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Identification and Electrophysiological Evaluation of 2-Methylbenzamide Derivatives as $Na_v1.1$ Modulators

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2-Methylbenzamide derivatives as $Na_v1.1$ modulators

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ABSTRACT. Voltage-gated sodium channels (Na_v) are crucial to the initiation and propagation of action potentials (APs) in electrically excitable cells and during the past decades they have received considerable attention due to their therapeutic potential. Here, we report for the first time the synthesis and the electrophysiological evaluation of 16 ligands based on a 2-methylbenzamide scaffold that have been identified as $Na_v1.1$ modulators. Among these compounds, *N*,*N*'-(1,3-phenylene)bis(2-

methylbenzamide) (**3a**) has been selected and evaluated in ex-vivo experiments in order to estimate the activation impact of such a compound profile. It appears that **3a** increases the $Na_v1.1$ channel activity although its overall impact remains moderate. Altogether, our preliminary results provide new insights into the development of small molecules activators targeting specifically $Na_v1.1$ channels to design potential drugs for treating CNS diseases.

KEYWORDS: 2-methylbenzamide derivatives, $Na_v1.1$ modulator, voltage-gated sodium channels, brain slices, fast-spiking interneurons

Voltage-gated sodium channels[1] play a pivotal role in initiating and propagating action potentials (APs) in neurons, myocytes and endocrine cells.[2] Thus, Na^+ channels have been the focus of a vast body of research including drug discovery activities.[3] Indeed, various sodium channel inhibitors have successfully been developed as human drugs and are currently approved for treating a variety of indications such as pain, epilepsy, migraine, bipolar disorders and cardiac disrhythmia.[4] By contrast, sodium channel activators are also known but have not previously been considered relevant in therapeutic settings mostly due to a high perceived risk of toxicity and convulsions. It has been clearly established that sodium channels play an important role in neuronal excitability by initiation and propagation of APs.[5] Interestingly, $Na_v1.1$ comprises the majority of the sodium current in GABAergic fast-spiking inhibitory interneurons that are immunoreactive for parvalbumin. Furthermore, it plays only a modest role in excitatory neurons due to the high redundancy of other types of sodium channels in these cells. Moreover, mouse models of reduced $Na_v1.1$ channel expression have revealed several phenotypes such as autistic-like behaviors, cognitive deficits, severe epilepsy and unexpected death. Accordingly, it has been well recognized that an important number of diseases, especially Dravet syndrome, are closely related with loss of function mutations in $Na_v1.1$ channels.[6] Jensen and co-workers recently published that selective activators of the $Na_v1.1$ sodium channel may hold therapeutic potential within diseases such as epilepsy, schizophrenia and Alzheimer's disease.[7] Different classes of nonselective sodium channel activators are well known including the pyrethroid insecticides

exemplified by allethrin and deltamethrin, the steroid-like alkaloid-based toxins veratridine and batrachotoxin, as well as a number of peptide-toxins isolated from diverse organisms such as spiders, scorpions and sea anemones.[8] From a drug discovery perspective non-selective Na^+ channel activators are considered non-viable due to general overall neuronal activation introducing a state of hyperexcitability and thereby a number of epileptogenic related adverse effects. Some degree of selectivity is therefore a likely prerequisite for bringing a Na^+ channel activator to the market. So far, no claims or descriptions have been made for specific $Na_v1.1$ activators.

In order to identify such activators, a high-throughput screening (HTS) of a small-molecule library (~ 55000 compounds) has been performed at a single concentration (30 μM) using a voltage protocol and veratridine and mexiletine as assay controls. Several derivatives having a 1,3-diamidobenzene core[9] and even more precisely containing a 2-methylbenzamide moiety were identified and confirmed as hits. Herein, we wish to present the design of 16 ligands bearing a 2-methylbenzamide unit in order to study the structure-activity relationship (SAR) of such a class compound as well as their electrophysiological evaluation on a HEK cell line with stable expression of $Na_v1.1$ channels. Furthermore, derivative **3a** was selected for brain slice experiments in order to understand the overall impact of the compound profile.

Chemistry

The synthetic pathway to obtain 2-methylbenzamide derivatives **3a**–**j** is outlined in Scheme 1. Benzene-1,3-diamine **1** reacted with one equivalent of 2-methylbenzoyl chloride in the presence of triethylamine (TEA) to furnish phenylamine **2** and bisbenzamide **3a** in 45% and 35% yields, respectively. Treatment of **2** with the appropriate acyl chlorides in a mixture of DCE–DCM provided the corresponding bis-benzamide derivatives **3b**–**i** in 83–99% yields.[10] Similarly, sulfonamide analogue **3j** was obtained from the reaction of **2** with 2-methylbenzenesulfonyl chloride in 89% yield.

The reaction sequence depicted in Scheme 2 was used as a convenient route to the compounds **3k**–**n**. After treatment of aminophenol **4a** with 2-methylbenzoyl chloride in the presence of TEA and a catalytic amount of DMAP ester **3k** was isolated in 89% yield. Following a similar protocol as above,

aminopyridine **4b** and benzene-1,4-diamine **7** led to benzamide derivatives **3l** and **3n** in 66% and 94% yields, respectively. Finally, the diamide derivative **3m** was synthesized in a two-step protocol: aminobenzoic **5** was first acylated providing carboxylic acid **6** in 82% yield. Subsequently, amidation with *o*-toluidine, HATU as coupling reagent and TEA in dry DMF gave **3m** in 58% yield. In addition to the series of the 14 ligands detailed above, amide derivative **3o** was prepared in two steps in which one of the usual 2-methylbenzamide moieties was replaced by a tetrazole amide-bioisostere. Thus, tetrazole **8** furnished after C–H activation aniline **9** in 16% yield which was subsequently acylated to lead to tetrazole **3o** in 87% yield. Moreover, constrained analogue **3p** was prepared in a three-steps procedure in order to test how the introduction of conformational restraint to a moderately flexible lead molecule would influence the activating properties with regards to the $Na_v1.1$ channel. Thus, nitroindoline **10** was first acylated furnishing amide **11** which the nitro group was reduced with a slight excess of $Na_2S_2O_4$ in the presence of K_2CO_3. Subsequent acylation of the resulting intermediate with 2-methylbenzoyl chloride afforded indoline **3p** in 21% yield over three steps (Scheme 2).

