Bioorganic & Medicinal Chemistry 17 (2009) 3642–3648

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

2,4(5)-Diarylimidazoles as inhibitors of hNa$_V$1.2 sodium channels: Pharmacological evaluation and structure–property relationships

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ARTICLE INFO

Article history:
Received 28 October 2008
Revised 27 March 2009
Accepted 31 March 2009
Available online 10 April 2009

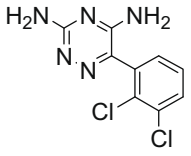
Keywords:
Sodium channel blockers
Electrophysiology
Diarylimidazoles
Physico-chemical properties
hNa$_V$1.2

ABSTRACT

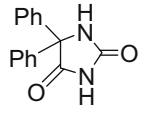
Sodium (Na) channels continue to represent an important target for the development of novel anticonvulsants. We have synthesized and evaluated a series of 2,4(5)-diarylimidazoles for inhibition of the human neuronal Na$_V$1.2 Na channel isoform. Starting with the unsubstituted lead compound previously published **3**, SAR studies were performed introducing substituents with different physico-chemical properties. Lipophilicity (log $D_{7.4}$) and basicity (pK_a) of the compounds were measured and submitted for QSPR investigations. Some of the active compounds described had IC_{50} values that were considerably lower than our lead compound. In particular, the *m*-CF_3 disubstituted **22** was the most active compound, inhibiting hNa$_V$1.2 currents within the nanomolar concentration range (IC_{50} = 200 nM). In comparison, lamotrigine and phenytoin, two clinically used anticonvulsant drugs known to inhibit Na channels, had IC_{50}'s values that were greater than 100 μM.

1. Introduction

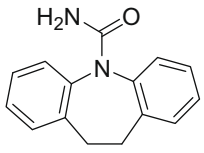
Epilepsy is a devastating neurological disorder that afflicts approximately 2% of the world's population.[1] Current treatment options for epilepsy focus on the suppression of seizures with the use of antiepileptic drugs (AEDs) which act on a diverse number of molecular targets, including voltage-gated ion channels (sodium, calcium and potassium), ligand-gated ion channels (mainly receptors for GABA and glutamate), neurotransmitters (mainly GABA and glutamate) and their receptors, neurotransmitter transporters and enzymes.[2,3] Particular focus has been directed toward targeting voltage-gated sodium (Na) channels since they play a fundamental role in establishing and regulating the excitability of neurons within the central nervous system (CNS). The Na channel isoforms Na$_V$1.1, Na$_V$1.2, Na$_V$1.3 and Na$_V$1.6 are known to be widely expressed within the CNS.[4] These isoforms mediate the fast transient Na current that generate the upstroke of the action potential in excitable cells, and in some neurons also induce a late persistent Na current. Changes in the expression pattern and behavior of these isoforms are known to occur in epilepsy and could play a role in seizure generation and spread.[5–7] The Na$_V$1.2 is an important isoform for drug targeting since it is abundantly expressed within the CNS, with specific expression along neuronal axons, close to presynaptic release sites.[8] Furthermore, mutations in *SCN2A*, the gene encoding Na$_V$1.2, have been identified in patients with generalized epilepsy.[9] In view of this, it is not surprising that a number of the clinically available AEDs, including lamotri-



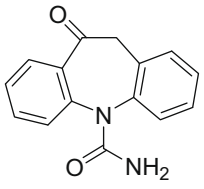
lamotrigine



phenytoin



carbamazepine



oxcarbazepine

Chart 1. Clinically used anticonvulsant drugs.

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doi:10.1016/j.bmc.2009.03.067

gine, phenytoin, carbamazepine and oxcarbazepine (Chart 1), target Na channels as a major mechanism of their action and have been shown to inhibit $Na_V1.2$ Na channel currents.[3,10–12]

We recently reported the synthesis and evaluation of a novel series of 2,4(5)-diarylimidazoles against $hNa_V1.2$ Na channel currents. These compounds were characterized by a 4(5) -phenylimidazole moiety with different rings as substituents in the C2 position (Fig. 1).[13] The derivative with two phenyl rings connected to the central heterocyclic moiety resulted in an attractive lead compound for further optimization by virtue of its activity against $Na_V1.2$ and of the simple modulability of the benzene portion (compound **3**). Starting from this lead, we have synthesized two subsets of derivatives (**4**–**11**: substitutions on the phenyl ring at C2 position; **12**–**19**: substitutions on the phenyl ring at C4 position), varying their physico-chemical properties (ionization, lipophilicity) through the introduction of different substituents in *meta* and *para* position of both the phenyl rings of the lead compound **3**.

The molecules discussed herein were prepared using a previously described simple and straightforward procedure (Scheme 1).[14] Compounds were evaluated for inhibition of $hNa_V1.2$ Na channel currents by patch-clamp electrophysiology. Relevant physico-chemical properties ($\log D_{oct,7.4}$ and pK_a) were also measured employing the shake-flask technique and the potentiometric pH-metric method, respectively. These properties were employed in quantitative structure–property relationship (QSPR) analysis.

