Spring 4-14-2018

SK Channel Modulators as Drug Candidates and Pharmacological Tools

Razan Orfali
Chapman University, orfal101@mail.chapman.edu

SK Channel Modulators as Drug Candidates and Pharmacological Tools

A Thesis by

Razan Saud Orfali

Chapman University

Irvine, CA

School of Pharmacy

Submitted in partial fulfillment of the requirements for the degree of

Master in Pharmaceutical Sciences

April 2018

Committee in charge:

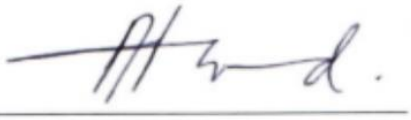
Miao Zhang, Ph.D., Chair

Aftab Ahmed, Ph.D.

Kamaljit Kaur, Ph.D.

The dissertation/thesis Of Razan Saud Orfali, is approved.

Miao Zhang, Ph.D., Chair



Aftab Ahmed, Ph.D.

Kamaljit Kaur, Ph.D.

April 2018

SK Channel Modulators as Drug Candidates

and Pharmacological Tools

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my mentor Dr. Miao Zhang who has taught me electrophysiology and invested so much time and energy supporting my advancement through graduate school. He has taught me more than I could ever give him credit for.

Similar, profound gratitude goes to Dr. Young Woo Nam for his scientific training, great support, and friendship. Both Dr. Zhang and Dr. Young have shown, by example, what great scientists (and people) should aspire to be.

I am grateful to all of those with whom I have had the pleasure to work with during my graduate education. I would also like to acknowledge and express my appreciation to my thesis committee members: Dr. Aftab Ahmed and Dr. Kamaljit Kaur, who have provided me extensive personal and professional guidance, as well as taught me a great deal about both scientific research and life.

This work would not have been possible without the financial support of the Saudi Arabian Cultural Mission Scholarship and Chapman University School of Pharmacy. I am especially indebted to Dr. Keykavous Parang who has been supportive of student's goals.

Finally, I would like to give special thanks to my parents for their constant guidance and support**.** Most importantly, I wish to thank my loving and supportive husband, Alla, and my wonderful princess, Mariam, who gave me the inspiration to continue and finish my graduate education.

ABSTRACT

SK Channel Modulators as Drug Candidates

and Pharmacological Tools

by Razan Saud Orfali

The small- and intermediate-conductance Ca^{2+}-activated K^+ (SK/IK) channels play a fundamental role in the regulation of neurons in the central nervous system. In animal models, SK/IK channel positive modulators have been shown to be effective in reducing the symptoms of neurological diseases such as ataxia. Ataxia is a lethal neurological rare disease characterized by lack of balance and incoordination of muscle movements, often as a result of cerebellar or spinocerebellar neurodegeneration. SK/IK channel modulators have been developed over the past few decades. Currently available modulators are often weak in potency. Lack of knowledge about the binding site for the compounds is the main reason hindering the development of more potent and effective therapeutics targeting SK channels. Dr. Zhang and his colleagues recently discovered the binding pocket for these positive modulators of SK/IK channels. This pocket is located at the interface between the channel and calmodulin. Dr. Zhang and his colleagues performed screening of a large number of compounds *in silico*, to find those fitting into the binding pocket. I performed electrophysiological recordings to evaluate the efficacy and the potency of these modulators on SK2 channels. We discovered a correlation between the total binding energy values calculated from the structures and the potencies determined from electrophysiological recording.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

SK channel: small conductance Ca^{2+} activated potassium channel

CaM: calmodulin

CaMBD: Calmodulin binding domain

AHP: afterhyperpolarization

PAM: Positive allosteric modulator

PC: Purkinje cells

DCN: deep cerebellar nuclei (DCN)

SCA: Spinocerebellar Ataxia

Eint: interaction Energy

CHAPTER 1

INTRODUCTION

1.1 Ataxia

Ataxia, a Greek word that means 'loss of order,' is used medically to describe a devastating neurological disorder characterized by lack of muscle control during voluntary movements, difficulties with gait, problems with clarity of speech, and intention tremor, often as a result of cerebellar or spinocerebellar neurodegeneration (Carlson et al., 2009). The cause of such neurodegeneration can be from brain tumor, multiple sclerosis, alcoholism or a congenital genetic defect (Carlson et al., 2009; Matilla-Dueñas et al., 2010; Orr, 2012). There are more than fifty different types of hereditary ataxias taking place during childhood or adulthood. Most of ataxias progress over a number of years, and the patients eventually lose the ability to swallow and breathe smoothly, which can be fatal. The most common type of ataxia is a cerebellar ataxia that is caused by a dysfunction of the cerebellum or in one of its pathways. Spinocerebellar ataxia (SCA) is an inherited form of ataxia in an autosomal-dominant pattern. SCA is caused by irregular function of the spinocerebellum, the portion of the cerebellar cortex that receives somatosensory signals from the spinal cord (Orr, 2012; Orr and Zoghbi, 2007). Currently, twenty-eight autosomal dominant SCAs have been described (Matilla-Dueñas et al., 2010). The autosomal dominant SCAs are typically progressive, late-onset, and often lethal neurodegenerative illnesses.