Results and discussion

To obtain a thorough biological understanding and re-confirmation of hits identified during the primary screen various electrophysiological approaches were performed. Since the primary screen was performed in an automated setting (Ion works/Barracuda) that does not allow for electrophysiological experiments in a quality that can match manual patch clamp with giga seals, the first attempt was to confirm the hits under conditions where a true giga seal can be obtained between the cell and the recording pipette.[11] This condition allows for a very precise determination, or clamp, of the cell membrane that can subsequently be controlled. Especially for ion channels that conduct large amount of currents and in addition show fast activation and inactivation kinetics, like voltage-gated Na^+ channels, reliable continuous control of the membrane potential is a prerequisite to fully characterize compound interactions with the channel. As an alternative to manual patch clamp experiments the automated Q-patch was applied where it is also possible to obtain giga ohm seals comparable to manual patch

clamp.[12] $Na_v1.1$ channels - stable expressed in HEK cells - were activated by depolarizing voltage steps from – 120 mV to + 70 mV in 10 mV increments to obtain current–voltage (I–V) curves showing the expected voltage dependent activation revealed as an inward current deflection followed by fast inactivation as demonstrated in Figure 1. For more detailed compound evaluation a more simple protocol was applied, i.e. with only 6 voltage steps from – 40 mV to + 10 mV (with 10 mV increments). Each depolarizing step was activated from – 120 mV and lasted for 20 ms and was separated with 500 ms. A holding potential (V_{hold}) of – 80 mV was used between each protocol with 6 voltage steps (see Figure 2). A modulator of the $Na_v1.1$ channels can exert its action in a number of ways and the most relevant mode of action and associated electrophysiological profile for obtaining biological activity is at present unknown. We chose to focus on 1) impact on peak current, 2) total area under the curve (AUC) for the time duration of the depolarizing pulse and 3) the compound´s ability to decrease inactivation velocity. The later parameter is addressed by determining the tau value of the inactivation current decay. In this setting a slowing of the inactivation, and thereby more conducted current, is revealed by an increase in the tau value. As summarized in Table 1, compounds **3a**, **3c**–**j**, **3l**, **3n** and **3p** from this 2-methylbenzamide family had a profile with a reduced peak current, but a compromised inactivation revealed as an increased tau value. As an example, peak current, AUC and tau values (in %) relative to control at – 10 mV for benzamide **3a** were 52, 81 and 124, respectively (see Figure 3). Unfortunately, no clear picture appeared with respect to the substitution pattern on the aromatic ring. However, ortho- and meta-substituted rings **3a** and **3c**–**f**, unsubstituted ring compound **3g**, derivatives bearing aliphatic moieties **3h**–**i**, sulfonamide **3j**, pyridine derivative **3l**, 1,4-substituted benzamide **3n** and constrained indoline **3p** did provide novel active analogues displaying a noteworthy trend for this class of compounds: the total AUC was significantly reduced but much less than the peak current while the tau value was considerably increased. Tetrazole **3o**, ester **3k** and amide **3m** were nearly inactive with no noticeably effects on the three parameters of our designed electrophysiological protocol (see Table 1 for more details).

Voltage-gated sodium channels share high similarities of sequence, large expression in neurons (except $Na_v1.4$ which is expressed mainly in skeletal muscle and $Na_v1.5$ which is found primarily in cardiac muscle) and biophysical characteristics[13] In order to quantify the selectivity profile of the compounds the same electrophysiological protocol described above was applied using benzamide **3a** on $Na_v1.2$, -1.4, -1.5, -1.6 and -1.7 channels - stable expressed in HEK cells. As depicted in Table 2, peak values were significantly less reduced and tau values were significantly lower while the AUC values remained almost constant when compared with the values obtained for $Na_v1.1$ channel. In addition, intrinsic hepatic clearances (Cl_{int}) of 8 synthesized compounds were also estimated for both rat and human after incubation of the corresponding compound in rat or human liver microsomes; apart from derivative **3p**, $Cl_{int,rat}$ and $Cl_{int,human}$ were relatively high with values between 3 and 38 L/kg/h (rat) and between 0.9 and 6.3 L/kg/h (human), respectively. Moreover, PgP efflux and permeability was investigated for a selection of compounds in the MDR1-MDCK cell assay. MDCK ratio[14] of derivatives **3a**, **3c**, **3d** and **3l** were 0.45, 0.52, 0.31 and 0.64, respectively showing that this class of compound has a small or modest efflux ratio (see Supporting Information for more details).

The biological nature of a compound profile as described above is difficult to predict. To gain more insight into whether an overall inhibitory, neutral or activation impact of these compounds was prevailing, ex-vivo experiments with brain slices were performed (see Supporting Information for more details). In brief, whole cell patch clamp were performed on parvalbumin containing interneurons with basket cell appearance in the hippocampal pyramidal cell layer. As illustrated in Figure 4A, cells were initially challenged by ramps starting with resting membrane potentials from – 70 mV that were gradually depolarized. The experiments were performed in the absence (control) and presence of $Na_v1.1$ modulator **3a** and as summarized data in Figure 4B application of **3a** shifted the threshold for firing APs to a more negative value. The reason for this is believed to be the activation of $Na_v1.1$ channels that facilitate the neuron to generate APs. To obtain a more thorough evaluation of the $Na_v1.1$ modulators ability to modify interneuron firing frequency depolarizing steps with different amplitude were provided to the neuron to provoke APs (see Figure 4C). As observed, **3a** was able to increase the number of

evoked APs even though the effect was most prominent after weak depolarizations that render a larger window available for further activity increase. Data for application at 30 and 100 μM of benzamide **3a** are depicted in Figure 4D. For input-output and threshold experiments a number of time matched control were performed to make certain that the observed effects were not due to sliding baseline as a function of time (data not shown).

