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2,4(5)-Diarylimidazoles: Synthesis and biological evaluation of a new class of sodium channel blockers against $hNa_v1.2$

Mirko Rivara [a], Aparna R. Baheti [b], Marco Fantini [a], Giuseppe Cocconcelli [c], Chiara Ghiron [c], Christopher L. Kalmar [b], Natasha Singh [b], Ellen C. Merrick [b], Manoj K. Patel [b], Valentina Zuliani [a,*]

[a] Dipartimento Farmaceutico, Università degli Studi di Parma, V.le G.P. Usberti, 27/A, I-43100 Parma, Italy
[b] Department of Anesthesiology, University of Virginia, Charlottesville, VA 22908, USA
[c] Siena Biotech S.p.A., Via Fiorentina, 1, I-53100 Siena, Italy

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ABSTRACT

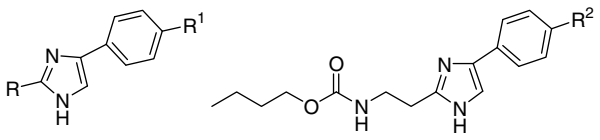
A small family of novel 2,4(5)-diarylimidazoles were prepared through a simple and efficient synthesis and evaluated as potential inhibitors of $hNa_v1.2$ sodium channel currents. One member of this series (**4**) exhibited profound inhibition of $Na_v1.2$ currents, emerging as a promising lead compound for further structure–activity relationship studies for the development of novel sodium channel blockers.

Epilepsy is a common and devastating neurological disorder characterized by recurrent spontaneous seizures within the brain. Current treatment options for patients with epilepsy involve seizure suppression through the use of a myriad of currently available anticonvulsant drugs (AEDs). Unfortunately, a substantial proportion of patients (~30%) continue to experience seizures even in the presence of optimal doses of AEDs. These patients are considered pharmaco-resistant.[1] In addition, many patients that achieve seizure control with medications suffer from medication induced neurotoxicity, sedation, and cognitive side effects.[2] Since voltage-gated sodium (Na) channels play a critical role in the initiation and propagation of action potentials in excitable cells they remain a promising target for the development of new AEDs.

In this letter, we have focused on the synthesis and biological evaluation of a series of imidazoles with functionality in the C2 and C4 positions for potency against Na channels. The imidazole ring system is an important substructure and is found in several pharmacologically active compounds.[3–7] Furthermore, imidazole derivatives are known to be modulators of the Na channel.[8] Structure–activity relationships (SARs) regarding imidazoles substituted in the 4(5) position with a phenyl ring and in the 2 position with an alkyl chain have led to the compounds reported in Chart 1: the derivative with an unsubstituted (R_2 = H) phenyl ring in the 4(5) position and an ethylcarbamate in the 2 position has been previously shown to be a potent site-2 Na channel blocker.[9]

Starting with these compounds, we have extended the SARs by replacing the alkyl chains in position C2 with either a phenyl or a heterocycle ring, characterized by different physico-chemical properties, to develop a novel class of Na channel blockers. Considering that the unsubstituted phenyl ring seems to be the best moiety at the C4 position of the imidazole, we varied the C2 position employing our synthetic protocol, previously shown to be effective in the preparation of a variety of 2,4(5)-diarylimidazoles through one-pot parallel synthesis.[10]

The compounds reported in Table 1 (**3–10**) were prepared through parallel synthesis, starting from the phenylglyoxal **1**, benzaldehydes **2**, ammonium acetate as an ammonia source and a polar protic solvent, such as methanol at room temperature, to afford the best yields in 2,4(5)-diarylimidazoles **3–10** (Scheme 1).



R = alkyl chain
R^1 = H, F, *tert*-butyl, isobutyl, phenyl
R^2 = H, F, Br, CF_3, pyrolidine, phenyl, alkyl chains

Chart 1. Alkylarylimidazoles with Na channel activity.

* Corresponding author.
E-mail address: valentina.zuliani@unipr.it (V. Zuliani).

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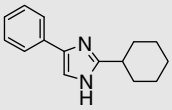
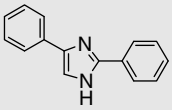
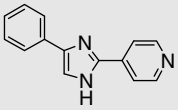
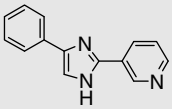
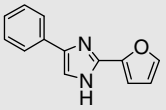
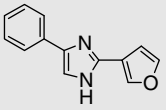
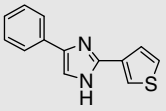
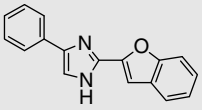
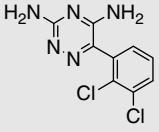
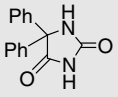
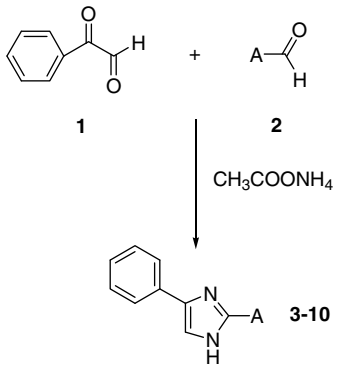
Table 1
Electrophysiogical evaluation of compounds for efficacy against $hNa_V1.2$

No	Compound	Percent block of $hNa_v1.2$ current at 10 μM[a] ($n = 4$)	Percent block of $hNa_v1.2$ current at 100 μM[a] ($n = 4$)
3		26.7 ± 6.1	91.5 ± 1.8
4		7.8 ± 3.2	80.5 ± 3.7
5		8.0 ± 3.3	40.7 ± 3.5
6		4.7 ± 3.8	22.5 ± 8.5
7		2.7 ± 1.1	37.7 ± 3.9
8		11.9 ± 7.9	27.3 ± 10.4
9		5.1 ± 1.8	70.9 ± 2.6
10		37.7 ± 8.2	72.4 ± 10.8
11		12.1 ± 2.6	35.1 ± 3.9
12		10.6 ± 0.7	21.2 ± 3.3

[a] See Ref. 12 for assay details.

