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Structure–activity relationship (SAR) investigations of tetrahydroquinolines as BKCa agonists

Vijay K. Gore [a,*], Vu V. Ma [a], Ruoyuan Yin [b], Joe Ligutti [b], David Immke [b], Elizabeth M. Doherty [a], Mark H. Norman [a]

[a] Department of Chemistry Research and Discovery, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1799, USA
[b] Department of Neuroscience, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1799, USA

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ABSTRACT

The membrane bound large-conductance, calcium-activated potassium channel (BKCa) is an important regulator of neuronal activity. Here we describe the identification and structure–activity relationship of a novel class of potent tetrahydroquinoline BKCa agonists. An example from this class of BKCa agonists was shown to depress the spontaneous neuronal discharges in an electrophysiological model of migraine.

BKCa, the large-conductance calcium-activated potassium channel, is an important regulator of neuronal excitability and synaptic transmission. It has been postulated that BKCa channels constantly monitor the electrical and metabolic state of the cell based on a unique activation mechanism whereby both membrane depolarization and intracellular calcium combine to open the channel.[1] Activation of BKCa channels results in a large potassium ion efflux that causes the cell membrane to be rapidly hyperpolarized, thereby reducing the neuronal excitability and decreasing the intracellular calcium load. It has been recently shown that BKCa channels are important regulators of neuronal firing in the trigeminovascular (TNC) pathway which is central to the pathogenesis of migraine.[2] Therefore, we sought to determine if a selective BKCa agonist could provide a novel way to treat migraine headaches.

During the past few years, various classes of BKCa agonists (Fig. 1) have been described, and their chemistry and pharmacology have been reviewed.[3,4] The benzimidazolone derivatives NS004 (**1**) and NS1619 (**2**) are the prominent chemotypes among the small molecule BKCa channel openers that have been studied in detail, both in vitro and in vivo.[5,6] Other class of structurally related agonists are the 3-aryl oxindole based analogs, represented by the fluorooxindole Maxipost (**3**)[7] and a series of 3-substituted-4-arylquinolin-2-ones (**4**) of BKCa-channel openers have also been disclosed by researchers at Bristol-Meyers Squibb.[8]

As a result of our screening efforts, we identified the tetrahydroquinoline **5** as a novel BKCa agonist. Compound **5** showed weak agonistic activity (2.2 ± 0.7-fold increase in K^+ current at 10 μM concentration) in a patch–clamp electrophysiological assay conducted in Chinese hamster ovary (CHO) cells stably over-expressing the human BKCa channel. We initiated a structure–activity relationship (SAR) investigation based on compound **5** to identify analogs with improved potency and to enhance our understanding of the biological activity of this structural class of BKCa agonists. In this paper, we describe a SAR data set that provides insights into this novel class of BKCa agonists.

The majority of compounds required for our SAR study were prepared by the Grieco three-component cycloaddition reaction[9] shown in Scheme 1. The reactions involved the condensation of aldehydes (**6**), cycloalkenes (**7**), and anilines (**8**) in the presence of trifluoroacetic acid (TFA) as the catalyst to generate the tetrahydroquinoline scaffold (**I**). The cycloaddition reaction resulted in formation of the *cis*–*trans* isomer (*cis* ring-fused as shown in structure **I**) as the major diastereomer (>95%). The compounds initially prepared included modifications adjacent to the tetrahydroquinoline nitrogen (R). Accordingly, the condensations of aldehydes **6a**–**6p** with cyclopentadiene **7a**, and aniline **8a** were carried out to yield the intermediate esters **9**–**24** as shown in Scheme 2. These intermediate esters were hydrolyzed with TFA to provide the final products **5** and **25**–**39**.

Schemes 3–5 outline the synthesis of analogs with modifications to the central tetrahydroquinoline core. Condensation of 2,4-dichlorobenzaldehyde (**6m**) with alkenes **7b**–**7d**, and *tert*-butyl-4-aminobenzoate (**8a**) were carried out to yield the intermediate esters **40**–**42** that were then hydrolyzed with TFA to give the final products, **43**–**45**.

* Corresponding author. Tel.: +1 805 447 4823; fax: +1 805 480 1337.
E-mail address: vgore@amgen.com (V.K. Gore).