1.2 Treatment of Ataxia

There is no cure that can specifically treat ataxia or the symptoms of ataxias. However, understanding the disease can be helpful to treat, cure, or prevent it. The cerebellum coordinates motor movement and helps maintain balance and posture. It has three deep nuclei that provide the main output of the cerebellum. In neurodegenerative ataxias, neuronal death usually occurs as a result of prolonged period of neuronal dysfunction (Shakkottai et al., 2011). The cerebellar Purkinje cells (PCs) are affected in many types of ataxias (Carlson et al., 2009; Orr, 2012; Shakkottai et al., 2004). PCs are the only output source of cerebellar cortex, and they primarily promote inhibitory signals to the deep cerebellar nuclei (DCN). The dysfunction of pacemaking activity in PCs is one of the primary causes for the symptom of early stage ataxia (Kasumu et al., 2012b; Shakkottai et al., 2011). As this malfunction of PCs would be expected to cause DCN hyperexcitability. The modulation of the DCN firing rate by PC input is believed to be responsible for coordination of movement. Therefore, a direct relationship between increased DCN firing rate and ataxia can be concluded (Shakkottai et al., 2004). Disruptions of regular cerebellar PCs activities have been identified in studies with mouse models of Episodic Ataxia EA2 (Walter et al., 2006), Cerebellar Ataxia (Gao et al., 2012), Spinocerebellar Ataxia SCA3 (Shakkottai et al., 2011) and SCA2 (Kasumu et al., 2012b). However, cerebellar ataxia could be caused without disturbance of PCs output. One approach to increase the firing rate of the DCN is to selectively block the SK channels. Cerebellar ataxias were observed in a transgenic (Tg) mice that expressed a dominant negative isoform of SK channel (Shakkottai et al., 2004). Hence, in cerebellar ataxias, the pharmacological

activation of SK channel can serve neuroprotective roles (Hosy et al., 2011). Here, using electrophysiology, the potency of two positive modulators on the SK2 channels was demonstrated.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Overview

Ion channels are the essential elements of the excitable cells that are responsible for the electrical signaling in nerves, muscles, and synapses. They are macromolecular pores that conduct and carry ions through the cell membrane. These channels open or close their pores to permit or block the ion flow in response to extracellular stimuli such as a membrane potential change (Wei and Lee, 2006).

2.2 Small conductance Ca^{2+}-activated potassium SK channels

Ataxias are often disabling with no symptomatic therapy available. Consequently, it seems appropriate to search for more effective approaches. The dysfunction of PCs leads to hyperexcitability of the Deep Cerebellar Nuclei (DCN), which results in cerebellar ataxia. The SK channels are a unique group of potassium ion channels that are activated by intracellular Ca2+ ions (Stocker, 2004; Kovalevskaya et al., 2013). Potassium efflux through these channels modulates cell excitability. Thus, SK channels have been recognized as potential therapeutic drug targets for ataxia. SK channels have tetrameric architecture, and each channel subunit is consisted of six transmembrane α-helical domains (Zhang et al., 2013). Ca2+ binds to the calcium sensor calmodulin (CaM). Calcium binding to calmodulin prompts conformational changes in the channel resulting in channel opening. SK channels are so called because of their small single-channel conductance, whereas the IK channel has an intermediate single-channel conductance and is thus named an ‘intermediate-

conductance' channel. SK1, SK2 and SK3, are the three types of SK channels that are expressed in the neurons, and their activation is involved in afterhyperpolarization that regulates the firing frequency of action potentials for many types of neurons (Sailer et al., 2004). SK channels emerged as one of the principle ion channels involved in the pacemaking of PCs. Among the three subtypes of SK channels, SK2 is the predominant subtype expressed in PCs (Cingolani et al., 2002; Sailer et al., 2004).

2.3 SK Channels Positive modulators (PAM)

Over the past decade, a significant number of compounds have been developed targeting SK/IK channels. 1-EBIO (1-ethyl-2-benzimidazolinone), the first positive modulator was identified twenty years ago, potentiates SK/IK channel activity, and modulates neuronal excitability (Devor et al., 1996). DCEBIO was developed from structural optimization of 1-EBIO, and it has about twenty-fold higher potency than 1-EBIO (Cui et al., 2014). NeuroSearch A/S, a Scandinavian biopharmaceutical company has developed a relatively potent IK/SK channels positive modulator NS309 (3-oxime-6,7-dichloro-1H-indole-2,3-dione) (Strøbaek et al., 2004). 1-EBIO and NS309 are non-selective positive modulators. These modulators cannot activate the SK/IK channels without the presence of Ca2+. Accordingly, they are termed as SK/IK channel positive modulators because they enhance the Ca2+ sensitivity of the SK/IK channels. Despite this progress, many modulators often suffer from low potency (Pedarzani and Stocker, 2008). Limited knowledge about the binding site for the compounds reflected in delaying the development of more effective agents targeting SK channels.

2.4 The Binding Pocket

A binding pocket exists for SK channels positive modulators at the SK channel/CaM interface. Dr. Zhang and his colleagues discovered this binding site several years ago(Zhang et al., 2012). Two FDA approved SK modulators (Chlorzoxazone and Riluzole) in addition to bound in Calmodulin DCEBIO also shared the same binding pocket in SK channels. Channel amino acid residues A477 and L480 interact with the modulators (fig.1A). Fig.1B shows a three-dimensional structure of calmodulin binding domain (CaMBD) and CaM in complex with riluzole. Calmodulin binding domain strongly interacts with N-lobe of calmodulin. Also, two calcium ions were N-lobe. The protein structure from SK2 fragment complexed with NS309 (PDB: 4J9Z) which we determined previously can be used as the model for search of potent SK2 channel modulators (Cui et al., 2014). Significantly, positive modulators have been shown to share the same binding site at the CaM/CaMBD interface with different potency, including 1-EBIO and NS309 (fig. 1). A structured analysis of the CaM/CaMBD complexed with various compounds could be used to facilitate drug discovery targeting SK channels. The determination of potency of some known PAMs is described in fig.2.