Once synthesized, compounds were tested at 10 and 100 μM for inhibition of the $hNa_V1.2$ current, a Na channel isoform expressed in the brain (Table 1). We also tested two clinically used anti-epileptic drugs, lamotrigine (**11**) and phenytoin (**12**), for comparison. In agreement with other studies, the presence of a cyclohexyl in the C2 position of the imidazole core led to a potent Na channel blocker in compound **3**.[7,8] In addition, derivatives with an aryl ring in position C2 (**4–10**) exhibited block of $hNa_V1.2$ currents with differing potencies (2.7–37.7% at 10 μM and 22.5–80.5% at 100 μM). In comparison, lamotrigine and phenytoin were less potent at 100 μM than compounds **3**, **4**, **9**, and **10**. The data obtained for this small series of compounds illustrate an interesting profile of activity, which appears to correlate with the lipophilicity of the substituents introduced in the C2 position of the imidazole. For example, derivatives having polar rings, such as a pyridine, (**5** and **6**) or a furan (**7** and **8**) were shown to have lower potencies.



A: phenyl, 3-pyridine, 4-pyridine, cyclohexyl, 2-furan, 3-furan, 2-benzofuran, 3-thiophene

Scheme 1. Synthesis of the 2,4(5)-diarylimidazoles **3**–**10**.[11]

Potency increased when the furan ring was replaced with the more lipophilic thiophene (compound **9**), and this trend being more evident at the higher concentration of 100 μM. However, the most profound differences were observed with addition of a phenyl ring, either alone or condensed with a heterocycle. For example, comparison of **7** with **10** confirms that addition of a lipophilic group is necessary for increased inhibition of the Na channel current, both at 10 and at 100 μM.

In summary, we report here the synthesis and the biological evaluation of eight new Na channel antagonists against the neuronally expressed human Na channel isoform, $hNa_V1.2$. Each compound has a 4(5)-phenylimidazole core that can incorporate changes at the C2 position of the central heterocyclic moiety. All compounds effectively inhibited the $hNa_V1.2$ current, with those containing a lipophilic group in the C2 position being most potent. Thus, compound **4** represents an attractive lead compound for further optimization by virtue of the simple modulability of the phenyl ring, toward development of further SAR studies, to generate novel anticonvulsant drugs.

Acknowledgments

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11. To a mixture of arylaldehyde (0.70 mmol) and ammomium acetate (3.41 mmol) in methanol (3.5 mL) was added, over a period of 10 min, a solution of phenylglyoxal monohydrate (0.70 mmol) in methanol (3.8 mL). The reaction mixture was stirred overnight at room temperature, then the solvent was evaporated and the residue was partitioned between saturated aqueous

$NaHCO_3$ solution (20 mL) and methylene chloride (20 mL). The organic phase was dried over Na_2SO_4 and the solvent was removed in vacuo. The isolation of the target compounds from the crude reaction mixture was obtained using SCX-2 column (2 g, 30–90 μm, loading 0.4 meq/g). The column is prewashed with DCM/methanol = 1:1 (10 mL), the side products were eluted with methanol (10 mL) and then the desired 2,4(5)-arylimidazoles were eluted with a methanolic ammonia 5% w/w solution (10 mL).

12. *Sodium channel electrophysiology:* Human embryonic kidney (HEK) cells stably expressing human $Na_v1.2$ were a kind gift from Dr. H.A. Hartmann (University of Baltimore, Maryland, USA) and were grown in DMEM/F12 media (Invitrogen, Corp., CA, USA) supplemented with 10% fetal bovine serum, penicillin (100 U/mL), streptomycin (100 μg/mL), and G418 (500 μg/mL; Sigma, MO, USA). Cells were grown in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Sodium currents were recorded using the whole-cell configuration of the patch clamp recording technique with an Axopatch 200 amplifier (Axon Instruments, Foster City, CA). All voltage protocols were applied using pCLAMP 9 software (Axon, USA) and a Digidata 1322A (Axon, USA). Currents were amplified and low pass filtered (2 kHz) and sampled at 33 kHz. Borosilicate glass pipettes were pulled using a Brown-Flaming puller (model P87, Sutter Instruments Co., Novato, CA) and heat polished to produce electrode resistances of 0.5–1.5 MW when filled with the following electrode solution (in mM); CsCl 130, $MgCl_2$ 1, MgATP 5, BAPTA 10, Hepes 5 (pH adjusted to 7.4 with CsOH). Cells were plated on glass coverslips and superfused with solution containing the following composition (in mM): NaCl 130, KCl 4, $CaCl_2$ 1, $MgCl_2$ 5, Hepes 5, and glucose 5 (pH adjusted to 7.4 with NaOH). Compounds were prepared as 100 mM stock solutions in dimethyl sulfoxide (DMSO) and diluted to desired concentration in perfusion solution. The maximum DMSO concentration used was 0.3% and had no effect on current amplitude. All experiments were performed at room temperature (20–22 °C). After establishing whole-cell, a minimum series resistance compensation of 75% was applied. Sodium currents were elicited by a depolarizing step from a holding potential of −100 to +10 mV for a duration of 25 ms at 15 s intervals. Test compounds were applied after a 3 min control period and continued until a steady state current amplitude was observed. All data represents percentage mean block ± standard error of the mean (SEM).