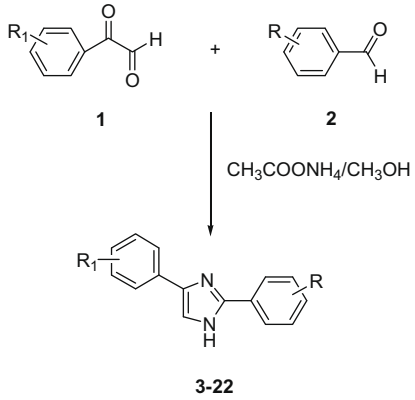
2. Results and discussion

2.1. Pharmacological activity

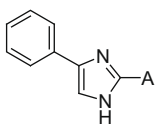
All compounds were initially screened for activity against the human $Na_V1.2$ Na channel isoform at 10 μM and 100 μM. Table 1 highlights the correlation between ring substitutions and inhibition of $hNa_V1.2$ channel currents. For comparison purposes, two clinically used anti-epileptic drugs, lamotrigine (**23**) and phenytoin (**24**) were also tested. The table shows that many of the substituted derivatives synthesized demonstrated greater activity against $hNa_V1.2$ compared to the unsubstituted lead compound **3** and to the clinically used AEDs lamotrigine and phenytoin.

At a concentration of 100 μM all of the compounds reported showed either comparable or greater activity against $hNa_V1.2$ Na currents when compared to the reference compounds lamotrigine (**23**) and phenytoin (**24**), and only few of them proved to be less active than our lead compound **3**. At the lower concentration of 10 μM, compounds **4**, **6**, **8**, **10, 11**, **16**, **18**, **19** exhibited greater block of $Na_V1.2$ compared to our lead compound **3**. Values ranged between 30.1% and 92.5% versus 7.8% as recorded for compound **3**. Furthermore, a significant difference in the inhibition of the $hNa_V1.2$ current was observed for the two subsets of compounds synthesized (**4**–**11** and **12**–**19**). For compounds **4–9**, substitution at the *para*-position was correlated with greater activity against $hNa_V1.2$ compared to substitution at the *meta*-position (compounds **4**, **6**, **8** vs compounds **5**, **7**, **9**). Furthermore, the greatest inhibition of $hNa_V1.2$ was observed for the compounds substituted with a nitro or chloro group in the *para* position (**4**, **8**: a 7–10-fold increase in activity compared to compound **3**), while substitution with a methoxy group, as in compound **6,** increased the activity by almost fourfold**.** While an increase in activity was generally observed for both electron-withdrawing and donating groups in the *para* position, it is unclear why the introduction of a trifluoromethyl led to the *meta*-substituted compound being more potent than the *para*-substituted one (**11** and **10**, respectively).

Compounds **12–19** were synthesized to explore the effects of adding substituents to the phenyl ring at position C4 of the imidazole. In general, the impact of this substitution pattern at the lower concentration seemed to be less favorable for activity against $hNa_V1.2$. For example, substitution at the *meta*-position (compounds **13**, **15**, **17**) led to compounds with activity against $hNa_V1.2$



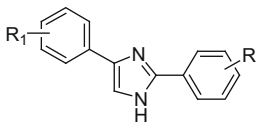
Scheme 1. Synthesis of the 2,4(5)-diarylimidazoles **3**–**22**.



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

Figure 1. 4(5)-Phenylimidazoles having in the C2 position different rings.

Table 1
Electrophysiological evaluation of compounds for efficacy against $hNa_v1.2$



3-22

No.	R	R_1	Percent block of $hNa_v1.2$ current at 10 μM ($n = 4$)	Percent block of $hNa_v1.2$ current at 100 μM ($n = 4$)
3	H	H	7.8 ± 3.2	80.5 ± 3.7
4	*p*-NO_2	H	57.1 ± 9.6	92.6 ± 4.3
5	*m*-NO_2	H	N.A.[a]	20.4 ± 7.5
6	*p*-OCH_3	H	30.1 ± 4.4	89.9 ± 2.7
7	*m*-OCH_3	H	N.A.[a]	41.7 ± 2.3
8	*p*-Cl	H	92.5 ± 2.1	100
9	*m*-Cl	H	7.5 ± 2.8	92.2 ± 4.3
10	*p*-CF_3	H	22.7 ± 3.5	66.8 ± 15.6
11	*m*-CF_3	H	49.2 ± 10.2	95.2 ± 1.8
12	H	*p*-NO_2	N.A.[a]	30.3 ± 3.3
13	H	*m*-NO_2	15.6 ± 2.8	85.8 ± 3.6
14	H	*p*-OCH_3	N.A.[a]	33.3 ± 4.1
15	H	*m*-OCH_3	14.2 ± 9.5	35.6 ± 2.2
16	H	*p*-Cl	31.5 ± 3.4	96.6 ± 1.1
17	H	*m*-Cl	16.8 ± 1.9	93.9 ± 0.5
18	H	*p*-CF_3	91.6 ± 3.1	100
19	H	*m*-CF_3	82.9 ± 3.8	100
20	*p*-Cl	*m*-CF_3	27.5 ± 4.8	43.6 ± 8.3
21	*p*-NO_2	*m*-CF_3	86.9 ± 2.8	100
22	*m*-CF_3	*m*-CF_3	100	100
23	Lamotrigine		12.1 ± 2.6	35.1 ± 3.9
24	Phenytoin		10.6 ± 0.7	21.2 ± 3.3

[a] NA: Not active.

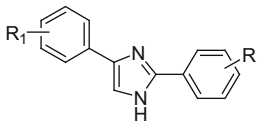
that was comparable to the lead compound **3** and the clinically active compounds lamotrigine (**23**) and phenytoin (**24**). In contrast to *para*-position substitution of NO_2 or OCH_3 on the phenyl ring at position C2, the same substitutions on the phenyl ring at position C4 were detrimental for activity against $hNa_v1.2$, leading to complete lack of inhibition at 10 μM (**12**, **14**). Two exceptions were the CF_3 derivative compounds **18** and **19**, that demonstrated greater inhibition of $hNa_v1.2$ Na currents even at 10 μM (91.6% and 82.9%, respectively) compared to the other compounds in the series (**12–17**).

