Published in final edited form as:

Bioorg Med Chem Lett. 2012 October 15; 22(20): 6401–6404. doi:10.1016/j.bmcl.2012.08.067.

Inhibition of Na_v1.6 sodium channel currents by a novel series of 1,4-disubstituted-triazole derivatives obtained via copper-catalyzed click chemistry

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Abstract

We have synthesized and evaluated a series of 1,4-disubstituted-triazole derivatives for inhibition of the rat Na_v1.6 sodium channel isoform, an isoform thought to play an important role in controlling neuronal firing. Starting from a series of 2,4(1*H*)-diarylimidazoles previously published, we decided to extend the SAR study by replacing the imidazole with a different heterocyclic scaffold and by varying the aryl substituents on the central aromatic ring. The 1,4-disubstituted 1,2,3-triazoles were prepared employing the copper-catalyzed azide-alkyne cycloaddition (CuAAC). Many of the new molecules were able to block the rNa_v1.6 currents at 10 μM by over 20%, displaying IC₅₀ values ranging in the low micromolar, thus indicating that triazole can efficiently replace the central heterocyclic core. Moreover, the introduction of a long chain at C4 of the central triazole seems beneficial for increased rNa_v1.6 current block, whereas the length of N1 substituent seems less crucial for inhibition, as long as a phenyl ring is not directly connected to the triazole. These results provide additional information on the structural features necessary for block of the voltage-gated sodium channels. These new data will be exploited in the preparation of new compounds and could result in potentially useful AEDs.

Keywords

sodium channels; patch clamp electrophysiology; 1,4-triazoles; rNa_v1.6; click chemistry

Voltage gated sodium channels (VGSCs) are membrane protein complexes that allow the passive flow of sodium ions across biological membranes.¹ VGSCs open on membrane depolarization, causing further depolarization of the membrane and the initiation of an action potential. As such, VGSCs play a critical role in regulating cellular excitability and controlling many of the physiological processes associated with neuronal activity.^{2–4} Alterations in the behaviour of VGSCs have been associated in the pathophysiology of epilepsy, a chronic neurological disorder characterized by recurrent unprovoked seizures.

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Supplementary Material

Supplementary data associated with this article can be found, in the online version.

These seizures occur when a brief, strong surge of electrical activity affects either one hemisphere (partial seizure) or both hemispheres of the brain (generalized seizure). In particular, mutations in VGSC genes *SCN1A* (encoding the Na_v1.1 α -subunit), *SCN2A* (encoding the Na_v1.2 α -subunit), *SCN1B* (encoding the auxiliary β 1-subunit), *SCN3A* (encoding the Na_v1.3 α -subunit) and *SCN9A* (encoding the Na_v1.7 α -subunit) have been reported in brain tissues of humans with epileptic syndromes.^{5–8} In animal models of epilepsy, the Na_v1.6 sodium channel isoform, which is heavily expressed with the central nervous system, has been shown to be upregulated.^{9,10} The Na_v1.6 isoform is thought to play an important role in controlling neuronal firing. Na_v1.6 channels have a particularly low threshold for spike initiation and are abundantly expressed along the axon initial segment (AIS), the site of action potential initiation. They have been shown to contribute to the spike trigger and also to regulate the repetitive discharge properties of hippocampal CA1 pyramidal neurons. Mice lacking Na_v1.6 have lower instantaneous firing rates and maximal firing rates in retinal ganglion cells (GCs).^{11,12} Impaired Na_v1.6 function in SCN8A (encoding the Na_v1.6 α -subunit) mutant mice ameliorates seizure harshness in a severe myoclonic epilepsy of infancy (SMEI) mouse model.^{13,14} In another study, the same mutant mice displayed considerable resistance to the initiation and development of kindling, a well-established model of abnormal plasticity leading to prolonged seizures and to epilepsy.⁹ It was also found that kindling is associated with higher expression of Na_v1.6 sodium channels in hippocampal CA3 neurons. All of these findings suggest that selective pharmacological inhibition of Na_v1.6 sodium channels could increase the efficacy of antiepileptic drugs providing them with an improved therapeutic profile.