To enable correct identification of fast-spiking parvalbumin expressing interneurons some electrophysiological experiments were performed with biocytin in the pipette. After successful recordings biocytin was injected into the cell and the slice was fixed for later identification. Slices were then co-stained for biocytin and parvalbumin to confirm that the compound had actually exerted its effect at a parvalbumin expressing interneuron (see Supporting Information for more details).

Conclusions

In summary, we have discovered novel 2-methylbenzamide derivatives that act as $Na_v1.1$ modulators. Among them, 2-methylbenzamide derivative **3a** has been demonstrated in ex-vivo experiments to act as a functional activator of fast-spiking hippocampal interneurons. Even though its overall activation impact was moderate, addition of **3a** clearly resulted in an increase of activity of the $Na_v1.1$ channel. These preliminary yet promising results open up a novel and challenging area of investigations on pharmacological activators of $Na_v1.1$ channels since such compound may have important therapeutic potential in the treatment of various CNS diseases. Further studies concerning discovery of subtype selective small molecule activators of the $Na_v1.1$ channel are currently in progress in our laboratories.

Experimental

1.1 Chemistry - Material and methods

Starting materials and reagents were obtained from commercial suppliers and used without further purifications. Syringes which were used to transfer anhydrous solvents or reagents were purged with nitrogen prior to use. Yields refer to isolated compounds estimated to be > 95 % pure as determined by ^{1}H NMR and LC–MS. Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} plates from Merck (Germany). Visualisation was accomplished by UV lamp (254 nm). Flash column chromatography was performed on chromatography grade, silica 60 Å particle size 35–70 micron from Fisher Scientific using the solvent system as stated. Melting points were determined on a Büchi apparatus and are uncorrected. HRMS were performed on a Bruker MicroTOF instrument. ^{1}H NMR spectra were recorded on a Bruker 600-Avance-III spectrometer equipped with a 5 mm TCI cryoprobe operating at 600 MHz using $CDCl_3$ or DMSO-d_6 as solvents and TMS as internal standard. Coupling constants (*J* values) are given in Hertz (Hz). Multiplicities of ^{1}H NMR signals are reported as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; dt, doublet of triplet; tt, triplet of triplets; ddd, doublet of doublet of doublets; m, multiplet; br, broad signal. LC–MS data were obtained on a PE Sciex API150EX instrument equipped with an ion-spray source and Shimadzu LC–8A/SLC–10A LC system: column, 30 mm × 4.6 mm Waters Symmmetry C18 column with 3.5 μm particle size; solvent system, A = water–TFA (100:0.05) and B = water–acetonitrile–TFA (5:95:0.03) (TFA = trifluoroacetic acid); method, linear gradient elution with 90% A to 100% B in 2.4 min and then 10% B in 0.4 min, with a flow rate of 3.3 mL/min. Total time including equilibration was 2.8 min. Injection volume was 10 μL from a Gilson 215 autosampler.

1.2 Synthesis and analytical data of representative compounds

3-Methoxy-*N*-(3-(2-methylbenzamido)phenyl)benzamide 3e.

To a solution of phenylamide **2** (143 mg, 0.63 mmol, 1 equiv) in a mixture DCE–DCM (5:1, 12 mL) at room temperature was added TEA (97 μL, 0.70 mmol, 1.1 equiv). The reaction mixture was cooled to 0

°C and 3-methoxybenzoyl chloride (93 μL, 0.66 mmol, 1.05 equiv) was added dropwise over 1 min. After few min the reaction mixture was allowed to warm up to room temperature and was stirred at this temperature for 2 h and concentrated in vacuo. The crude material was purified by column chromatography on silica gel using a gradient elution (EtOAc–heptane, 1:1 to 3:2) to afford diamide **3e** (222 mg, 97%) as a white solid; R_f = 0.54 (EtOAc–heptane, 1:1); mp 163.9–165.6 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.35 (br s, 1H), 10.16 (br s, 1H), 8.27–8.34 (m, 1H), 7.50–7.57 (m, 3H), 7.43–7.47 (m, 2H), 7.37–7.42 (m, 2H), 7.28–7.33 (m, 3H), 7.16 (ddd, J = 8.2 and 2.6 and 0.9, 1H), 3.84 (s, 3H), 2.39 (s, 3H); LC–MS (m/z) 361.3 (MH^+); RT = 0.70; purity (UV, ELSD): 100%; 100%; HRMS $C_{26}H_{32}N_3O_3$ [$M+H_2NEt_2^+$] calc 434.2438, found 434.2440.

***N*,*N*'-(Pyridine-2,6-diyl)bis(2-methylbenzamide) 3l.**

To a solution of aminopyridine **4b** (400 mg, 3.67 mmol, 1 equiv) in a mixture DCE–DCM (5:1, 48 mL) at room temperature was added TEA (1.07 mL, 7.70 mmol, 2.1 equiv). The reaction mixture was cooled to 0 °C and 2-methylbenzoyl chloride (0.98 mL, 7.51 mmol, 2.05 equiv) was added dropwise over 3 min. After few min the reaction mixture was allowed to warm up to room temperature and was stirred at this temperature for 22 h and concentrated in vacuo. The crude material was purified by column chromatography on silica gel using a gradient elution (EtOAc–heptane, 3:5 to 3:4) to afford the desired diamide **3l** (840 mg, 66%) as a colourless oil which slowly crystallized as a white solid when stored in the fridge overnight; R_f = 0.44 (EtOAc–heptane, 3:5); ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.51 (s, 2H), 7.79–7.93 (m, 3H), 7.41–7.48 (m, 2H), 7.38 (dt, J = 7.5 and 1.3, 2H), 7.23–7.32 (m, 4H), 2.39 (s, 6H); LC–MS (m/z) 346.1 (MH^+); RT = 0.73; purity (UV, ELSD): 96%; 100%; HRMS $C_{25}H_{31}N_4O_2$ [$M+H_2NEt_2^+$] calc 419.2442, found 419.2453.