In view of these findings, we synthesized three novel hybrid compounds, maintaining the *m*-CF_3 substitution on the phenyl ring in the C4 of the imidazole, and introducing on the other benzene substituents (*p*-Cl, *p*-NO_2, *m*-CF_3) that clearly demonstrate enhance activity against $hNa_v1.2$ Na channel currents (**20–22**). These compounds were designed and synthesized to explore the possibility of an additional effect of these substitutions, leading to more active compounds. In agreement with the data previously shown, two of the new molecules synthesized (**21** and **22**) were more active against hNav1.2, exhibiting profound inhibition of Na currents. In contrast, compound **20**, that combine two of the best features of the R and R_1 substitutions (*p*-Cl, *m*-CF_3) showed very little activity against hNav1.2. Values recorded were similar to those obtained for lamotrigine (**23**) and phenytoin (**24**).

To further evaluate the activity of our most active compounds (**8**, **18–22**) different concentrations were tested against hNav1.2 and compared with our lead compound and the reference compounds lamotrigine and phenytoin (**3**, **23, 24**; Fig. 2). Calculated IC_{50}'s are shown in Table 2. All the compounds tested were full antagonists and most of them had IC_{50} values in the micromolar range between 1.4 and 2.7 μM. All compounds, except compound **20**, had greater potency when compared to the two clinically used compounds lamotrigine (**23**) and phenytoin (**24**). Moreover, the hybrid compound **22** exhibited profound inhibition of hNav1.2, having nanomolar activity (IC_{50} = 200 nM), thus demonstrating the importance of the *m*-CF_3 substitution on both the phenyl rings connected to the central imidazole core.

Correlations between pharmacological activity and experimental physico-chemical properties of the monosubstituted compounds **3–19**, for example, ionization behavior, modeled by pK_a values, and apparent lipophilicity at pH 7.4, modeled by distribution coefficients (log $D_{oct,\ 7.4}$) were sought; however, no apparent correlation pattern was observed. In general, for both C2 and C4 substituted derivatives, substitutions with electron-withdrawing substituents, leading to reduced basicity of the central imidazole ring, was favorable for increased activity against $hNa_v1.2$ (e.g., compounds **4**, **8**, **16**, **18**). Introduction of a OCH_3 group in the *para*-position of the phenyl ring in C2 (**6**), led to a 0.3 log unit increase in pK_a, and caused a greater inhibition of $hNa_v1.2$ with respect to compound **3**, in contrast with the same substitution on the benzene in the C4 position of imidazole ring (**14**). It is unclear why substituting with CF_3 afforded greater inhibition of $hNa_v1.2$. It is possible that the CF_3 substitution allows for a more stable interaction with the Na channel binding pocket, allowing for stabilization of the inactivated state.

Table 2
IC_{50} Values of some selected compounds for potency against $hNa_v1.2$



3, **8**, **18-22**

No.	R	R_1	IC_{50} (μM)	*n*
3	H	H	40.6	3–5
8	*p*-Cl	H	1.4	4
18	H	*p*-CF_3	1.4	4–7
19	H	*m*-CF_3	2.7	4
20	*p*-Cl	*m*-CF_3	>100	5–6
21	*p*-NO_2	*m*-CF_3	2.5	4
22	*m*-CF_3	*m*-CF_3	0.2	3–5
23	Lamotrigine		>100	3
24	Phenytoin		>100	5

n = Number of cells tested.

2.2. Ionization constants (pK_a) and lipophilicity (log $D_{oct,\ 7.4}$)

We also investigated the ionization and the lipophilicity of the series of 2,4(5)-diarylimidazoles synthesized **3–19**. The experi-

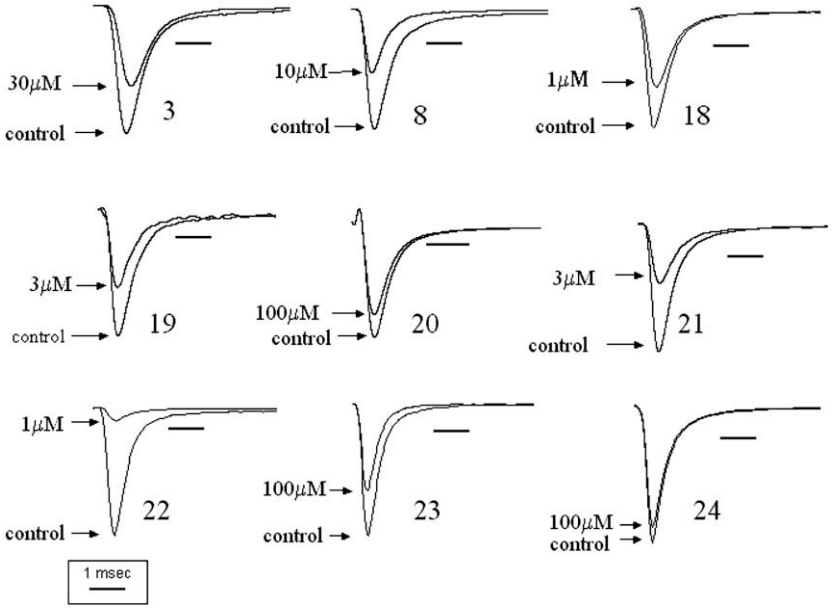
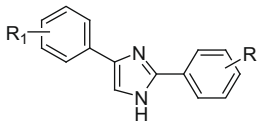


Figure 2. Example traces demonstrating block of $hNa_v1.2$ sodium channel currents.