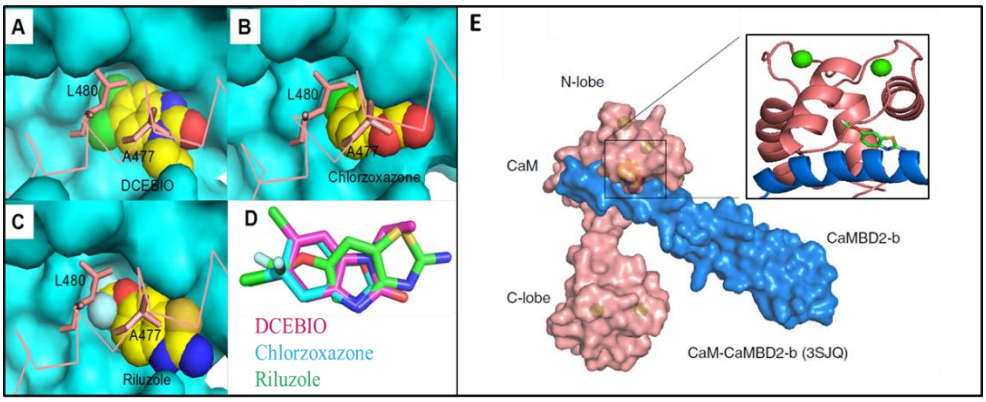


Figure 1. Shared binding pocket by DCEBIO. (A), Chlorzoxazone (B) and Riluzole (C) in the CaM (cyan) complex with SK2-a channel (salmon). (D) Overlay of the conformations of three compounds obtained from their respective protein crystal structures. (E) A space-filled model of the CaMBD and CaM in complex with Riluzole.

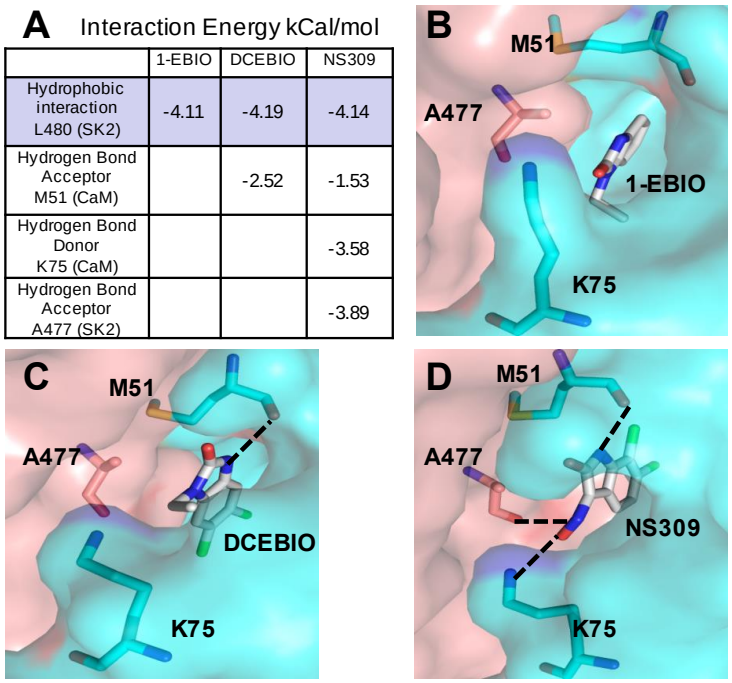


Figure 2. Structural basis for determining the potency of known SK modulators. (A) The interaction energy with major residues in the binding pocket for 1-EBIO, DCEBIO and NS309. (B) Lack of the hydrogen bonds between 1-EBIO (gray) and the receptor (magenta). (C) Formation of one hydrogen bond (yellow dash lines) between DCEBIO (gray) and M51 (cyan). (D) Formation of three hydrogen bonds (yellow dash lines) between NS309 (gray) and A477, M51 and K75 (yellow).

CHAPTER 3

METHODOLOGY

The identification of potent and selective SK modulators could alleviate the symptom of ataxia, in addition to providing pharmacological tools to probe the important roles of SK channels in the pacemaking of cerebellar PCs during the development of ataxia. The computer-based approach is able to screen a large number of compounds in silico, to find those fitting into the binding pocket. Electrophysiology expertise would be critical in characterizing the new modulators in detail.

3.1 Virtual High Throughput Screening (vHTS) for SK channel modulators.

The protein structure from SK2-a fragment complexed with NS309 (PDB: 4J9Z) determined previously was used as the model for search of novel SK2 channel modulators. The vHTS screening was performed by Dr. Meng Cui in Northeastern University School of Pharmacy. The binding pocket of compounds 11 and 14 were determined by Dr. Young Woo Nam. The interaction energy (E_{int}) was calculated from the crystal structures by Dr. Cui in Northeastern University School of Pharmacy.

3.2 Electrophysiology:

SK2 channels were expressed in HEK293 cells. A calcium phosphate method was used for co-transfection of rat SK2 cDNA in pIRES2-AcGFP1 vector, together with rat CaM cDNA in pcDNA3.1(+) vector. Channel activities were recorded from GFP positive cells 1-2 days after transfection (figure.3-A).

Patch clamp experiments were performed with a Multiclamp Axon 200B amplifier (Molecular Devices) at room temperature. pClamp 10.6.2 (Molecular Devices) was used for data analysis and acquisitions. The resistance of the patch electrodes ranged from 3–7 MΩ. The pipette solution contained 140 mM KCl, 10 mM HEPES, 1 mM MgSO4, at pH 7.4. The bath solution contained 140 mM KCl and 10 mM HEPES, at pH 7.2. EGTA (1 mM) and HEDTA (1 mM) were mixed with Ca^{2+} to obtain free Ca^{2+} of indicated concentrations, calculated using the software by Chris Patton of Stanford University (http://www.stanford.edu/~cpatton/maxc.html)

Currents were recorded using an inside-out patch configuration. The intracellular face was initially exposed to a zero-Ca^{2+} bath solution, and subsequently to bath solutions with increasing 0.1 μM Ca^{2+}. Currents were recorded by repetitive 1 sec-voltage ramps from -100 mV to +100 mV from a holding potential of 0mV. One minute after switch bath solutions, ten sweeps, with a one-second interval, were recorded at each compound concentration in the presence of 0.1 μM Ca^{2+}. The integrity of the patch was examined by switching the bath solution back to the zero-Ca^{2+} buffer.