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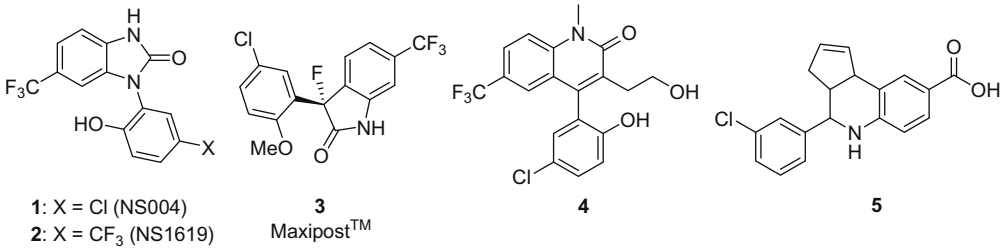


Figure 1. BKCa agonists.

Additional modifications to the oxidation state of the tetrahydroquinoline ring system are outlined in Scheme 4. Saturated analogs **48** and **49** were prepared by the hydrogenolysis of intermediates **40** and **21** with Adam's catalyst followed by hydrolysis with TFA. Alternatively, compound **21** was treated with Boc-anhydride followed by dihydroxylation using OsO_4–*N*-methylmorpholine-*N*-oxide (NMO). Subsequent deprotection of the Boc group and hydrolysis of the *tert*-butyl ester with TFA yielded the dihydroxylated analog **50**. The aromatization of intermediate **47** was carried out by 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) oxidation followed by deprotection using TFA to afford the 2,3-dihydro-1H-cyclopenta[*c*]quinoline analog **51**.

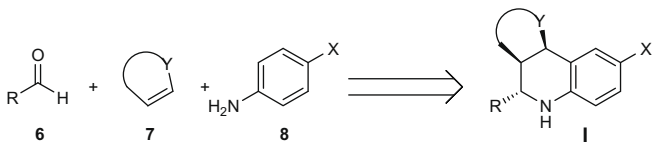
An alternative synthetic route was required to prepare the analog devoid of the fused cyclopentyl ring on the tetrahydroquinoline core (Scheme 5). Methyl quinoline-6-carboxylate **52** was converted to its corresponding *N*-oxide using *m*-CPBA followed by chlorination with phosphorus oxychloride to yield the methyl 2-chloroquinoline-6-carboxylate. Suzuki coupling with 2,4-dichlorophenylboronic acid provided intermediate **53**. Compound **53** was subjected to hydrogenolysis in the presence of Adam's catalyst followed by basic saponification to give the final truncated product **54**.

Schemes 6 and 7 show the synthesis of analogs with modifications to the carboxylic acid group. Grieco three-component condensations of 2,4-dichlorobenzaldehyde (**6m**) with cyclopenta-1,3-diene (**7a**), and anilines **8b–8e**[10] and were carried out to provide tetrahydroquinolines **55**, **57**, and **59**–**60**. The intermediate ester **55** was subjected to basic hydrolysis to give compound **56** and lithium aluminum hydride (LAH) reduction of methyl ester **57** afforded the alcohol derivative **58**.

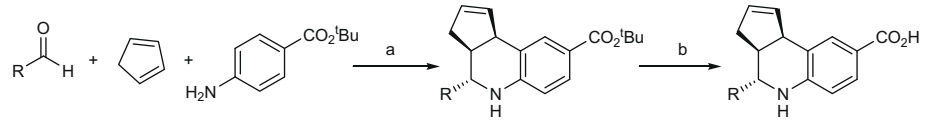
Finally, carboxylic acid replacements **61** and **62** were synthesized from compound **36** as shown in Scheme 7. Coupling of acid **36** with methoxylamine hydrochloride or methanesulfonamide in the presence of 1-(3-dimethylaminopropyl))-3-ethylcarbodiimide (EDC) resulted in the formation of compounds **61** and **62**, respectively.

The compounds prepared in this investigation were tested for their ability to evoke potassium current as a measure of agonism in the PatchXpress electrophysiology assay (Molecular Devices)[11] using CHO cells expressing human BKCa channel. The assay results shown in the Tables 1–3 represent the functional activity reported as a ratio (fold increase) of the maximum current observed compared to control[12] at 10 or 3 μM compound concentrations. The fold-increase value represents the average of at least two independent cell-recording experiments with a minimum of two cells ($n \geqslant 2$) in each experiment. The higher the fold increase, the more potent is the BKCa agonist in this system. The SAR investigations were carried out in the three regions: **A** (phenyl group modifications), **B** (central core changes), and **C** (carboxylic acid replacements) as highlighted in Figure 2.