In a recent report, a series of 2,4-(*1H*)-diarylimidazoles were identified as sodium channel blockers (Fig. 1).^{15,16}

These derivatives carry a central imidazole moiety with two phenyl rings attached to positions 2 and 4, respectively. Different substituents were introduced on each phenyl ring to vary the physico-chemical properties (ionization, lipophilicity, steric hindrance) of the resulting molecules. Some of the compounds presented in those studies had IC₅₀ values within the nanomolar-micromolar range against another neuronal expressed sodium channel isoform, Na_v1.2 and showed anticonvulsant activity in an acute *in vivo* seizure rodent model (MES: Maximal Electoshock Seizure test) with low sedative and ataxic side effects when tested in a rat rotorod test.¹⁷

Starting from this interesting series of sodium channel blockers, we decided to extend the SAR study on that class of compounds by replacing the imidazole with a different heterocyclic scaffold and by varying the aryl substituents on the central ring. The SAR analysis of these new series of compounds should provide us with additional information about the structural features necessary to inhibit voltage-gated sodium channels, providing new compounds that may have AED activity. The new molecules were designed by replacing the imidazole ring with a triazole, because of its interesting properties from a medicinal chemistry standpoint, such as good stability to metabolic degradation and a straightforward synthetic route. Interestingly, several drugs contain the 1,2,3-triazole moiety; among them, tazobactam, an inhibitor of bacterial beta-lactamases, various cytostatic and antiproliferative agents and, most importantly, rufinamide, an anticonvulsant medication developed in 2004.^{18–21}

In view of the fact that the Na_v1.6 sodium channel has been heavily implicated in epilepsy as well as other common CNS disorders,¹⁴ we decided to test these new derivatives (at a concentration of 10 μ M) for activity against the Nav1.6 isoform stably expressed in Human Embryonic Kidney (HEK 293) cells. We also tested two diarylimidazoles compounds previously shown to have activity against hNav1.2, on Na_v1.6 for comparison purposes.

The 1,4-disubstituted 1,2,3-triazoles reported in Table 1 were prepared according to Schemes 1 and 2, employing the copper-catalyzed azide-alkyne cycloaddition (CuAAC), a widely used synthetic strategy characterized by high efficiency and a simple workup procedure.^{22,23} With a one-pot reaction, starting from the appropriate alkyne and azido-derivative or bromo-derivative, copper(II) sulfate and sodium ascorbate, used for the *in situ* generation of Cu(I) catalyst, after heating the reactions mixture overnight at 65 °C in *t*BuOH/H₂O (1:1), we were able to obtain the desired products with yields ranging from 70 to 96% (see Supplementary Data).

Compounds **1–14** were evaluated for their ability to inhibit Na_v1.6 sodium channel currents at a concentration of 10 μM (see Supplementary Data).

The 1,4-triazole derivatives **1–12** were screened for activity against the rNa_v1.6 sodium channel isoform at 10 μM. Table 1 shows the correlation between triazole substitutions and inhibition of rNa_v1.6 channel currents. For comparison purposes, we also tested two diarylimidazoles, previously shown to have differing activity against hNa_v1.2 (**13**, **14**). At a concentration of 10 μM, compound **13** exhibited a small block of Na_v1.2 (7.8%) while compound **14** had a greater block (30.1%). Results reported in Table 1 showed similar behaviour of both compounds **13** and **14** against hNa_v1.2 and rNa_v1.6 at 10 μM (16.0% block for **13** and 21.7% block for **14**). The new triazole derivatives **1–12**, synthesized with the aim to study a new central heterocycle with different substituents, inhibited rNa_v1.6 currents between 5.8% and 25.9% at 10 μM, thus showing comparable potencies with the diarylimidazole blockers. Dose-response curves were generated for compounds **2**, **4**, **6**, **9** and **12** and the calculated IC₅₀'s are reported in Table 1. IC₅₀ values ranged between 28.5 μM and 125.4 μM. These findings suggest that the presence of a phenyl ring directly connected to the central triazole is less favorable for inhibition of rNa_v1.6, as demonstrated by the percent block of compounds **1**, **3**, **4** and **10**. Two exceptions were the phenyl-triazole derivatives **2** and **7**, which demonstrated greater inhibition of rNa_v1.6 currents compared to the previous compounds. An interesting pattern can be observed by considering the different substituents introduced in position 1 of the triazole. In particular, the substituent in R (phenyl for **1**, **2**, **3**, benzyl for **4**, **5**, **6**; phenylethyl for **7**, **8**, **9** and phenylethanone for **10**, **11**, **12**) correlates with an increase in inhibitory activity with the lengthening of the chain in the substituent at position 4 (R¹). In fact, as shown in Table 1, the most potent molecule for each group possesses a phenoxypropyl-chain in R¹ (**6**, **9** and **12**). Nevertheless, considering the first group (compounds **1**, **2**, **3**), it is possible to observe that compound **2**, having a phoxymethylchain, is the most active, behaving as an outlier. The three phenoxypropyl-derivatives (**6**, **9** and **12**) display comparable inhibitory activity against rNa_v1.6 currents, independently of the substituent carried on position 1 of the triazole ring.

