely, the gain-of-function mutations in Nav1.7 underlie inherited erythromelalgia, paroxysmal extreme pain disorder and idiopathic small fiber neuropathies [5,9]. These lines of evidence indicate that Nav1.7 is a promising target for pain therapy. Ralfinamide (Fig. 1), a Nav1.7 inhibitor, was developed by Newron Pharmaceuticals, it also shows inhibition toward to Cav2.2 and *N*-methyl-D-aspartate (NMDA) receptor [10-14]. Ralfinamide was under phase III clinical trial for low back pain before discontinued [15]. Nav1.7 is a promising target for pain therapy, so optimizing the structure of ralfinamide may lead to a compound with higher Nav1.7 inhibition and better *in vivo* efficacy in antinociception.

In this study, three series of novel α-aminoamides derivatives were designed and synthesized for investigation of their structure–activity relationship (SAR). Started with investigation of the influence of the length of carbon linkage between the α-amino group and the aryl group (Fig. 1, **I**), then, we modified the α-aminoamide groups (Fig. 1, **II**) before various substituents were introduced into the benzyl group for investigation of the SAR (Fig. 1, **III**). Seventeen novel α-aminoamides derivatives were obtained and their inhibitions of human Nav1.7 currents were tested in whole-cell patch clamp recording assay. A lead compound was further evaluated for its *in vitro* metabolic stability and *in vivo* antinociception.

The syntheses of α-aminoamides derivatives were depicted in Scheme 1. Detailed procedures and compound characterizations can be found in Supporting information. 4-Hydroxyphenethyl alcohol as a starting material reacted with 2-fluorobenzyl bromide in acetone to give compound **2** and then it was oxidized by the Dess-Martin periodinane to yield the compound **3** [16,17]. Compound **3** reacted further with L-alaninamide hydrochloride in the presence of sodium cyanoborohydride to yield the target compound **4**. The compound **6** was achieved by the reaction of methyl 3-(4-hydroxyphenyl) propionate with 2-fluorobenzyl bromide in acetonitrile, subsequently through the hydrolysis of carboxylic ester to produce the compound **7**. Compound **7** was reduced with lithium aluminum hydride to the alcohol **8**, which was oxidized by Dess-Martin periodinane to yield the compound **9**. The target compound **10** was obtained by the reaction of compound **9** with L-alaninamide hydrochloride. 4-(4-Hydroxyphenyl)butanoic acid was refluxed with 2-fluorobenzyl bromide in acetone to afford the compound **12**. Compound **12** was reduced with lithium aluminum