3.3 Data analysis:

To construct the dose–response curve, the current amplitudes were normalized at maximal concentration. The normalized currents were plotted as a function of the concentrations of each compound. Relative currents at -100 mV were plotted as a function of compound concentrations. Half-activation compound concentration (EC_{50}) and Hill coefficients were determined by fitting the data points obtained from

individual experiments to a standard dose-response curve (y = 100/(1 + (x/EC50)^-Hill)).

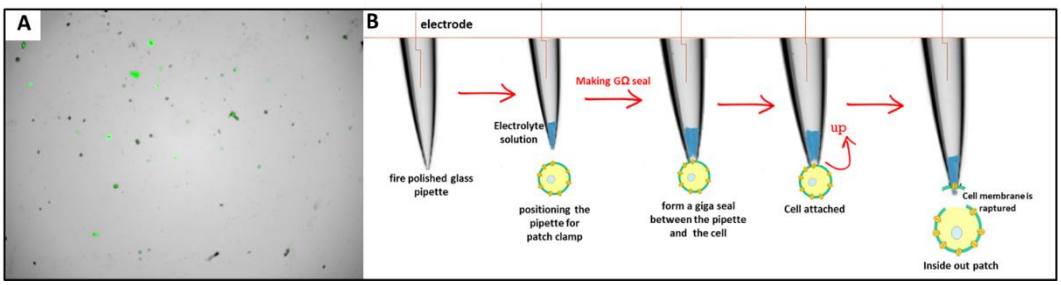


Figure 3. The general principle of patch-clamp electrophysiological recording technique. (A) SK2 channels were co-transfected with a Green Fluorescent Protein (GFP) in HEK293 cells. (B) Diagram illustrating the methods of performing electrophysiological recordings with the inside-out configuration.

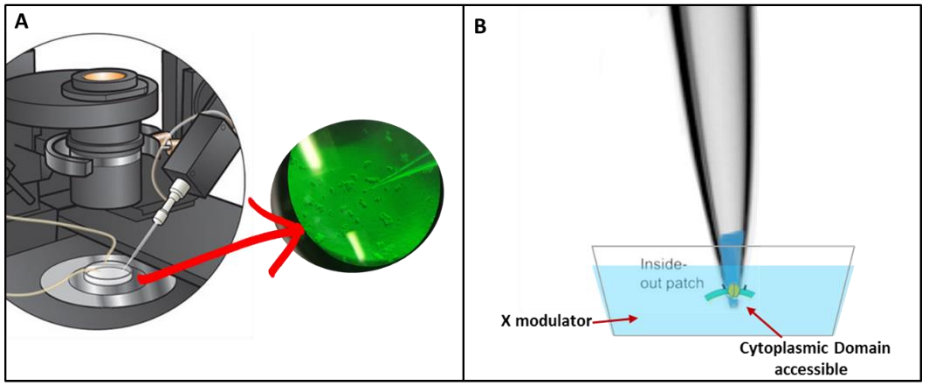


Figure 4. Diagram illustrating the method of testing compounds using the electrophysiological recordings. (A) The cells and the pipette under the microscope. (B) The intracellular side of the channel is exposed to drug in different concentrations applied to the bath.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Activation of SK2 channels by Ca^{2+}

Dose dependent activation of SK2 channels by Ca^{2+} was measured first. The raw current traces are indicated in fig. 5A. The threshold Ca^{2+} concentration was determined to be 0.1 μM as shown in fig.5B. This threshold Ca^{2+} concentration is a minimal concentration of Ca^{2+} that is required for the modulators. As such, all following experiments were performed in the presence of 0.1 μ M Ca^{2+}.

4.2 Potentiation of SK2 channels by Compounds #11 and #14

Before I started in the lab, Dr. Cui in Northeastern University has performed the vHTS, Dr. Zhang and other lab members has purchased and screened the top 100 hit compounds from the vHTS. Dr. Zhang and other lab members has identified compound #11 and compound #14 as two novel SK channel positive modulators.

After I started in the lab, in order to determine the dose dependent potentiation of SK2 channels by these compounds, SK2 channels were expressed in HEK293 cells, and their responses to compound #11 and compound #14 were recorded in the presence of 0.1 μM Ca^{2+} in the bath solution.

4.3 Compound #11

SK2 channels showed very minimal activation upon application of a 0.1 μM Ca^{2+} solution (Fig. 6B). The channel activity was potentiated by compound #11 with

increasing concentrations, from 0.3 μM up to 300 μM. The maximal response to 300 μM of compound 11 in the presence of 0.1 μM Ca^{2+} is 94.60 ± 3.03% of the maximal current induced by μM Ca^{2+} (Fig. 7). Compound 11 dose dependently potentiate the SK2 channel activity (Fig. 7A), with the EC_{50} of 3.87±0.88μM (Fig. 7B) and a Hill coefficient of 1.40±0.14 (n=8).

4.4 Compound #14

SK2 channels opened slightly upon application of a 0.1 μM Ca^{2+} solution (Fig. 6C). The channel activity is further potentiated by compound #14 with increasing concentrations, from 3μM up to 600 μM. In the presence of 0.1 μM Ca^{2+}, the maximal response to 600 μM of compound 14 is 92.03± 3.44% of the maximal current induced by μM Ca^{2+} (Fig. 7). Compound 14 dose dependently potentiate the SK2 channel activity (Fig. 7A), with the EC_{50} of 33.24±4.80μM (Fig. 7B) and a Hill coefficient of 1.40±0.15 (n=9).