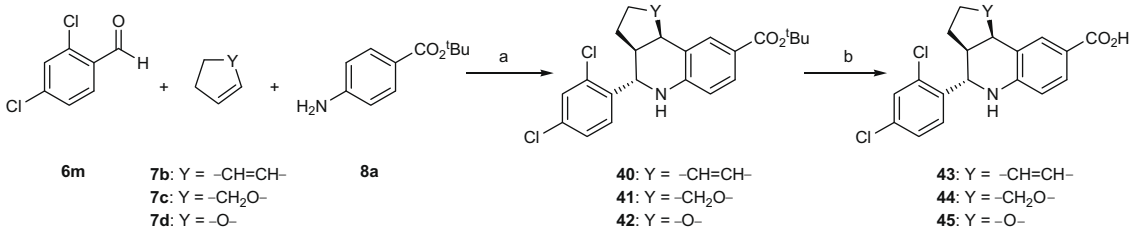
We first examined the effects on SAR of region **A** and initially evaluated the compounds at a 10 μM concentration (Table 1). Removal of the 3-chlorophenyl group in compound **5**, or replacing it with *tert*-butyl group, (compounds **25** and **26**, respectively) resulted in complete loss of activity. The phenyl analog (**27**) showed a moderate loss of potency, but the activity was retained when the



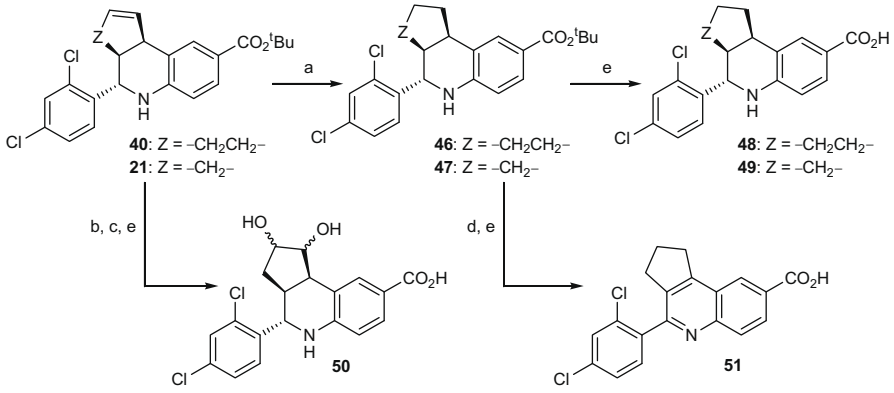
Scheme 1. General synthesis of tetrahydroquinoline scaffold (**I**).



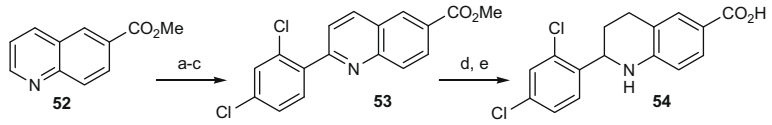
Scheme 2. Synthesis of compounds **5**, **25**–**39**. Reagents and conditions: (a) TFA, MeCN, 0 °C 8 h; (b) TFA, room temperature, 2 h, ~60% (over two steps).



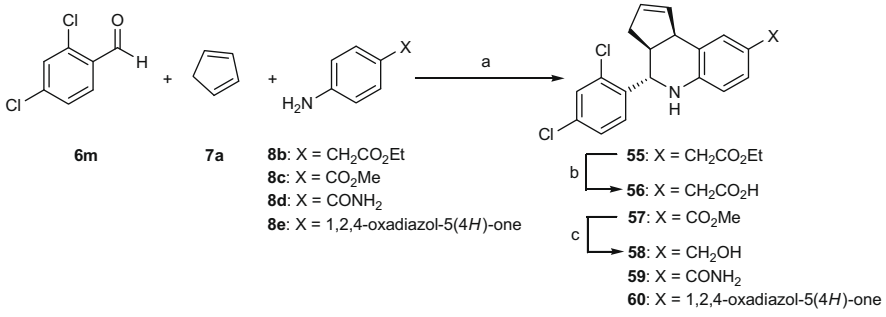
Scheme 3. Synthesis of compounds **43**–**45**. Reagents and conditions: (a) TFA, MeCN, 0 °C 8 h; (b) TFA, room temperature, 2 h, ~60% (over two steps).