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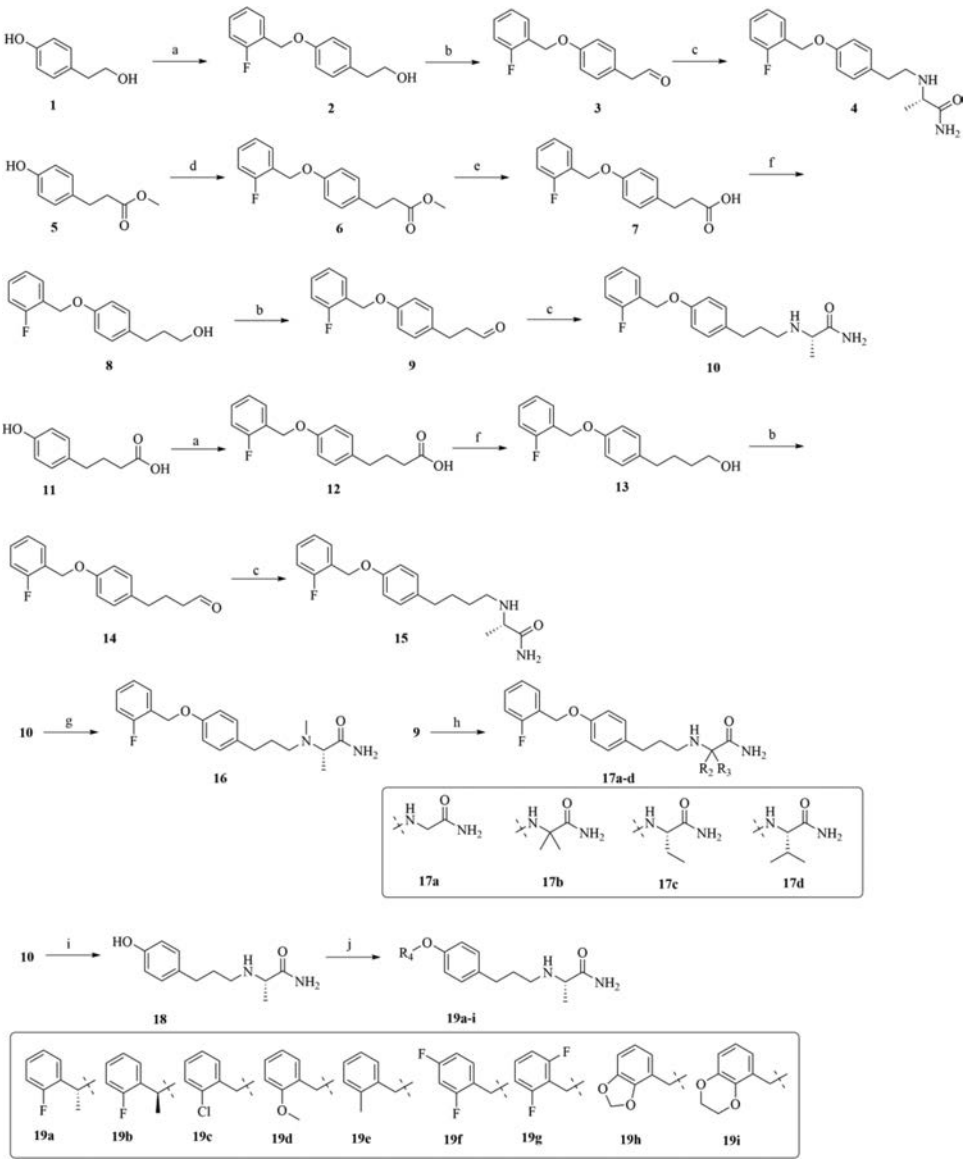

Scheme 1. Synthesis of α-aminoamides derivatives. Reaction conditions: (a) 2-Fluorobenzyl bromide, K_2CO_3, acetone, reflux, 63%–96%. (b) Dess-Martin periodinane, CH_2Cl_2, r.t., 43%–88%. (c) L-Alaninamide hydrochloride, $NaBH_3CN$, MeOH, AcOH, r.t. to 60 °C, 43%–69%. (d) 2-Fluorobenzyl bromide, K_2CO_3, acetonitrile, 90 °C, 70%. (e) NaOH, MeOH, THF, 60 °C, 93%. (f) $LiAlH_4$, THF, 80 °C, 60%. (g) 37% Formaldehyde (aq), $NaBH_3CN$, MeOH, AcOH, r.t., 88%. (h) Appropriate α-aminoamides hydrochloride, $NaBH_3CN$, MeOH, AcOH, r.t., 44%–71%. (i) Hydrogen, 5% palladium on activated carbon, EtOH, 45 °C, 90%. (j) Substituted benzyl alcohol derivatives, PPh_3, *di-tert*-butyl azodicarboxylate (DBAD), THF, 90 °C, 18%–57%.

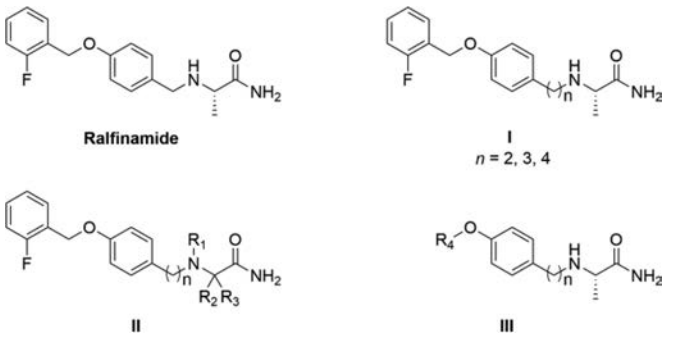

Fig. 1. Chemical structures of ralfinamide and its designed compounds (**I**, **II** and **III**).

hydride and then it was oxidized by the Dess-Martin periodinane to yield the compound **14**. The compound **14** reacted further with L-alaninamide hydrochloride to give the target compound **15**. The target compound **16** was achieved by the reaction of compound **10** with formaldehyde. The compound **9** reacted with appropriate α-aminoamides hydrochloride to yield the target compounds **17a**–**d**. The compound **18** was obtained by removing the *ortho*-fluorobenzyl group from compound **10**, which reacted further with a series of substituted benzyl alcohol to yield the target compounds **19a**–**i**.