***N*,*N*'-(1,4-phenylene)bis(2-methylbenzamide) 3n.**

To a solution of benzene-1,4-diamine **7** (450 mg, 4.16 mmol, 1 equiv) in a mixture DCE–DCM (8:3, 22 mL) at room temperature was added TEA (1.27 mL, 9.15 mmol, 2.2 equiv). The reaction mixture was cooled to 0 °C and 2-methylbenzoyl chloride (1.14 mL, 8.74 mmol, 2.1 equiv) was added dropwise over 2 min. After few min the reaction mixture was allowed to warm up to room temperature and was stirred

at this temperature for 1.5 h then at 50 °C for 1 h. The resulting mixture was cooled to room temperature and filtered. The resulting precipitate was washed successively with DCM, DCE and water then freeze-dried overnight to afford the desired diamide **3n** (1.35 g, 94%) as a white solid; R_f = 0.52 (EtOAc–heptane, 1:1); ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.28 (s, 2H), 7.70 (s, 4H), 7.46 (d, J = 7.4, 2H), 7.39 (dt, J = 7.9 and 1.2, 2H), 7.26–7.33 (m, 4H), 2.38 (s, 6H); LC–MS (m/z) 345.3 (MH^+); RT = 0.69; purity (UV, ELSD): 95%; 93%; HRMS $C_{26}H_{32}N_3O_2$ [$M+H_2NEt_2^+$] calc 418.2489, found 418.2485.

1.3 Electrophysiology - Methods

A HEK cell line with stable expression of $Na_v1.1$ channels was used for in-vitro electrophysiological recordings in patch clamp mode. I–V curves for both activation and inactivation was performed in order to confirm the expected characteristic of $Na_v1.1$ channels, both with manual patch clamp and with automated patch clamp (Ion Works and Q-Patch). The first HTS screening of a small library was performed on the Ion Works platform, but the following evaluation of new compounds from medicinal chemistry efforts was performed with a Q-patch16x in single hole mode. It is possibly to obtain giga ohm seals with the Q-patch, which make higher quality recordings more comparably to manual patch. For electrophysiological recordings HEK cells were harvested with detachin (5–10 min) and applied to the Q-patch with a cell density between 1 and 3 million cells per mL. The cell suspension could be used up to 4-5 hours after harvesting with good performance. For evaluation of the effects of compound on the peak current, AUC and tau values a protocol with 6 voltage steps were constructed. The voltage steps were depolarizing steps from – 40 mV up to + 10 mV (with 10 mV increments). Each depolarizing step was activated from –120 mV and lasted for 20 ms and was separated with 500 ms. A V_{hold} of – 80 mV was used between each protocol with 6 voltage steps (Figure 2). The single protocol with 6 voltage steps was run approximately every min. For test of compounds in a single concentration the voltage protocol was run 6 times in control (~ 6 min) followed by drug application and 6 voltage run in drug (~ 6 min) and finally wash out and 6 voltage run again (~ 6 min). Effects of compounds were measured

relative to control values before drug application for each cell. For more details concerning all the cell lines, see Supporting Information.

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LIST OF ABBREVIATIONS

The following abbreviations are used: DCM: dichloromethane; DCE: 1,2-dichloroethane; EtOAc: ethyl acetate; TEA: triethylamine; THF: tetrahydrofuran; DMAP: 4-dimethylaminopyridine; HATU: 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate; DMF: *N*,*N*-dimethylformamide; NMP: *N*-methyl-2-pyrrolidone; AUC: area under the curve; MDCK: Madin-Darby Canine Kidney; CNS: central nervous system; AP: action potential; current–voltage: I–V; V_{hold}: holding potential; Cl_{int}: intrinsic hepatic clearance.

SUPPORTING INFORMATION

Full experimental details for all synthesized compounds, ^{1}H and ^{13}C NMR spectra of selected compounds, brain slice experiments and co-staining protocols as well as table containing intrinsic clearance and MDCK ratio. This material is available free of charge via the Internet at http://pubs.acs.org.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interest.

AUTHORS CONTRIBUTIONS

H.S.J., K.D., J.F.B., M.G. and N.S. conceived and designed the project. F.C. carried out the organic synthesis and analyzed all the compounds. K.F. carried out and analyzed the in-vitro experiments. P.H.L. carried out and analyzed the co-staining experiments. D.L., H.L. and C.Y. carried out and analyzed the ex-vivo experiments. F.C., K.F., M.G. and N.S. wrote the manuscript with the help of D.L., H.L. and C.Y.

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(11) A seal between cell and recording pipette with very high resistance (1 giga ohm or more) is called a giga seal.

(12) This has been evaluated with Na^+ channels to perform at a quality level comparable to manual recordings.

(13) Goldin, A. L.; Barchi, R. L.; Cadwell, J. H.; Hofmann, F.; Howe, J. R.; Hunter, J. C.; Kallen, R. G.; Mandel, G.; Meisler, M. H.; Netter, Y. B.; Noda, M.; Tamkun, M. M.; Waxman, S. G.; Wood, J. N.; Catterall, W. A. (2000) Nomenclature of Voltage-gated Sodium Channels. *Neuron 28*, 365–368.