Scheme 4. Synthesis of compounds **48**–**51**. Reagents and conditions: (a) THF/EtOH, PtO_2, H_2, room temperature, 12 h; (b) THF, $(Boc)_2O$, DMAP, room temperature, 12 h; (c) cat. OsO_4, NMO, aq dioxane; (d) DDQ, DCM; (e) TFA, DCM, room temperature, 2 h, 35–65%.



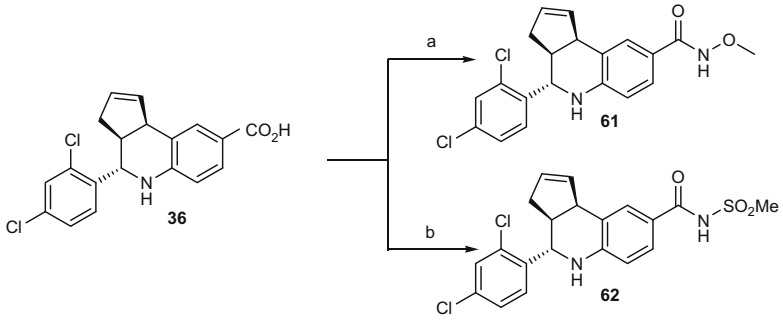
Scheme 5. Synthesis of compound **54**. Reagents and conditions: (a) *m*-CPBA, DCM; (b) $POCl_3$, DCM, 18% (over two steps); (c) 2,4-dichlorophenylboronic acid, $Pd(PPh_3)_4$, DME, 1 M Na_2CO_3; (d) THF/MeOH, AcOH, PtO_2, H_2, room temperature, 12 h, 50%; (e) aq NaOH, MeOH, THF, 69% (over three steps).



Scheme 6. Synthesis of compounds **55**–**60**. Reagents and conditions: (a) TFA, MeCN, 0 °C 8 h; (b) NaOH (1 N), EtOH, room temperature, 16 h, 67%; (c) LAH, 0 °C, THF, 49% (over two steps).

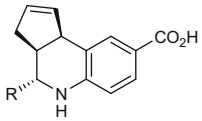
3-chloro group was replaced with 3-bromo or 3-methyl substituents (**28** and **29**, respectively). On the other hand, the regio-isomeric analogs, 2-chlorophenyl (**30**) and 4-chlorophenyl (**31**), showed a two- to threefold improvement in activity, clearly illustrating that analogs with ortho or para substituents are favored over the meta-substituted derivatives. Replacing the 4-chloro group with the more sterically demanding 4-trifluoromethyl (**32**) or 4-bromo groups (**33**) showed cellular activity comparable to **31**. Next we prepared a series of disubstituted analogs (**34**–**37**). The 2,3-dichloro analog (**34**) did not show any improvement in activity but the 2,4- and 3,4-disubstituted analogs (**35**–**37**) showed a five- to sixfold enhancement in potency relative to **5**. Heteroaromatic substitutions such as the 3,5-dichloropyridin-2-yl analog **38** and the pyrazolyl analog **39** did not provide any improvement in the activity. Taken together these SAR results suggest that the region **A** substituents occupy a large hydrophobic pocket in the receptor.

Due to the significant boost in potency obtained in the first set of analogs, the remaining derivatives were tested at a lower concentration (3 μM). Based on the SAR studies for region **A**, we retained the 2,4-dichlorophenyl group in the subsequent compounds and examined modifications to the central core (region



Scheme 7. Synthesis of compounds **61** and **62**. Reagents: (a) $H_2NOMe{\cdot}HCl$, EDC, HOBt, DIPEA, DMF, DCM, 50%; (b) EDC, DMAP, $MeSO_2NH_2$, $CHCl_3$, 39%.

Table 1
Structures and assay results of analogs **5** and **25–39**



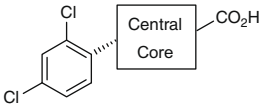
Compound	R	Cell assay[a] fold increase at 10 μM concentration
5	3-ClPh	2.2 ± 0.7
25	H	0.6 ± 0.2
26	*t*-Bu	0.8 ± 0.1
27	Ph	1.2 ± 0.2
28	3-BrPh	2.6 ± 0.1
29	3-MePh	3.1 ± 0.9
30	2-ClPh	4.9 ± 1.6
31	4-ClPh	7.3 ± 3.9
32	4-CF_3Ph	6.0 ± 2.9
33	4-BrPh	8.9 ± 3.3
34	2,3-DiClPh	2.7 ± 0.5
35	3,4-DiClPh	12.1 ± 4.9
36	2,4-DiClPh	11.2 ± 3.5
37	2,4-DiMePh	8.9 ± 1.2
38	3,5-Dichloropyridin-2-yl	2.5 ± 1.4
39	1-Methyl-1H-pyrazol-4-yl	1.0 ± 0.3