All synthesized compounds (**4**, **10**, **15**, **16**, **17a**–**d** and **19a**–**i**) were tested for their inhibition of human Nav1.7 channel currents using whole-cell patch clamp recordings technique (Supporting information) [18,19]. Ralfinamide was selected as reference compound. As summarized in Table 1 and Fig. 2, all compounds exhibited a dose-dependent inhibition of Nav1.7 currents with half maximal inhibitory concentration (IC_{50}) values ranging from 2.9 μmol/L to 21.4 μmol/L. Among them, the compound **19h** (IC_{50} = 2.9 μmol/L) was the most potent with about 12-fold more potency than ralfinamide (IC_{50} = 35.2 μmol/L). The compounds **4**, **10** and **15** showed better potency than ralfinamide, indicating that increasing

Table 1
Inhibition of Nav1.7 channel currents by α-aminoamides derivatives in whole-cell patch clamp assay.

Compound	IC_{50} (μmol/L)[a]	Compound	IC_{50} (μmol/L)
4	11.1 ± 2.7	**19b**	15.4 ± 2.5
10	5.0 ± 0.9	**19c**	9.9 ± 1.9
15	9.9 ± 1.4	**19d**	11.7 ± 2.0
16	18.4 ± 4.2	**19e**	10.2 ± 2.1
17a	10.1 ± 2.1	**19f**	12.0 ± 2.6
17b	15.3 ± 2.0	**19g**	14.0 ± 0.6
17c	9.3 ± 2.5	**19h**	2.9 ± 0.8
17d	21.4 ± 5.2	**19i**	7.5 ± 1.2
19a	12.1 ± 3.3	Ralfinamide	35.2 ± 2.8

[a] Data are obtained using manual patch clamp electrophysiology and are presented as the mean ± standard error of mean (SEM), n = 4–8

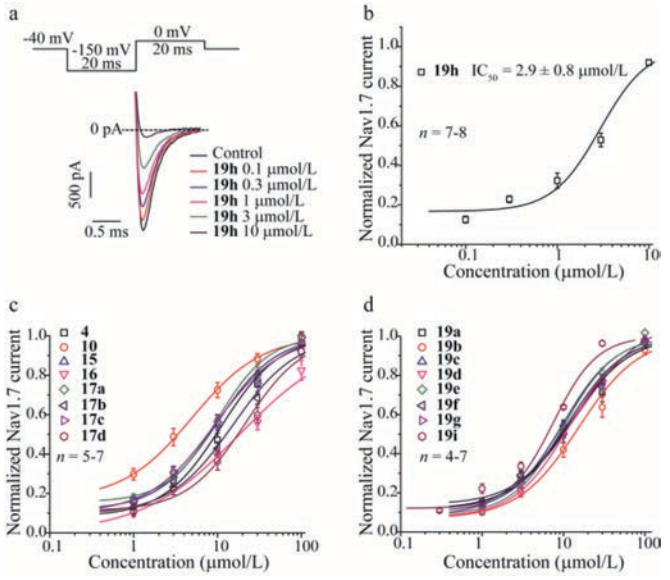

Fig. 2. The dose-dependent inhibition of Nav1.7 currents by α-aminoamides derivatives in whole-cell patch clamp recordings of HEK293 cells stably expressed Nav1.7 channel. (a) Top panel, voltage protocol used in compound inhibition experiments. To activate Nav1.7 from a holding voltage of −40 mV, a 20 msec prepulse to −150 mV is applied to recover the current from inactivation followed by a 0 mV pulse to open the channel with 1 s intervals. Bottom panel, representative current traces elicited by 0 mV before and after applications of compound **19h**. The dashed line represents zero current level. (b) Dose-response curve of compound **19h** was fitted using logistic function with IC_{50} of 2.9 ± 0.8 μmol/L, n = 7–8. (c) and (d) Curves were fitted using logistic function for concentration-dependent inhibition of Nav1.7 by α-aminoamides derivatives, n = 4–8. All data were expressed as the mean ± SEM.