(14) Membrane permeability of selected derivatives was examined and obtained in an MDCK cell system expressing human MDR1 [ABCB1, P-glycoprotein (P-gp)] as previously described.[15]

(15) Blom, S. M.; Rottländer, M.; Kehler, J.; Bundgaard, C.; Schmidt, N.; Jensen, H. S. (2014) From Pan-Reactive K_v7 Channel Opener to Subtype Selective Opener/Inhibitor by Addition of Methyl Group. *PLoS One 9*, e100209.

FIGURE CAPTIONS

Figure 1. $Na_v1.1$ channels stable expressed in HEK cells were activated by stepping the membrane potential from – 120 mV to + 70 mV in 10 mV steps from a holding potential of -100 mV. (A) Raw data traces. (B) I–V curve for activation. Traces measured on the Q-patch in single hole mode.

Figure 2. Screening protocol. $Na_v1.1$ channels stable expressed in HEK cells were activated by stepping the membrane potential from – 120 mV in steps with 10 mV increments from – 40 mV to + 10 mV. Screening of test compounds were performed by evaluation of the effect induced by test compounds at – 10 mV on the peak, AUC and tau values. V_{hold} between the step protocol was – 80 mV. Traces were measured on the Q-patch in single hole mode.

Figure 3. Effect of compound **3a** at 30 μM on the current trace activated to – 10 mV. **3a** showed inhibition of the peak current and the AUC, but **3a** also induced a decrease in the inactivation (tau value) (see also Table 1). Control (gray trace) and compound **3a** (black trace) are showed in Figure 3A. For comparison control trace and time matched DMSO trace is shown in Figure 3B. DMSO did not induce any effect either on peak, AUC or tau (see also Table 1).

Figure 4. Compound **3a** increases neuronal excitability in a fast-spiking interneuron recorded in the stratum pyramidal in hippocampal CA1 field. (A) Top: A current ramp evoked repetitive spike firing in the baseline pre-drug period. Bottom: following perfusion of **3a** (100 μM) for 10 mins, the same current ramp now induced firing at a more negative membrane potential. V_{hold} = – 70 mV. (B) Group data from 5 interneurons tested showed that **3a** was able at 100 μM (but not at 30 μM) to elicit firing at a more negative membrane potential suggesting that **3a** lower the threshold for firing. (C) Left top and bottom: raw voltage traces recorded from an interneuron showed the evoked firing by a *weak* (200 pA) rectangular pulse before and after perfusion of **3a**. It was able to increase the excitability of the interneuron. Right top and bottom: baseline firing evoked by a *stronger* depolarizing pulse was also enhanced by **3a**. (*Weak* depolarization is defined as the depolarizing pulses used that evoked 5~10

spikes and *strong* depolarization is defined as the depolarizing pulses used that evoked 13~21 spikes). (D) Time course of neuronal excitability change in 5 interneurons following **3a** perfusion. Note that **3a** (at 30 and 100 μM) can induce more firing of interneurons evoked by a weak current pulse than from a stronger current pulse. See Supporting Information for more details.

SCHEME TITLES

Scheme 1. Synthesis of phenylamide **2**, diamides **3a**–**i** and sulfonamide **3j**. Reagents and conditions: (i) 2-MePhCOCl (1 equiv), TEA (1.05 equiv), DCE, 0 °C to rt, **2**: 45%, **3a**: 35%; (ii) RCOCl (1.05 equiv), TEA (1.1 equiv), DCE–DCM (5:1), 0 °C to rt, **3b**: 83%, **3c**: 91%, **3d**: 96%, **3e**: 97%, **3f**: 96%, **3g**: 99%, **3h**: 99%, **3i**: 97%; (iii) 2-MePhSO_2Cl (1.05 equiv), TEA (1.1 equiv), dry THF, 0 °C to rt, 89%.

Scheme 2. Synthesis of ester **3k**, diamides **3l**–**n**, tetrazole **3o** and indoline **3p**. Reagents and conditions: (i) 2-MePhCOCl (2.05 equiv), TEA (2.1 equiv), DMAP (0.04 equiv), DCE–DCM (5:1), 0 °C to rt, **3k**: 89%; (ii) 2-MePhCOCl (2.05 equiv), TEA (2.1 equiv), DCE–DCM (5:1), 0 °C to rt, **3l**: 66%; (iii) 2-MePhCOCl (1.05 equiv), TEA (1.1 equiv), $CHCl_3$, rt, **6**: 82%; **3o**: 87%; (iv) 2-Me$PhNH_2$ (1.5 equiv), TEA (1.05 equiv), HATU (1 equiv), dry DMF, rt, 58%; (v) 2-MePhCOCl (2.1 equiv), TEA (2.2 equiv), DCE–DCM (8:3), 0 °C to rt then to 50 °C, 94%; (vi) 2-MePhI (1.1 equiv), Cs_2CO_3 (1.1 equiv), CuI (1 equiv), $Pd(OAc)_2$ (0.05 equiv), (*o*-furyl) $_3$P (0.1 equiv), dry CH_3CN, 65 °C then 90 °C, 16%; (vii) 2-MePhCOCl (1.05 equiv), TEA (1.1 equiv), dry THF, 0 °C to rt, 91%; (viii) a) K_2CO_3 (3 equiv), $Na_2S_2O_4$ (6 equiv), NMP–EtOH (5:7), 0 °C to 100 °C; b) 2-MePhCOCl (1.05 equiv), TEA (1.1 equiv), $CHCl_3$, 0 °C to rt, 23% over two steps.

TABLES TITLES

Table 1. Peak current, AUC and tau values for compounds **3a**–**p** tested at 30 μM on a HEK cell line with stable expression of $Na_v1.1$ channels.

Table 2. Peak current, AUC and tau values for compound **3a** tested at 30 μM on HEK cell lines with stable expression of $Na_v1.1$, -1.2, -1.4, -1.5, -1.6 and -1.7 channels.