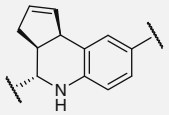
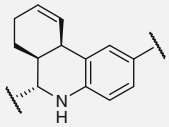
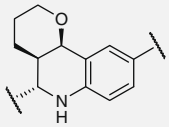
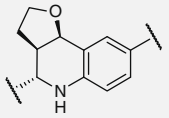
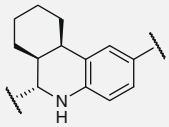
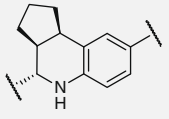
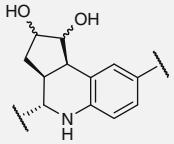
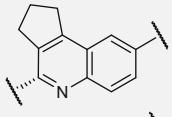
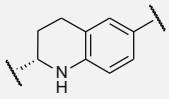
[a] The cellular assay was carried out in human BKCa channel expressed in CHO cells. Results are the average of two experiments with $n \geqslant 2$ cells.

B, Table 2). Replacement of the central tetrahydro-3*H*-cyclopenta[*c*]quinoline core of **36** with hexahydrophenanthridine **43** resulted in a moderate loss of activity. Additionally, introducing heteroatoms and polar groups in the central core, such as compounds **44**, **45**, and **50**, resulted in a further decrease in potency, suggesting that lipophilic functionalities in region **B** were preferred for activity. On the other hand, saturating the cyclohexenyl ring to give compound **48** did show modest activity, albeit less active than compound **43**. However, the corresponding cyclopentyl analog **49** demonstrated significant reduction in potency compared to its corresponding cyclopentenyl analog **36**. Complete removal of the cyclopentenyl ring to give compound **54** resulted in complete loss of activity, as did aromatization of the core (**51**). These results suggested that the central tetrahydro-3*H*-cyclopenta[*c*]quinoline ring (**36**) was preferred.

In the next phase of our SAR investigation, we explored the effect of various carboxylic acid modifications and isosteric replacements (region **C**, Table 3). Homologation of **36**–**56** was detrimental to the activity in the cell assay. Similarly, the methyl ester (**57**) and benzyl alcohol (**58**) analogs also showed no activity. Replacement of the carboxylic acid with an isostere such as oxadiazolone (**60**) showed moderate agonistic activity but were still weaker than its carboxylic acid counterpart (**36**). The other two isosteres,

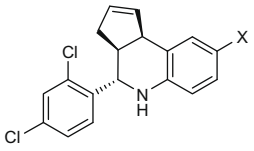
Table 2
Structures and assay results of analogs **36**, **43–45**, **48–51**, and **54**

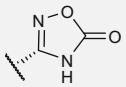


Compound	Central core	Cell assay[a] fold increase at 3 μM concentration
36		6.7 ± 2.9
43		4.5 ± 1.4
44		1.5 ± 0.2
45		1.0 ± 0.3
48		3.6 ± 0.8
49		4.6 ± 0.6
50		0.9 ± 0.02
51		1.0 ± 0.1
54		0.9 ± 0.6

[a] The cellular assay was carried out in human BKCa channel expressed in CHO cells. Results are the average of two experiments with $n \geqslant 2$ cells.

Table 3
Structures and assay results of analogs **36**, **56**–**58**, and **60**–**62**



Compound	X	Cell assay[a]fold increase at 3 μM concentration
36	CO_2H	6.7 ± 2.9
56	CH_2CO_2H	1.5 ± 0.4
57	CO_2Me	1.0 ± 0.12
58	CH_2OH	1.5 ± 0.2
60		3.4 ± 0.04
61	CONHOMe	2.2 ± 0.6
62	$CONHSO_2Me$	2.7 ± 1.5

[a] The cellular assay was carried out in human BKCa channel expressed in CHO cells. Results are the average of two experiments with $n \geqslant 2$ cells.

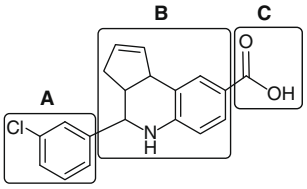


Figure 2. SAR strategy for tetrahydroquinolines.

acylamino methyl ether (**61**) and acyl sulfonamide (**62**) showed weak activity.