Table 3
Partition coefficients ($\log D_{oct,7.4}$), dissociation constants (pK_a) and substituent constants[15,16] employed for QSPR models



3-19

No.	R	R_1	pK_a	$\log D_{oct,7.4}$	σ	π
3	H	H	5.65 ± 0.09	3.72 ± 0.05	0	0
4	*p*-NO_2	H	4.74 ± 0.14	4.06 ± 0.06	0.78	−0.28
5	*m*-NO_2	H	4.92 ± 0.09	3.89 ± 0.02	0.71	−0.28
6	*p*-OCH_3	H	6.03 ± 0.12	3.62 ± 0.02	−0.27	−0.02
7	*m*-OCH_3	H	5.57 ± 0.03	3.84 ± 0.04	0.12	−0.02
8	*p*-Cl	H	5.37 ± 0.11	4.27 ± 0.18	0.23	0.71
9	*m*-Cl	H	5.12 ± 0.08	4.54 ± 0.04	0.37	0.71
10	*p*-CF_3	H	5.19 ± 0.10	4.90 ± 0.07	0.54	0.88
11	*m*-CF_3	H	5.11 ± 0.15	4.54 ± 0.02	0.43	0.88
12	H	*p*-NO_2	5.12 ± 0.12	3.95 ± 0.06	0.78	−0.28
13	H	*m*-NO_2	4.82 ± 0.05	3.90 ± 0.05	0.71	−0.28
14	H	*p*-OCH_3	5.83 ± 0.03	3.66 ± 0.01	−0.27	−0.02
15	H	*m*-OCH_3	5.58 ± 0.07	3.74 ± 0.02	0.12	−0.02
16	H	*p*-Cl	5.47 ± 0.04	4.53 ± 0.11	0.23	0.71
17	H	*m*-Cl	5.45 ± 0.07	4.76 ± 0.07	0.37	0.71
18	H	*p*-CF_3	5.31 ± 0.10	4.78 ± 0.03	0.54	0.88
19	H	*m*-CF_3	5.27 ± 0.15	4.72 ± 0.02	0.43	0.88

mentally determined constants pK_a and the $\log D_{oct,7.4}$ values are shown in Table 3, together with the substituent constants employed for the QSPR model, reported in the compilation of Skagerberg and in that of van de Waterbeemd and Testa.[15,16]

Compared to the pK_a of imidazole nucleus ($pK_a \sim 7$)[17], the electron-withdrawing effect of the aryl substitution at the 2- and 4(5)-position decreased the basicity of the central imidazole ring by 1.35 log units on average. As expected, the introduction of substituents in the *para*- and *meta*-positions of phenyl ring produced significative variations in the ionization behavior and lipophilicity of the set. For both subsets of 2- and 4(5)-substituted diarylimidazoles the variation in pK_a values could be correlated with the electronic constants (σ) of aromatic substituents:

$$pK_a = -1.15(\pm 0.09)\sigma + 5.67(\pm 0.04)$$
$$n = 9; \quad r^2 = 0.960; \quad s = 0.09; \quad F = 168.6 \qquad (1)$$

for 2-substituted diarylimidazoles

$$pK_a = -1.05(\pm 0.16)\sigma + 5.98(\pm 0.85)$$
$$n = 8; \quad r^2 = 0.865; \quad s = 0.13; \quad F = 44.9 \qquad (2)$$

for 4(5)-substituted diarylimidazoles

Thus for example, the introduction of electron-withdrawing NO_2 in the *para*-position (**4**) lowered the pK_a value of the imidazole ring by nearly 1 log unit, corresponding to a 10-fold increase in the concentration of unionized species of the imidazole nucleus at physiological pH (pH 7.4) compared to compound **3**.

Distribution coefficients at pH 7.4, accounting for lipophilicity of both ionized and unionized species of the imidazole nucleus[18] were roughly correlated to Hansch π values for aromatic substituents:

$$\mathrm{Log}\, D_{oct,7.4} = 0.81(\pm 0.11)\pi + 3.96(\pm 0.06)$$
$$n = 17; \quad r^2 = 0.783; \quad s = 0.22; \quad F = 54.03 \qquad (3)$$

Thus for example, the lipophilicity of unsubstituted **3** was increased by almost one unit with the introduction of the hydrophobic CF_3 (**10**, **18**). The introduction in the correlation equation of the substituent electronic constants σ *(Eq. 4)* or of experimental pK_a values (Eq. 5), measured by potentiometric pH-metric method, significantly improved the fitting, as suggested by log *D* definition.[15]

$$\log D_{oct,7.4} = 0.81(\pm 0.05)\pi + 0.56(\pm 0.08)\sigma + 3.76(\pm 0.04) \quad n = 17;$$
$$r^2 = 0.951; \quad s = 0.11; \quad F = 134.70 \qquad (4)$$

$$\log D_{oct,7.4} = 0.83(\pm 0.07)\pi - 0.46(\pm 0.10)pK_a + 6.43(\pm 0.54) \quad n = 17;$$
$$r^2 = 0.912; \quad s = 0.14; \quad F = 72.41 \qquad (5)$$

The combination of an intermediate lipophilicity with a molecular weight lower than 500 and with a reduced number of H-bond donors (less than 5) and acceptors (less than 10) allow to apply the 'drug-like' definition[19] to this set of derivatives.