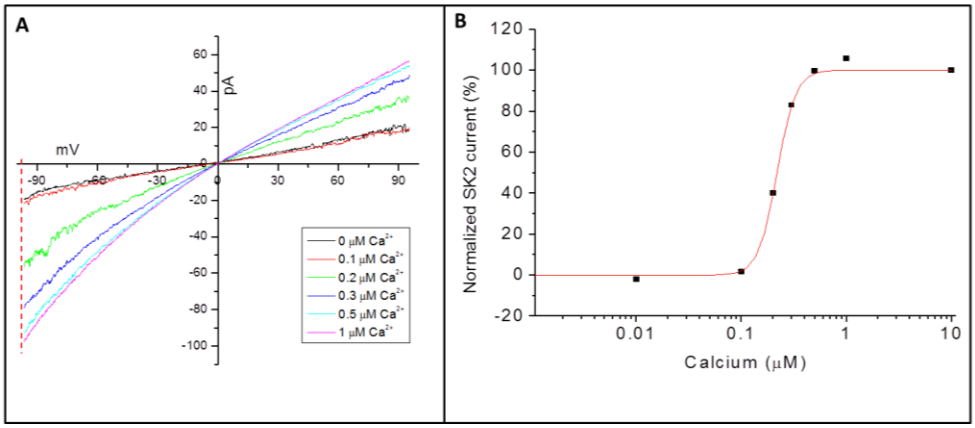


Figure 5. Activation of SK2 channels by Ca^{2+}. (A) Raw current traces from an inside-out patch with SK2 channels expressed in HEK293 cells. The SK2 channels are activated by the Ca^{2+} concentrations indicated. A voltage ramp from −100 mV to +100 mV was applied. (B) Dose dependent activation of SK2 channels by Ca^{2+}.

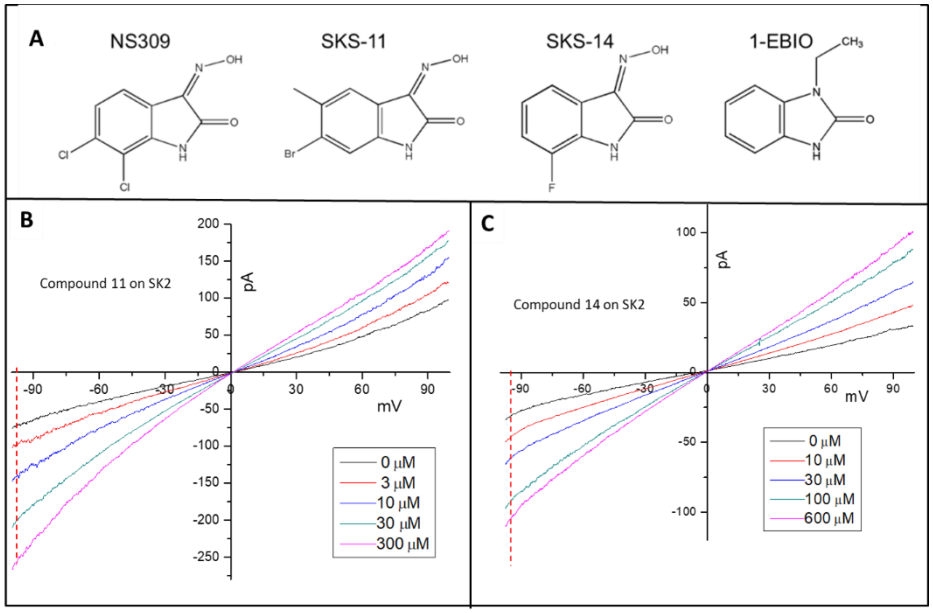


Figure 6. Potentiation of SK2 channel currents by positive allosteric modulators. (A) The chemical structures of NS309, 1-EBIO, SKS-11 and SKS-14. (B) Raw current traces from an inside-out patch with SK2 channels expressed in HEK293 cells. The SK2 channels are activated by 0.1μM Ca^{2+}, with subsequent potentiation by compound 11 at the concentrations indicated (all in the presence of 0.1 μM Ca^{2+}). A voltage ramp, from −100 mV to +100 mV, was applied. (C) Raw current traces from an inside-out patch with SK2 channels expressed. The SK2 channels are activated by 0.1 μM Ca^{2+}, with subsequent potentiation by compound 14 at the concentrations indicated (Nam et al., 2017).

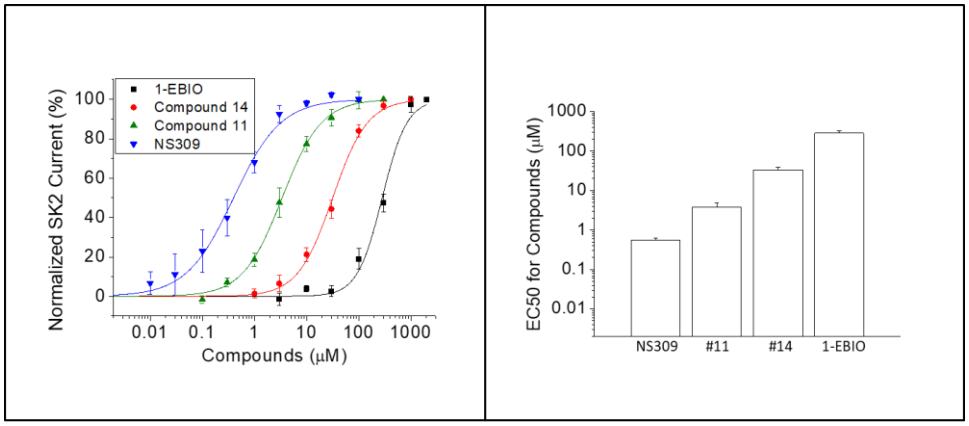


Figure 7. Potency of allosteric modulators. (A) Dose–response curves for 1-EBIO, compound 11, compound 14 and NS309 for their potentiation of the SK2 channel activities. (B) EC_{50}s of the potentiation of SK2 channel activities by 1-EBIO (n=6), compound 11(n=8), compound 14(n=9) and NS309(n=8). Note that the *y* axis is in the log scale.