the carbon chain length between the α-amino and the aryl group was beneficial for the bioactivity. Among the three compounds, the compound **10** (IC_{50} = 5.0 μmol/L) with ideal three carbon atoms of the chain length showed the most potent inhibition of Nav1.7 current. The compound **16** demonstrated weaker inhibition than the compound **10**, indicating that the methyl substitution on the α-amino group was not beneficial for the bioactivity. Removing the methyl group from α-position of aminoamides (compound **17a**) or adding methyl group to α-position of aminoamides (compound **17b**) reduced inhibitory activity, it also showed a steric effect of the substituents at α-position of aminoamides with a potency order of CH_3 (compound **10**) > CH_2CH_3 (compound **17c**) > $CH(CH_3)_2$ (compound **17d**). Introduction of a methyl group to the *orthro*-fluoro benzyl position (compounds **19a** and **19b**) led to a decrease potency with a little chirality effect. 2-Cl (compound **19c**), 2-OCH_3 (compound **19d**) and 2-CH_3 (compound **19e**) substitution on the phenyl ring led to a decreased efficiency. Alternatively, extra fluorine in either the *para*- or the *orthro*-position (compounds **19f** and **19g**) showed no improvement on Nav1.7 inhibition. Whereas substituents such as 1,2-methylenedioxy (compound **19h**) showed the best potency with IC_{50} = 2.9 μmol/L, which is better than a bulkier substituted group such as 1,2-ethylenedioxy (compound **19i**).

Table 2
In vitro metabolic half-life and predicted intrinsic clearance (CL_{int}) of compound **19h** in human and mouse liver microsomes.

Compound	Liver microsome	$T_{1/2}$ (min)	CL_{int} (μL min^{-1} mg^{-1})
19h	Human	177.2	9.8
	Mouse	36.9	147.7
Ralfinamide	Human	263.1	6.6
	Mouse	57.4	95.0

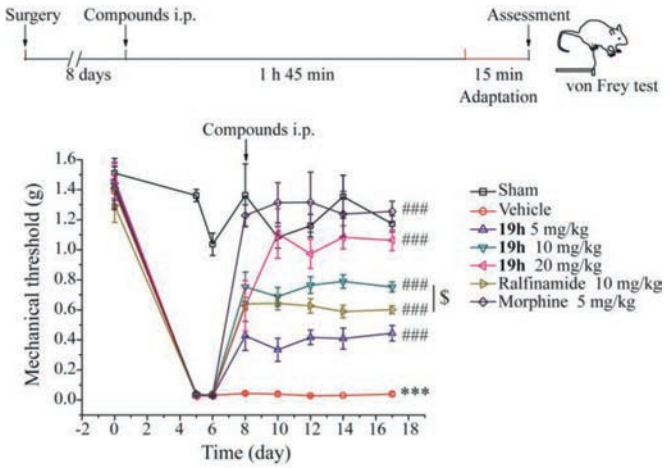

Fig. 3. The dose-dependent attenuation of nociception by compound **19h** in SNI-induced mechanical allodynia in mice. Top panel, a schematic time-course of paw mechanical withdrawal threshold (PMWT) measured by von Frey hair stimulation in mouse model of SNI. Bottom panel, a dose-dependent antinociception by **19h** (5, 10 and 20 mg/kg, i.p.), morphine (5 mg/kg) and ralfinamide (10 mg/kg) in SNI model. Two-way ANOVA followed by Bonferroni *post hoc* tests revealed a significant decrease of PMWT between group sham and group vehicle, $^{***}P < 0.001$; a significant increase of PMWT in **19h** (5, 10 and 20 mg/kg, i.p.) injected groups, compared with group vehicle, $^{###}P < 0.001$; and also a significant difference between group **19h** (10 mg/kg) and group ralfinamide (10 mg/kg), $^{\$}P < 0.05$. Data were expressed as the mean ± SEM and $^{***}P < 0.001$ (*vs.* Sham), n = 5–9.

It is important to study the metabolic stability of compound, because the most of the drugs are metabolized by cytochrome P450 (CYP450) in the liver [20-23]. The metabolic stability of the compound **19h** was tested in human and mouse liver microsomes (Table 2 and Supporting information), and **19h** appeared less stable than ralfinamide likely due to its higher clearance.