3. Conclusions

In this study we have synthesized and tested a series of 19 analogues (**4–22**) starting with a previously published lead compound scaffold (**3**). Substituents with different physico-chemical properties were introduced to the *meta*- and *para*-positions of both phenyl rings to evaluate the effects of benzene ring modulation on the inhibition of $hNa_v1.2$ Na channel currents. Many of the compounds synthesized (**4**, **6**, **8**, **11**, **16**, **18–22**) exhibited greater inhibition of $hNa_v1.2$ compared to the lead compound (**3**) and to two clinically used anticonvulsant drugs, lamotrigine (**23**) and phenytoin (**24**), with IC_{50} values in the nanomolar-micromolar range. In particular, the hybrid compound **22** exhibited profound activity against $hNa_v1.2$ recording an IC_{50} value of 200 nM, 500-fold more potent than lamotrigine and phenytoin. Despite the good correlation between the measured physico-chemical properties (pK_a and $\log D_{7.4}$) and the substituents constants (σ and π) of the monosubstituted compounds **3–19**, no clear correlation between the substituents and biological activities of the compounds could be established. It is possible that perhaps these compounds allow for more specific interactions between themselves and the amino acids within the binding pocket of the Na channel, stabilizing the channel in a non-conducting state. Future studies investigating the state selectivity of some of the more potent compounds may provide further insight into the mechanisms of action of these novel derivatives and their potential for AED activity.

4. Experimental

4.1. General methods

Melting points are not corrected and were determined using a Gallenkamp melting point apparatus. Final compounds were synthesized in parallel using Büchi Syncore® Reactor and were analyzed on a ThermoQuest (Italia) FlashEA 1112 Elemental Analyzer, for C, H, N. The percentages recorded were within ±0.4% of the theoretical values. The ^{1}H NMR spectra were recorded on a Bruker 300 Avance spectrometer (300 MHz); chemical shifts (δ scale) are reported in parts per million (ppm) relative to the central peak of the solvent. ^{1}H NMR Spectra are reported in order: multiplicity, approximate coupling constant (*J* value) in hertz (Hz) and number of protons; signals were characterized as s (singlet), d (doublet), t (triplet), m (multiplet), br s (broad signal). Abbreviations are the following: Ph, phenyl; Im, imidazolyl. The ^{13}C NMR spectra were recorded on a VARIAN Mercury Plus (100 MHz); chemical shifts (δ scale) are reported in parts per million (ppm). Mass spectra were recorded using a Applied Biosystem/MDS SCIEX, Foster City, CA, USA instrument. Selective isolation of either non-basic or basic compounds was obtained using ISOLUTE®

SCX-2 columns (Particles size: 30–90 μM). Reactions were monitored by TLC, on Kieselgel 60 F 254 (DC-Alufolien, Merck).

4.2. General procedure for the synthesis of 2,4(5)-diarylimidazoles 3–22

A solution of phenylglyoxal monohydrate (0.70 mmol) in methanol (3.8 mL) was added dropwise to a stirred suspension of arylaldehyde (0.70 mmol) and ammomium acetate (3.41 mmol) in methanol (3.5 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 mequiv/g). The column was 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). All the products were then crystallized as hydrochloride from abs. ethanol/diethyl ether.

4.2.1. 2,4(5)-Diphenylimidazole (3)[14,20]

83% Yield, mp 274–275 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.46 (t, J = 7.23, 1H, Ph), 7.55 (t, J = 7.68, 2H, Ph), 7.66 (t, J = 3.24, 3H, Ph), 8.05 (d, J = 7.35, 2H, Im), 8.30–8.33 (m, 3H, Ph+Im). ^{13}C NMR (100 MHz, DMSO-d_6): δ 117.3, 123.9, 126.6, 127.7, 128.1, 129.7, 129.8, 129.9, 132.6, 134.5, 145.0. MS (EI) 221 [M$^+$]. Anal. Calcd for ($C_{15}H_{12}N_2$·HCl): C, 70.17; H, 5.10; N, 10.91. Found: C, 69.85; H, 5.15; N, 10.59.

4.2.2. 2-(4-Nitrophenyl)-4(5)-phenylimidazole (4)[14,21]

68% Yield, mp 253–257 °C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.43 (t, J = 7.29, 1H, Ph), 7.53 (t, J = 7.74, 2H, Ph), 8.03 (d, J = 7.32, 2H, Ph), 8.31 (s, 1H, Im), 8.45–8.54 (m, 4H, Ph). ^{13}C NMR (100 MHz, DMSO-d_6): δ 118.6, 125.0, 126.4, 128.8, 129.7, 143.2, 149.0. MS (EI) 266 [M$^+$]. Anal. Calcd for ($C_{15}H_{11}N_3O_2$·HCl·1/2H_2O): C, 57.97; H, 4.21; N, 13.52. Found: C, 57.98; H, 4.15; N, 13.35.

4.2.3. 2-(3-Nitrophenyl)-4(5)-phenylimidazole (5)[14]

59% Yield, mp 269–272 °C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.45 (t, J = 7.50, 1H, Ph), 7.55 (t, J = 7.50, 2H, Ph), 7.94 (t, J = 8.10, 1H, Ph), 8.02 (d, J = 7.80, 2H, Ph), 8.31 (s, 1H, Im), 8.44 (d, J = 9.00, 1H, Ph), 8.74 (d, J = 7.80, 1H, Ph), 9.14 (s, 1H, Ph). ^{13}C NMR (100 MHz, DMSO-d_6): δ 118.0, 122.5, 126.2, 126.4, 129.7, 131.6, 133.9, 143.2, 148.9. MS (EI) 266 [M$^+$]. Anal. Calcd for ($C_{15}H_{11}N_3O_2$·HCl): C, 59.71; H, 4.01; N, 13.93. Found: C, 59.34; H, 3.75; N, 13.74.