4.5 The correlation between the interaction energy and the potency

Previously, Dr. Zhang's lab has determined the potency of other known SK channel modulators, such as 1-EBIO (EC_{50}= 285.99 ± 31.57μM, n=6), and NS309 (EC_{50}= 0.55±0.08μM, n=8). As such, we have four compounds with very diverse potency values on potentiating SK2 channels including 1-EBIO, compound 14, compound 11 and NS309, in the order of potency from low to high.

Previously, Dr. Zhang has determined the crystal structures of the binding pocket of 1-EBIO (PDB: 4G28) and NS309 (PDB: 4J9Z). Recently, Dr. Young Woo Nam in Zhang Lab determined the crystal structures of the binding pocket of compound 11 and 14 (data not shown). As such, our lab has four crystal structures available for the binding pocket of the four compounds mentioned above. Our collaborator, Dr. Cui in Northeastern University calculated the interaction energy between the individual compound with its binding pocket from these four crystal structures using the Discovery Studio program.

The correlation between the total interaction energy and the potency of these four compounds were shown in (Fig. 8). There is a strong correlation between these two with a correlation coefficient of ~0.99. The correlation remains between the electrostatic interaction energy and the potency of the compounds (fig. 9A), with a correlation coefficient of ~0.91. However, the correlation is lost between the van der Waals (VDW) interaction energy and the potency of the compounds (fig. 9B), with a correlation coefficient of ~0.74.

Despite the difference in the potency of the compounds, the efficacy of the compounds in potentiating SK2 channels are not significantly different from each other (fig. 10).

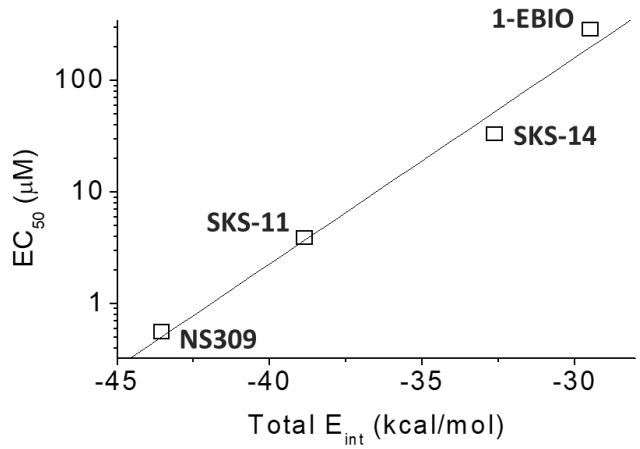


Figure 8. The Correlation between the interaction energy values with the EC_{50} values. The x-axis shows total interaction energy of each individual compound (1-EBIO, compound 11, compound 14 and NS309) with its binding pocket. The y-axis shows the potency (EC_{50} values) of the same group of compounds in potentiation of SK2 channels. The correlation coefficient is 0.99 (Nam et al., 2017).

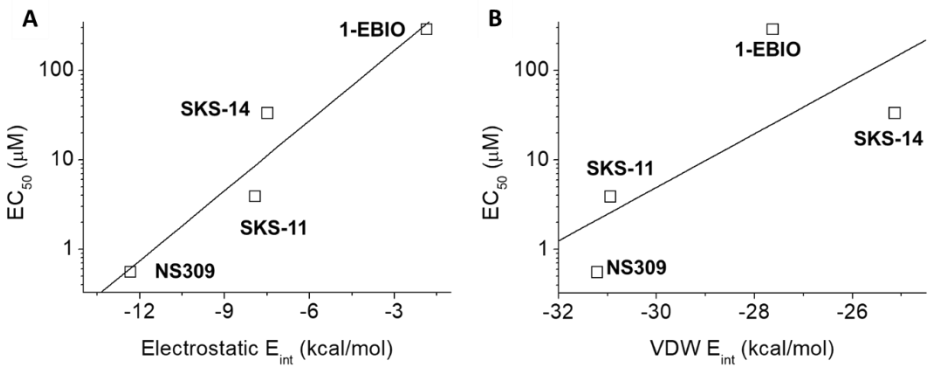


Figure 9. E_{int} between the compounds and their binding pocket (A) The correlation between the electrostatic Eint and the potency of NS309, SKS-11, SKS-14 and 1-EBIO (r = 0.91). (B) The lack of correlation between the VDW Eint and the potency of NS309, SKS-11, SKS-14 and 1-EBIO (r = 0.74). In (b,c and d), the y-axis is set in log-10 scale (Nam et al., 2017).

CHAPTER 5

CONCLUSION

The cerebellum plays an important role in motor control. Cerebellar PCs are affected in many types of ataxia. The early symptoms of ataxia may result from the dysfunction of PCs, and loss of firing precision. Based on these findings, drugs that restore the regular firing of PCs have been suggested as therapeutics for the symptom of ataxia patients. There are various ion channels that control the spontaneous electrical activity of PCs. SK channels emerged as one of the principle ion channels involved in the pacemaking of PCs (Womack and Khodakhah, 2003). SK2 is the predominant subtype expressed in PCs. SK channels modulate the afterhyperpolarization of the PCs that occurs after action potential generation. Therefore, SK channel positive modulators can be effective in alleviating some behavioral and neuropathological symptoms of ataxia in animal models (Shakkottai et al., 2011; Walter et al., 2006; Shakkottai et al., 2004; Kasumu et al., 2012a). Several small molecule modulators of SK channels have been identified previously. Riluzole, an SK channel modulator, yielded promising results in a phase II clinical trial of a mixed population of ataxic patients (Ristori et al., 2010). Despite these promising results, the potency of both agents is not satisfactory for widespread clinical application.