Table 1

Compounds	n[b]	Response in % (± SEM) relative to control at – 10 mV[a] Peak (%)	AUC (%)	Tau (%)
DMSO[c]	13	101 ± 1	102 ± 2	102 ± 2
3a	10	52 ± 2	81 ± 9	124 ± 6
3b	5	89 ± 1	100 ± 5	101 ± 4
3c	8	70 ± 5	83 ± 3	115 ± 3
3d	11	47 ± 3	81 ± 5	143 ± 7
3e	3	54 ± 3	86 ± 2	127 ± 8
3f	9	39 ± 2	51 ± 2	136 ± 14
3g	12	56 ± 3	83 ± 6	128 ± 3
3h	3	51 ± 1	74 ± 13	128 ± 4
3i	7	75 ± 2	93 ± 4	110 ± 6
3j	3	57 ± 2	70 ± 11	123 ± 6
3k	4	86 ± 2	92 ± 4	104 ± 6
3l	4	50 ± 12	80 ± 13	140 ± 11
3m	5	93 ± 1	96 ± 8	103 ± 10
3n	10	105 ± 1	112 ± 3	110 ± 2
3o	3	88 ± 2	86 ± 3	95 ± 2
3p	5	96 ± 2	112 ± 10	116 ± 9

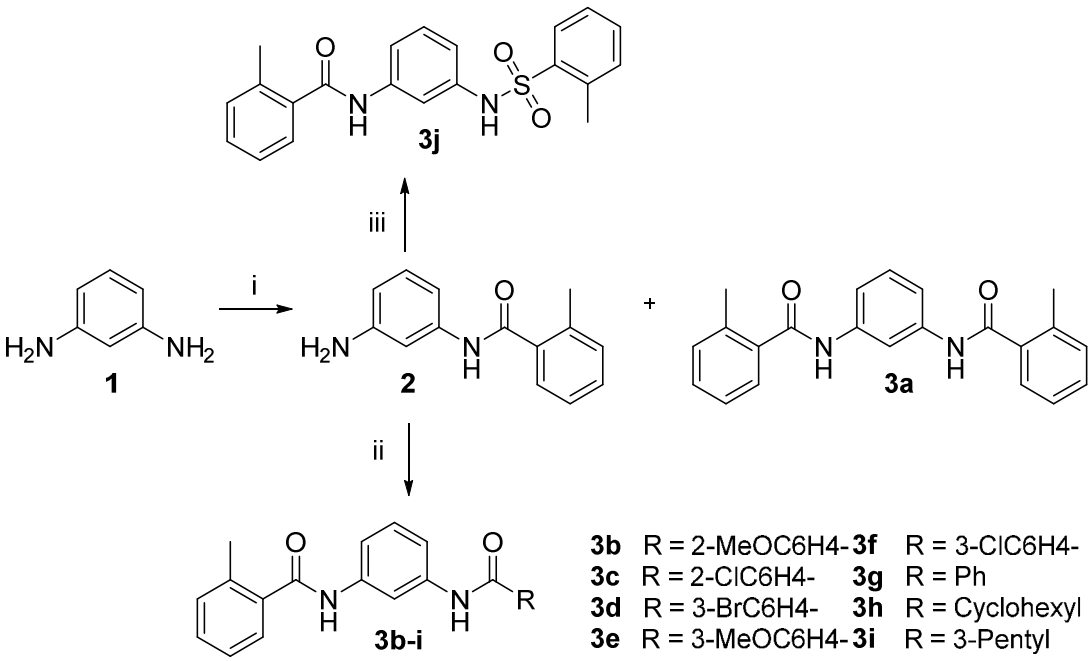
[a]: Performed at 30 μM; [b]: Number of individual experiments; [c]: DMSO control run on the same plates as the tested compounds.

Table 2

	n[b]	Response in % (± SEM) relative to control at – 10 mV[a]		
		Peak (%)	AUC (%)	Tau (%)
$Na_v1.1$	10	52 ± 2	81 ± 9	124 ± 6
$Na_v1.2$	4	71 ± 2	79 ± 2	97 ± 2
$Na_v1.4$	5	94 ± 5	79 ± 3	85 ± 5
$Na_v1.5$	4	82 ± 4	84 ± 6	102 ± 5
$Na_v1.6$	4	101± 6	98 ± 5	96 ± 1
$Na_v1.7$	4	93 ± 6	75 ± 2	84 ± 2

[a]: Performed at 30 μM; [b]: Number of individual experiments.

Scheme 1


3j
iii
i
1
2
3a
ii
3b-i
3b R = 2-MeOC6H4- 3f R = 3-ClC6H4-
3c R = 2-ClC6H4- 3g R = Ph
3d R = 3-BrC6H4- 3h R = Cyclohexyl
3e R = 3-MeOC6H4- 3i R = 3-Pentyl