4.2.4. 2-(4-Methoxyphenyl)-4(5)-phenylimidazole (6)[14,22]

57% Yield, mp 250–253 °C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.89 (s, 3H, OCH_3), 7.23 (d, J = 8.88, 2H, Ph), 7.45 (t, J = 7.29, 1H, Ph), 7.55 (t, J = 7.68, 2H, Ph), 8.01 (d, J = 7.38, 2H, Ph), 8.23 (s, 1H, Im), 8.25 (d, 2H, Ph). ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.3, 115.4, 116.2, 116.7, 126.5, 129.7, 129.9, 134.0, 145.3, 162.7. MS (EI) 251 [M$^+$]. Anal. Calcd for ($C_{16}H_{14}N_2O$·HCl): C, 67.02; H, 5.27; N, 9.77. Found: C, 66.78; H, 5.19; N, 9.55.

4.2.5. 2-(3-Methoxyphenyl)-4(5)-phenylimidazole (7)[14]

62% Yield, mp 215–217 °C (dec.) ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.91 (s, 3H, OCH_3), 7.21 (dd, J = 8.70, J = 2.40, 1H, Ph), 7.47 (t, J = 6.90, 1H, Ph), 7.52–7.59 (m, 3H, Ph), 7.88 (d, J = 7.80, 1H, Ph), 8.00 (s, 1H, Ph), 8.06 (d, J = 7.20, 2H, Ph), 8.29 (s, 1H, Im). ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.5, 113.2, 117.3, 118.8, 120.1, 125.1, 126.7, 127.7, 129.7, 129.8, 131.1, 134.6, 144.9, 160.3. MS (EI) 251 [M$^+$]. Anal. Calcd for ($C_{16}H_{14}N_2O$·HCl) C, 67.02; H, 5.27; N, 9.77. Found: C, 66.86; H, 5.20; N, 9.73.

4.2.6. 2-(4-Chlorophenyl)-4(5)-phenylimidazole (8)[14,23]

60% Yield, mp 263–265 °C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.45 (t, J = 7.20, 1H, Ph), 7.54 (t, J = 7.50, 2H, Ph), 7.77 (d, J = 8.70, 2H, Ph), 8.01 (d, J = 7.50, 2H, Ph), 8.27–8.30 (m, 3H, Ph+Im). ^{13}C NMR (100 MHz, DMSO-d_6): δ 117.5, 123.3, 126.5, 128.0, 129.7, 129.8, 130.0, 135.0, 137.2, 144.1. MS (EI) 255 [M$^+$]. Anal. Calcd for ($C_{15}H_{11}ClN_2$·HCl): C, 62.06; H, 4.17; N, 9.66. Found: C, 61.78; H, 4.01; N, 9.90.

4.2.7. 2-(3-Chlorophenyl)-4(5)-phenylimidazole (9)[14]

56% Yield, mp 218–219 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.45 (t, J = 7.35, 1H, Ph), 7.55 (t, J = 7.71, 2H, Ph), 7.66–7.70 (m, 2H, Ph), 7.99 (d, J = 7.35, 2H, Ph), 8.16–8.21 (m, 1H, Ph), 8.26 (s, 1H, Im), 8.36 (s, 1H, Ph). ^{13}C NMR (100 MHz, DMSO-d_6): δ 117.6, 126.2, 126.6, 127.7, 127.9, 129.7, 129.9, 131.8, 132.1, 134.6, 135.1, 143.6. MS (EI) 255 [M$^+$]. Anal. Calcd for ($C_{15}H_{11}ClN_2$·HCl·1/2H_2O): C, 60.19; H, 4.38; N, 9.37. Found: C, 59.88; H, 3.99; N, 9.26.

4.2.8. 2-(4-Trifluoromethylphenyl)-4(5)-phenylimidazole (10)[14]

74% Yield, mp 262–264 °C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.45 (t, J = 7.32, 1H, Ph), 7.54 (t, J = 7.68, 2H, Ph), 8.04 (dd, J = 8.73, J = 1.95, 4H, Ph), 8.31 (s, 1H, Im), 8.48 (d, J = 8.13, 2H, Ph). ^{13}C NMR (100 MHz, DMSO-d_6): δ 118.0, 125.8, 126.5, 126.8, 128.7, 129.7, 131.7, 135.8, 143.6. MS (EI) 289 [M$^+$]. Anal. Calcd for ($C_{16}H_{11}F_3N_2$·HCl): C, 59.18; H, 3.72; N, 8.63. Found: C, 58.98; H, 3.81; N, 8.22.

4.2.9. 2-(3-Trifluoromethylphenyl)-4(5)-phenylimidazole (11)[14]

66% Yield, mp 260–261 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.45 (t, J = 7.29, 1H, Ph), 7.55 (t, J = 7.71, 2H, Ph), 7.90 (t, J = 7.89, 1H, Ph), 7.99–8.03 (m, 3H, Ph), 8.30 (s, 1H, Im), 8.58 (d, J = 7.77, 1H, Ph), 8.67 (s, 1H, Ph). ^{13}C NMR (100 MHz, DMSO-d_6): δ 117.8, 124.8, 125.7, 126.5, 128.2, 128.5, 129.7, 130.4, 130.7, 131.1, 131.8, 135.4, 143.7. MS (EI) 289 [M$^+$]. Anal. Calcd for ($C_{16}H_{11}F_3N_2$·HCl): C, 59.18; H, 3.72; N, 8.63. Found: C, 59.17; H, 3.70; N, 8.27.

4.2.10. 2-Phenyl-4(5)-(4-nitrophenyl)imidazole (12)[14,21]

65% Yield, mp 285–290 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ *7.63–7.65* (m, 3H, Ph), 8.27–8.39 (m, 6H, Ph), 8.50 (s, 1H, Im). ^{13}C NMR (100 MHz, DMSO-d_6): δ 120.0, 124.9, 127.1, 127.9, 129.9, 132.3, 146.5, 147.5. MS (EI) 266 [M$^+$]. Anal. Calcd for ($C_{15}H_{11}N_3O_2$·HCl·1/2H_2O): C, 58.05; H, 4.23; N, 13.55. Found: C, 57.73; H, 4.28; N, 13.23.