The SK channel activator 1-EBIO has low potency, whereas NS309 is a potent SK channel modulator (Fig. 7A). Compound #11 and compound #14 were identified as positive modulators of SK2 channels. The purpose of this study is to characterize the modulators #11 and #14 in details. To achieve this goal, an inside-out patch method was used to record the currents as the inside surface of the SK2 channel was exposed to different drug concentrations applied to the bath. Compound #11 was applied in the range from 0.3 μM to 300 μM to the inside of the patch at a [Ca2+] of 0.1 μM resulted in a concentration-dependent potentiation of the SK2 current (Figure 6B). To quantify the dose dependence of compound #11, a number of experiments were performed and the current response in each experiment was normalized. The normalized data were averaged and plotted together with other positive modulators (fig 7A). Data were fitted by the Hill equation which yielded an EC_{50} value for SK2 potentiation of 3.87±0.88μM, a Hill coefficient of 1.40±0.14 (n=8) of compound #11. Figure. 7A depicts that compound #11 potently activated SK2 channels more than compound #14. Moreover, The EC_{50} of compound is less than the EC_{50} of compound #14. The EC_{50} indicates how much of the agent is needed to achieve the half-maximal response. The potency of the compound is inversely related to the EC_{50}. The more potent the agent the smaller the EC_{50} will be. Thus, compound # 11 can be considered as a highly potent SK channel modulator, but when compared to NS309, compound # 11 is less potent than the most potent modulator, NS309. Using electrophysiology, I evaluated the efficacy and potency of two positive modulators (compounds 11 and 14) on the SK2 channels. The efficacy values of these two compounds are not significantly different from the efficacy of 1-EBIO and NS309 as reported previously by Dr. Zhang

(Fig. 10), whereas the potency values of 1-EBIO, compound 14, compound 11 and NS309 are significantly different from each other (Fig. 7B). Utilizing the previous data from Dr. Zhang on the potency of two other modulators (1-EBIO and NS309) and the crystal structures of the binding pocket of the compounds determined by Dr. Nam, I found a correlation between the potency of these compounds and the interaction energy of the compounds to their binding pocket (fig. 8). These structure-activity relationship studies will provide valuable information on how the potency of the compounds can be improved through chemical modification, which will facilitate drug discovery targeting SK channels to combat ataxia. The results described in this thesis have been published in the Scientific Report journal on December 7, 2017 (Nam et al., 2017).

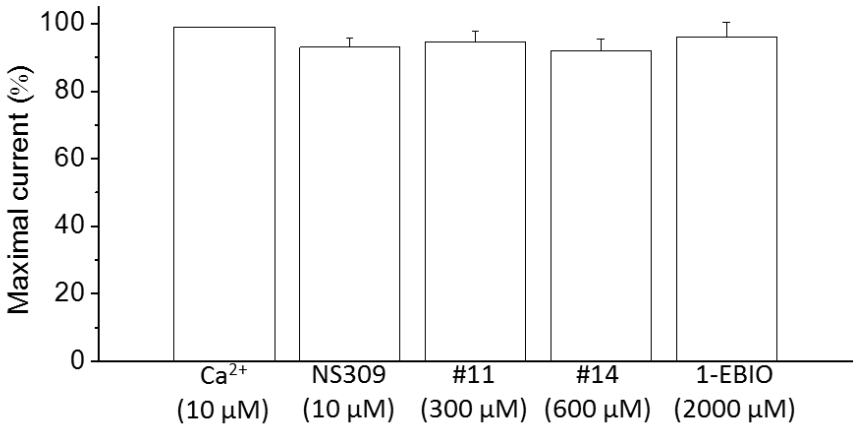


Figure 10. Efficacy of the compounds. The maximal response of the compounds is normalized to the maximal response of 10 μM Ca^{2+}. Efficacy induced by 1-EBIO(n=6), compound 11(n=8), compound 14(n=9) and NS309(n=8).

BIBLIOGRAPHY

Carlson, K.M., Andresen, J.M., Orr, H.T., 2009. Emerging pathogenic pathways in the spinocerebellar ataxias. Curr. Opin. Genet. Dev. 19, 247–253. https://doi.org/10.1016/j.gde.2009.02.009

Cingolani, L.A., Gymnopoulos, M., Boccaccio, A., Stocker, M., Pedarzani, P., 2002. Developmental regulation of small-conductance Ca2+-activated K+ channel expression and function in rat Purkinje neurons. J. Neurosci. Off. J. Soc. Neurosci. 22, 4456–4467. https://doi.org/20026415

Cui, M., Qin, G., Yu, K., Bowers, M.S., Zhang, M., 2014. Targeting the Small- and Intermediate-Conductance Ca2+-Activated Potassium Channels: The Drug-Binding Pocket at the Channel/Calmodulin Interface. Neurosignals 22, 65–78. https://doi.org/10.1159/000367896

Devor, D.C., Singh, A.K., Frizzell, R.A., Bridges, R.J., 1996. Modulation of Cl- secretion by benzimidazolones. I. Direct activation of a Ca(2+)-dependent K+ channel. Am. J. Physiol. 271, L775-784. https://doi.org/10.1152/ajplung.1996.271.5.L775

Gao, Z., Todorov, B., Barrett, C.F., van Dorp, S., Ferrari, M.D., van den Maagdenberg, A.M.J.M., De Zeeuw, C.I., Hoebeek, F.E., 2012. Cerebellar ataxia by enhanced Ca(V)2.1 currents is alleviated by Ca2+-dependent K+-channel activators in Cacna1a(S218L) mutant mice. J. Neurosci. Off. J. Soc. Neurosci. 32, 15533–15546. https://doi.org/10.1523/JNEUROSCI.2454-12.2012

Hosy, E., Piochon, C., Teuling, E., Rinaldo, L., Hansel, C., 2011. SK2 channel expression and function in cerebellar Purkinje cells. J. Physiol. 589, 3433–3440. https://doi.org/10.1113/jphysiol.2011.205823

Kasumu, A.W., Hougaard, C., Rode, F., Jacobsen, T.A., Sabatier, J.M., Eriksen, B.L., Strøbæk, D., Liang, X., Egorova, P., Vorontsova, D., Christophersen, P., Rønn, L.C.B., Bezprozvanny, I., 2012a. Selective positive modulator of calcium-activated potassium channels exerts beneficial effects in a mouse model of spinocerebellar ataxia type 2. Chem. Biol. 19, 1340–1353. https://doi.org/10.1016/j.chembiol.2012.07.013