Scheme 2

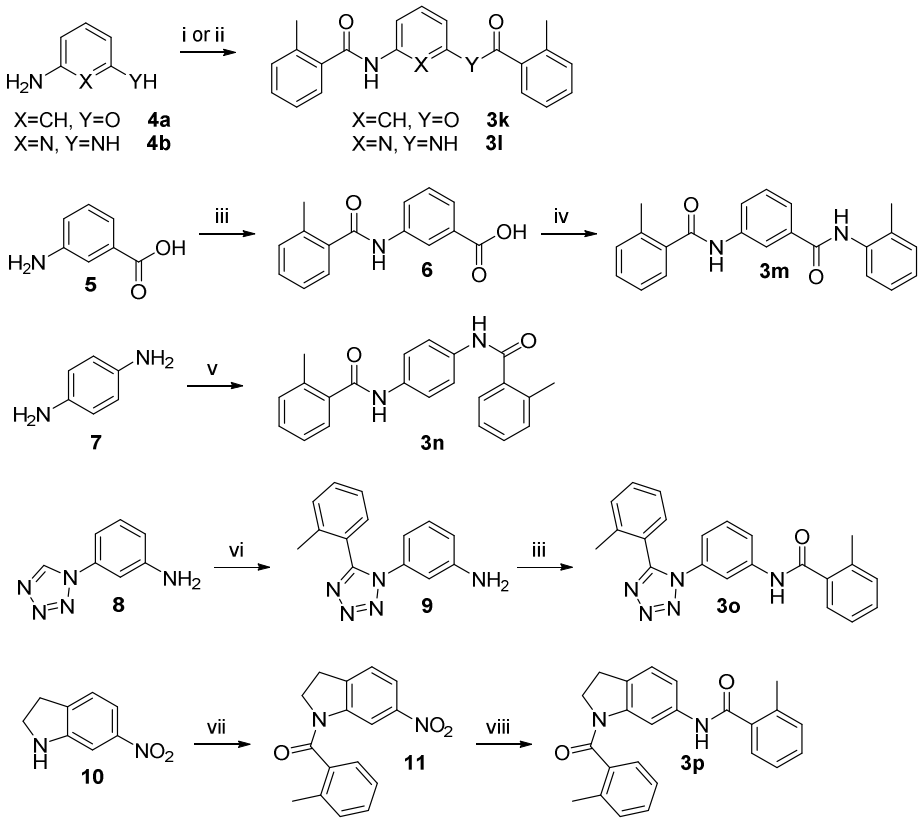

i or ii
X=CH, Y=O 4a
X=N, Y=NH 4b
X=CH, Y=O 3k
X=N, Y=NH 3l
iii
iv
5
6
3m
v
7
3n
vi
iii
8
9
3o
vii
viii
10
11
3p

Figure 1

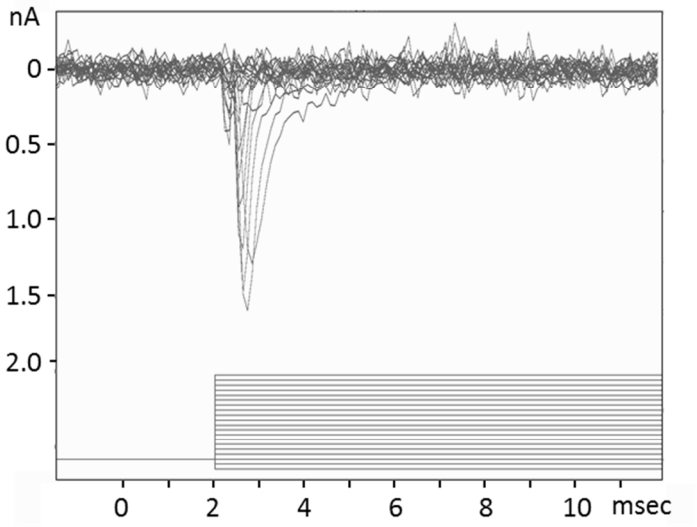


Figure 2

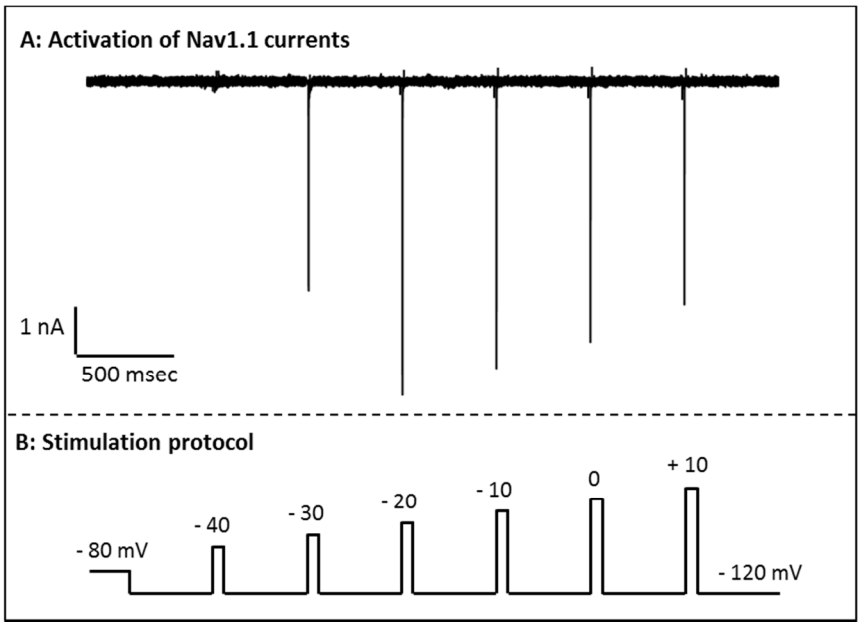

A: Activation of Nav1.1 currents
1 nA
500 msec
B: Stimulation protocol
- 80 mV
- 40
- 30
- 20
- 10
0
+ 10
- 120 mV