4.2.11. 2-Phenyl-4(5)-(3-nitrophenyl)imidazole (13)[14,24]

52% Yield, mp 270–273 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ *7.65–7.67* (m, 3H, Ph), 7.83 (t, J = 8.01, 1H, Ph), 8.26–8.32 (m, 3H, Ph), 8.52–8.53 (m, 2H, Ph+Im), 8.92 (s, 1H, Ph). ^{13}C NMR (100 MHz, DMSO-d_6): δ 119.0, 120.8, 124.0, 124.5, 128.0, 129.9, 131.3, 132.5, 133.1, 145.8, 149.0. MS (EI) 266 [M$^+$]. Anal. Calcd for ($C_{15}H_{11}N_3O_2$·HCl): C, 59.71; H, 4.01; N, 13.93. Found: C, 59.31; H, 3.98; N, 13.57.

4.2.12. 2-Phenyl-4(5)-(4-methoxyphenyl)imidazole (14)[14,25]

70% Yield, mp 255–259 °C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.84 (s, 3H, OCH_3), 7.12 (d, J = 9.00, 2H, Ph), 7.66–7.68 (m, 3H, Ph), 7.94 (d, J = 9.00, 2H, Ph), 8.16 (s, 1H, Im), 8.20–8.23 (m, 2H, Ph). ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.0, 115.1, 116.0, 120.1, 124.0, 128.0, 128.2, 129.9, 132.5, 134.6, 144.5, 160.6. MS (EI) 251 [M$^+$]. Anal. Calcd for ($C_{16}H_{14}N_2O$·HCl): C, 67.02; H, 5.27; N, 9.77. Found: C, 66.68; H, 5.32; N, 9.64.

4.2.13. 2-Phenyl-4(5)-(3-methoxyphenyl)imidazole (15)[14,26]

57% Yield, mp 228–231 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.87 (s, 3H, OCH_3), 7.02 (dd, J = 8.19, J = 1.98, 1H, Ph), 7.44 (t, J = 8.07, 1H, Ph), 7.60 (d, J = 7.92, 1H, Ph), 7.65–7.70 (m, 4H, Ph), 8.29–8.33 (m, 3H, Ph+Im). ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.2, 112.0, 115.7, 117.5, 118.8, 124.0, 128.2, 129.0, 129.9, 130.9, 132.6, 134.5, 145.0, 160.4. MS (EI) 251 [M^+]. Anal. Calcd for ($C_{16}H_{14}N_2O$·HCl): C, 67.02; H, 5.27; N, 9.77. Found: C, 66.70; H, 5.11; N, 9.55.

4.2.14. 2-Phenyl-4(5)-(4-chlorophenyl)imidazole (16)[14,27]

76% Yield, mp 277–280 °C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6): δ *7.61–7.67* (m, 5H, Ph), 8.07 (d, J = 8.58, 2H, Ph), 8.27–8.30 (m, 2H, Ph), 8.32 (s, 1H, Im). ^{13}C NMR (100 MHz, DMSO-d_6): δ 117.8, 124.0, 126.9, 128.1, 128.3, 129.7, 130.0, 132.6, 133.6, 134.3, 145.2. MS (EI) 255 [M^+]. Anal. Calcd for ($C_{15}H_{11}ClN_2$·HCl·1/2H_2O): C, 60.19; H, 4.38; N, 9.37. Found: C, 59.87; H, 3.99; N, 9.35.

4.2.15. 2-Phenyl-4(5)-(3-chlorophenyl)imidazole (17)[14]

83% Yield, mp 253–257 °C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6): δ *7.49–7.60* (m, 2H, Ph), 7.66 (t, J = 3.30, 3H, Ph), 8.04 (d, J = 7.59, 1H, Ph), 8.21 (s, 1H, Ph), 8.29–8.32 (m, 2H, Ph), 8.39 (s, 1H, Im). ^{13}C NMR (100 MHz, DMSO-d_6): δ 118.2, 124.1, 125.1, 126.1, 128.1, 129.4, 130.1, 129.9, 131.6, 132.6, 133.3, 134.5, 145.4. MS (EI) 255 [M^+]. Anal. Calcd for ($C_{15}H_{11}ClN_2$·HCl): C, 62.06; H, 4.17; N, 9.66. Found: C, 61.70; H, 4.12; N, 9.57.

4.2.16. 2-Phenyl-4(5)-(4-trifluoromethylphenyl)imidazole (18)[14,25]

65% Yield, mp 265–268 °C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6): δ *7.65–7.67* (m, 3H, Ph), 7.91 (d, J = 7.65, 2H, Ph), 8.28 (m, 4H, Ph), 8.44 (s, 1H, Im). ^{13}C NMR (100 MHz, DMSO-d_6): δ 118.9, 124.4, 126.6, 127.0, 128.1, 130.0, 132.5, 133.6, 145.9. MS (EI) 289 [M^+]. Anal. Calcd for ($C_{16}H_{11}F_3N_2$·HCl·1/2H_2O): C, 57.65; H, 3.93; N, 8.41. Found: C, 57.27; H, 3.74; N, 8.12.