Kasumu, A.W., Liang, X., Egorova, P., Vorontsova, D., Bezprozvanny, I., 2012b. Chronic suppression of inositol 1,4,5-triphosphate receptor-mediated calcium signaling in cerebellar purkinje cells alleviates pathological phenotype in spinocerebellar ataxia 2 mice. J. Neurosci. Off. J. Soc. Neurosci. 32, 12786–12796. https://doi.org/10.1523/JNEUROSCI.1643-12.2012

Kovalevskaya, N.V., van de Waterbeemd, M., Bokhovchuk, F.M., Bate, N., Bindels, R.J.M., Hoenderop, J.G.J., Vuister, G.W., 2013. Structural analysis of calmodulin binding to ion channels demonstrates the role of its plasticity in regulation. Pflugers Arch. 465, 1507–1519. https://doi.org/10.1007/s00424-013-1278-0

Matilla-Dueñas, A., Sánchez, I., Corral-Juan, M., Dávalos, A., Alvarez, R., Latorre, P., 2010. Cellular and molecular pathways triggering neurodegeneration in the spinocerebellar ataxias. Cerebellum Lond. Engl. 9, 148–166. https://doi.org/10.1007/s12311-009-0144-2

Nam, Y.-W., Orfali, R., Liu, T., Yu, K., Cui, M., Wulff, H., Zhang, M., 2017. Structural insights into the potency of SK channel positive modulators. Sci. Rep. 7, 17178. https://doi.org/10.1038/s41598-017-16607-8

Orr, H.T., 2012. Cell biology of spinocerebellar ataxia. J Cell Biol 197, 167–177. https://doi.org/10.1083/jcb.201105092

Orr, H.T., Zoghbi, H.Y., 2007. Trinucleotide Repeat Disorders. Annu. Rev. Neurosci. 30, 575–621. https://doi.org/10.1146/annurev.neuro.29.051605.113042

Pedarzani, P., Stocker, M., 2008. Molecular and cellular basis of small--and intermediate-conductance, calcium-activated potassium channel function in the brain. Cell. Mol. Life Sci. CMLS 65, 3196–3217. https://doi.org/10.1007/s00018-008-8216-x

Ristori, G., Romano, S., Visconti, A., Cannoni, S., Spadaro, M., Frontali, M., Pontieri, F.E., Vanacore, N., Salvetti, M., 2010. Riluzole in cerebellar ataxia: a randomized, double-blind, placebo-controlled pilot trial. Neurology 74, 839–845. https://doi.org/10.1212/WNL.0b013e3181d31e23

Sailer, C.A., Kaufmann, W.A., Marksteiner, J., Knaus, H.-G., 2004. Comparative immunohistochemical distribution of three small-conductance Ca2+-activated potassium channel subunits, SK1, SK2, and SK3 in mouse brain. Mol. Cell. Neurosci. 26, 458–469. https://doi.org/10.1016/j.mcn.2004.03.002

Shakkottai, V.G., Chou, C., Oddo, S., Sailer, C.A., Knaus, H.-G., Gutman, G.A., Barish, M.E., LaFerla, F.M., Chandy, K.G., 2004. Enhanced neuronal excitability in the absence of neurodegeneration induces cerebellar ataxia. J. Clin. Invest. 113, 582–590. https://doi.org/10.1172/JCI20216

Shakkottai, V.G., do Carmo Costa, M., Dell'Orco, J.M., Sankaranarayanan, A., Wulff, H., Paulson, H.L., 2011. Early changes in cerebellar physiology accompany motor dysfunction in the polyglutamine disease spinocerebellar ataxia type 3. J. Neurosci. Off. J. Soc. Neurosci. 31, 13002–13014. https://doi.org/10.1523/JNEUROSCI.2789-11.2011

Stocker, M., 2004. Ca(2+)-activated K+ channels: molecular determinants and function of the SK family. Nat. Rev. Neurosci. 5, 758–770. https://doi.org/10.1038/nrn1516

Strøbaek, D., Teuber, L., Jørgensen, T.D., Ahring, P.K., Kjaer, K., Hansen, R.S., Olesen, S.P., Christophersen, P., Skaaning-Jensen, B., 2004. Activation of human IK and SK Ca2+ -activated K+ channels by NS309 (6,7-dichloro-1H-indole-2,3-dione 3-

oxime). Biochim. Biophys. Acta 1665, 1–5. https://doi.org/10.1016/j.bbamem.2004.07.006

Walter, J.T., Alviña, K., Womack, M.D., Chevez, C., Khodakhah, K., 2006. Decreases in the precision of Purkinje cell pacemaking cause cerebellar dysfunction and ataxia. Nat. Neurosci. 9, 389–397. https://doi.org/10.1038/nn1648

Wei, C., Lee, S., 2006. Interaction between SK channel calmodulin binding domain and calmodulin. Oregon Health & Science Universityil.

Womack, M.D., Khodakhah, K., 2003. Somatic and dendritic small-conductance calcium-activated potassium channels regulate the output of cerebellar Purkinje neurons. J. Neurosci. Off. J. Soc. Neurosci. 23, 2600–2607.

Zhang, M., Pascal, J.M., Schumann, M., Armen, R.S., Zhang, J.-F., 2012. Identification of the functional binding pocket for compounds targeting small-conductance Ca^{2+}-activated potassium channels. Nat. Commun. 3, 1021. https://doi.org/10.1038/ncomms2017

Zhang, M., Pascal, J.M., Zhang, J.-F., 2013. Unstructured to structured transition of an intrinsically disordered protein peptide in coupling Ca^{2+}-sensing and SK channel activation. Proc. Natl. Acad. Sci. U. S. A. 110, 4828–4833. https://doi.org/10.1073/pnas.1220253110