Based on these SAR studies, we found that the disubstituted aromatic groups (e.g., 2,4-dichlorophenyl) provided the optimal substitution pattern alpha to the tetrahydroquinoline nitrogen. Introduction of heterocyclic rings and polar groups in the central tetrahydroquinoline ring (region **B**) was detrimental to the activity, while hydrophobic groups were well tolerated in the central core of the molecule. Replacement of the carboxylic acid group with various isosteres did not improve the activity.

Because of its excellent in vitro potency, we evaluated whether compound **36** would have a physiological effect on neuronal firing. An experiment was designed to measure the spontaneous action potentials in the rat trigeminal nucleus caudalis which is central to the neuronal pathway of migraine. The electrophysiology-based extracellular recording technique was used to evaluate the spontaneous excitability of native neurons in freshly sectioned trigeminal nucleus caudalis slices (male, Sprague–Dawley, 3–4 weeks old, 400 μM thickness). Figure 3A illustrates an exemplary recording of the slice electrophysiology experiment showing a dose-dependent reduction in the spontaneous firing frequency elicited by the continuous perfusion of compound **36** (0.1–10 μM) into the recording chamber. At the highest dose tested (10 μM) the average inhibition was ~75% (Fig. 3B; $n = 3$ slices). The inhibitory effect of compound **36** was reversed by co-addition of a selective BKCa antagonist, Iberiotoxin[13] (IBTX) (Fig. 3A and B), strongly suggesting that the event was BKCa-mediated and not due to an off-target effect.

In conclusion, through SAR studies based on compound **5**, we identified a novel class of tetrahydroquinoline analogs as potent BKCa agonists. We were able to achieve significant improvement in the PatchXpress electrophysiology assay compared to the initial screening-hit **5**. In addition, we demonstrated that analog **36** was

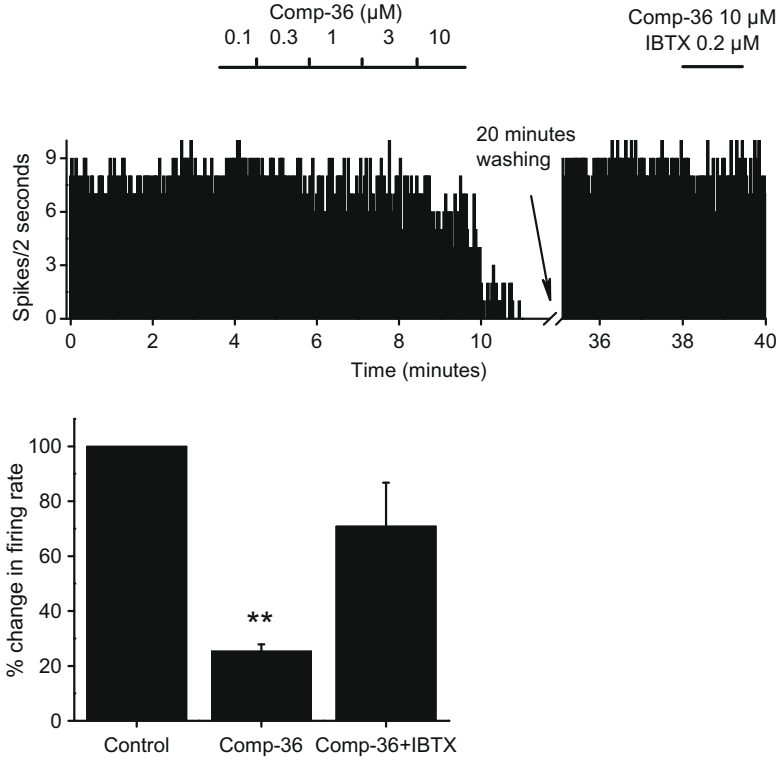


Figure 3. Compound **36** inhibited spontaneous action potentials (SAP) in the trigeminal nucleus caudalis slices of rat. (A) A histogram, compound **36** dose-dependently inhibited SAP. (B) Summary of the inhibition with compound **36** at 10 μM ($n = 3$) on SAP and reversal of the agonism induced by compound **36** with the BKCa antagonist Iberiotoxin (IBTX) (0.2 μM) ($n = 3$). $^{**}p = 0.01$, one way ANOVA, compared with control.

able to block spontaneous, BKCa-mediated neuronal firing in an electrophysiological model of migraine.

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