The spared nerve injury (SNI) model of neuropathic pain is characterized with a significant and continuous increase in mechanical sensitivity of mice [24]. Detailed procedures can be found in supporting information. The withdrawal threshold of the ipsilateral paw after surgery (0.03 ± 0.002 g) was significantly lower than the sham operation group (1.03 ± 0.07 g) on the sixth day after operation, indicating a successful establishment of mouse SNI model (Fig. 3). After intraperitoneal administrations of compound **19h** (5, 10 and 20 mg/kg) for 10 days, the average paw withdrawal threshold was dose-dependently increased to 0.44 ± 0.05 g, 0.75 ± 0.03 g and 1.06 ± 0.07 g, respectively, as compared with the value of 0.04 ± 0.001 g for vehicle control or 0.60 ± 0.03 g for ralfinamide at 10 mg/kg. In addition, the paw withdrawal threshold for the group with compound **19h** at 20 mg/kg (1.06 ± 0.07 g) was comparable to the sham operation group (1.17 ± 0.05 g) and morphine (1.25 ± 0.07 g) at 5 mg/kg.

Locomotor activity is commonly used for evaluation of psychostimulative or sedative effects. We next tested the effect of com-

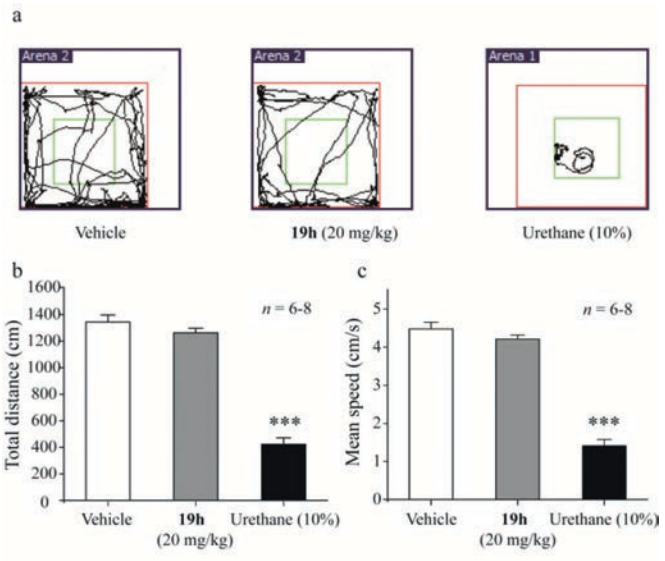

Fig. 4. Compound **19h** lack of altering locomotion. (a) Representative traces of mouse travels in the open field test after injections of saline, compound **19h** (20 mg/kg, i.p.) and urethane (10%, i.p.). (b) The total distance traveled in the open field for 5 min after injections of saline, compound **19h** (20 mg/kg, i.p.) and urethane (10%, i.p.). (c) The mean travel speed in open field for 5 min after injection of Saline, compound **19h** (20 mg/kg, i.p.) and urethane (10%, i.p.). One-way ANOVA followed by Tukey test, $n = 6–8$. All data were expressed as the mean ± SEM.

pound **19h** on locomotion by assessing the total travel distance and average speed in the open field test (Supporting information). As shown in Fig. 4, mice treated with 20 mg/kg compound **19h** (i.p.) had no significant differences in the total travel distance and the average speed, as compared with the vehicle control group. This result suggests that the compound **19h** induced-antinociception was less likely resulted from the sedation effect.

With ralfinamide as a starting point, we synthesized a variety of α-aminoamides derivatives that were evaluated against Nav1.7 channel using whole-cell patch clamp recordings. Among the tested compounds, the compound **19h** was the most potent with about 12-fold more potency than ralfinamide on inhibition of Nav1.7 channel. Our study provides valuable insights into the structure–activity relationship of ralfinamide analogues towards Nav1.7 channel. It gives a strategy to improve the Nav1.7 inhibition of ralfinamide analogues. The compound **19h** was more efficacious in antinociception in the mouse model of neuropathic pain induced by spared nerve injury compared with ralfinamide, although **19h** shows a slightly shorter half-life and faster clearance liver microsomes. The open field test result suggests that the compound **19h** had no alteration on locomotion in mice.

Declaration of competing interest

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2021.08.026.

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