The copper-catalyzed azide-alkyne cycloaddition (CuAAC) was employed to prepare 1,4-triazoles derivatives with different aryl substituents at the N1 and C4 positions. Although none of the newly synthesized compounds resulted in more active compounds compared to the reference compounds **13** and **14** at inhibiting rNa_v1.6 sodium channels, most of them were able to inhibit rNa_v1.6 currents at 10 μM by more than 20%. The newly synthesized sodium channel blockers displayed IC₅₀ values that were in the micromolar concentration range, indicating that triazole can efficiently replace the central heterocyclic core. Moreover, the introduction of a long chain at C4 of the central triazole seems beneficial rNa_v1.6 current inhibition, whereas the length of N1 substituent seems less crucial for this activity, as long as a phenyl ring is not directly connected to the triazole. These results provide additional informations on the structural features necessary for the block of the voltage-gated sodium channels. These new data could be useful on the design of future candidate therapeutics. Activity against Nav1.6 may lead to the generation of more effective and better tolerated anticonvulsant drugs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to the Centro Interdipartimentale Misura of the University of Parma for providing the NMR instrumentation. Funding from the National Institutes of Health NINDS R21NS061069-02 (M.K.P.) is gratefully acknowledged.

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Figure 1.
2,4(*1H*)-Diarylimidazoles

Scheme 1.

Reagents and conditions: (a) alkyne (2.0 mmol), azido-derivative (2.0 mmol), Na ascorbate (0.5 mmol), CuSO_4 (0.025 mmol), $t\text{BuOH/H}_2\text{O}$ (1:1) (6 mL), 65 °C, overnight, 70–88% yields.

Scheme 2.

Reagents and conditions: (a) alkyne (2.1 mmol), bromo-derivative (2.0 mmol), NaN₃ (2.1 mmol), Na ascorbate (0.5 mmol), CuSO₄ (0.1 mmol), *t*BuOH/H₂O (1:1) (6 mL), 65 °C, overnight, 72–96% yields.

Table 1 Electrophysiological evaluation of compounds **1–14** efficacy against rNa_v1.6

Comp	R	R ¹	Percent block of rNa _v 1.6 current at 10 μM (n = 4)	IC ₅₀ (μM)
1			6.2 ± 1.6	N.D.
2			16.0 ± 1.0	28.5
3			5.9 ± 1.3	N.D.
4			10.0 ± 2.1	125.4
5			11.0 ± 1.7	N.D.
6			26.0 ± 3.0	31.7
7			11.5 ± 1.5	N.D.
8			8.9 ± 1.2	N.D.
9			22.7 ± 3.1	52.0
10			3.9 ± 1.0	N.D.
11			13.6 ± 2.7	N.D.
12			22.7 ± 4.5	53.6

Comp	R	R ¹	Percent block of rNa _v 1.6 current at 10 μM (n = 4)	IC ₅₀ (μM)
13			16.0 ± 3.5	32.2
14			21.7 ± 2.2	19.6

N.D.: Not Determined