Figure 3

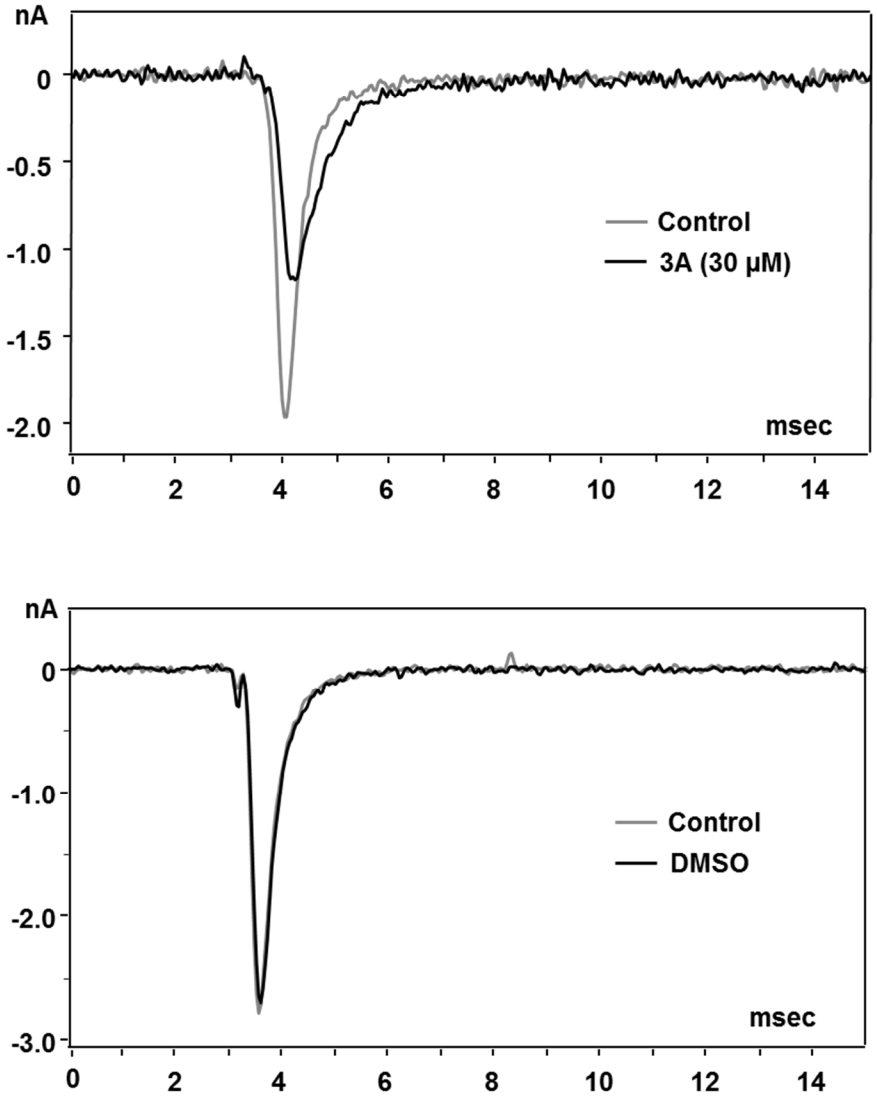
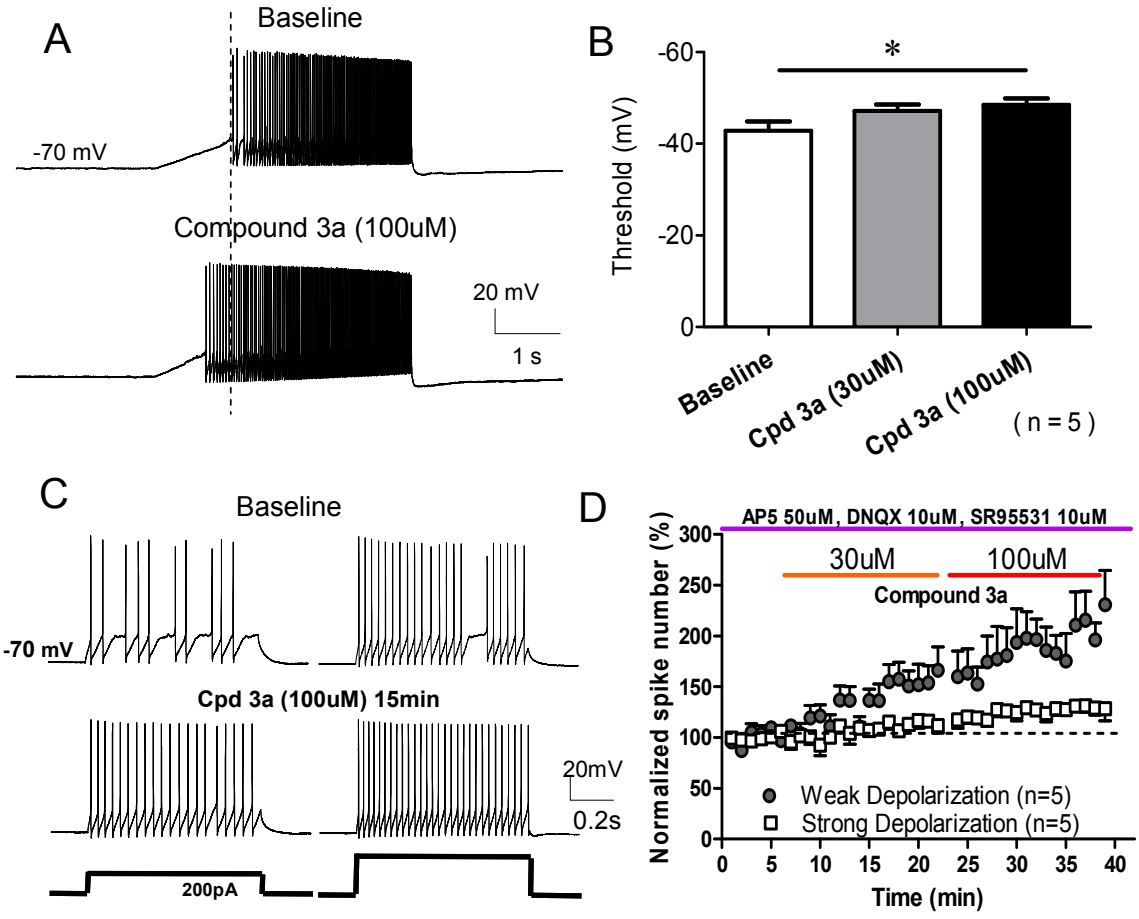

nA
0
-0.5
-1.0
-1.5
-2.0
Control
3A (30 μM)
msec
0
2
4
6
8
10
12
14
nA
0
-1.0
-2.0
-3.0
Control
DMSO
msec
0
2
4
6
8
10
12
14

Figure 4



Weak Depolarization (5~10 APs evoked in baseline)
Strong Depolarization(13~21 APs evoked in baseline)

Graphical abstract