4.2.17. 2-Phenyl-4(5)-(3-trifluoromethylphenyl)imidazole (19)[14,28]

81% Yield, mp 231–235 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ *7.66–7.68* (m, 3H, Ph), 7.75–7.82 (m, 2H, Ph), 8.27–8.31 (m, 2H, Ph), 8.36 (br s, 1H, Ph), 8.47 (s, 2H, Ph+Im). ^{13}C NMR (100 MHz, DMSO-d_6): δ 118.5, 123.0, 124.3, 125.9, 128.0, 129.9, 130.4, 130.8, 132.6, 133.4, 145.6. MS (EI) 289 [M^+]. Anal. Calcd for ($C_{16}H_{11}F_3N_2$·HCl): C, 59.18; H, 3.72; N, 8.63. Found: C, 58.84; H, 4.01; N, 8.65.

4.2.18. 2-(4-Chlorophenyl)-4(5)-(3-trifluoromethylphenyl)-imidazole (20)

71% Yield, mp 268–271 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ *7.73–7.78* (m, 4H, Ph), 8.29–8.35 (m, 3H, Ph), 8.43 (s, 2H, Ph+Im). ^{13}C NMR (100 MHz, DMSO-d_6): δ 115.5, 118.08, 122.3, 129.1, 129.5, 129.6, 130.3, 144.2. MS (EI) 323 [M^+]. Anal. Calcd for ($C_{16}H_{10}F_3N_2Cl$·HCl·1/2H_2O): C, 52.19; H, 3.29; N, 7.61. Found: C, 52.20; H, 3.17; N, 7.44.

4.2.19. 2-(4-Nitrophenyl)-4(5)-(3-trifluoromethylphenyl)-imidazole (21)

84% Yield, mp 255–256 °C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6): δ *7.71–7.73* (m, 2H, Ph), 8.27–8.43 (m, 7H, Ph+Im). ^{13}C NMR (100 MHz, DMSO-d_6): δ 119.1, 124.5, 127.4, 130.2, 143.7. MS (EI) 334 [M^+]. Anal. Calcd for ($C_{16}H_{10}F_3N_3O_2$·HCl): C, 51.98; H, 3.00; N, 11.37. Found: C, 51.73; H, 3.05; N, 10.99.

4.2.20. 2-(3-Trifluoromethylphenyl)-4(5)-(3-trifluoromethylphenyl)-imidazole (22)

67% Yield, mp 250–251 °C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6): δ *7.74–7.77* (m, 2H, Ph), 7.88 (t, 1H, Ph), 7.97 (d, 1H, Ph), 8.35–8.38 (m, 1H, Ph), 8.44 (d, 2H, Ph), 8.61 (d, 1H, Ph), 8.69 (s, 1H, Im). ^{13}C NMR (100 MHz, DMSO-d_6): δ 107.1, 118.2, 129.5, 129.7, 130.1, 130.4, 130.9, 143.6. MS (EI) 357 [M^+]. Anal. Calcd for ($C_{17}H_{10}F_6N_2$·HCl): C, 52.00; H, 2.82; N, 7.13. Found: C, 51.86; H, 2.94; N, 6.98.

4.3. Sodium channel electrophysiology

Human embryonic kidney cells (HEK) 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). Cel006Cs 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 MΩ 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 either 100 mM or 10 mM stock solutions in Dimethly sulfoxide (DMSO) and diluted to desired concentration in perfusion solution. The maximum DMSO concentration used was 0.1% 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 mV 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. Tonic block was assessed by comparing peak sodium current in drug free conditions to peak current when drug was present. Dose response data were fitted using the Hill equation:

$$I_{Na}/I_{Na\ peak} = 1/(1 + (C/IC_{50})^H)$$

where C is the drug concentration, IC_{50} is the concentration that blocks 50% of the current and H is the hill coefficient. All data represent percentage mean block ± standard error of the mean (S.E.M.).

4.4. Lipophilicity

log $D_{oct,7.4}$ Values were measured by the standard shake-flask technique with minor modifications. The two-phase partition system consisted of mutually saturated *n*-octanol and zwitterionic buffer 50 mM MOPS [3-(*N*-morpholino)propanesulfonic acid] pH 7.4. Ionic strength was adjusted at 0.15 M by KCl addition.

After compound addition, dissolution and partitioning at room temperature (25 ± 1 °C), each of the two partition phases was separated (1000 g, 10 min) and diluted with analytical grade CH_3OH before injection in a HPLC gradient system (Shimadzu) equipped with UV–vis SPD-10A detector and two LC-10AD pumps; the volume injected was 20 μl per run. The column was a Supelcosil LC-18-DB, 150 × 4.6 mm, 5 μm (Supelco). Mobile phases were mixtures of analytical CH_3CN and 0.5% H_3PO_4 in different proportions. Values for log $D_{7.4}$ were calculated from the ratio of the mean peak areas in the two phases, correcting

for instrumental attenuation and dilution; reported in Table 3 are the means of four different measurements together with their standard deviations.

4.5. Dissociation constants

Values for pK_a were determined by the pH-metric method[29] using a Sirius PCA200 instrument (Sirius Analytical Instruments, UK) equipped with a semi-micro combined electrode, two precision dispensers, a two-way valve for distributing titrants (0.5 M HCl, 0.5 M KOH), a temperature probe and a stirrer. Two precision dispensers (Metrohm) were employed for distributing other reagents (0.15 M KCl ionic strength adjusted water and methanol). The weighted samples (1–5 mg) were added manually to the glass vessel while the titrant and other reagents were supplied automatically. The temperature of the sample solution was kept at 25.0 ± 0.1 °C by a circulating water bath. None of the compound was sufficiently water-soluble for standard aqueous titration. Therefore, the pK_a values were determined by extrapolation to 100% aqueous, starting from 10 to 60 wt % methanol–water solutions, employing Yasuda–Shedlowsky method.[30]

Acknowledgements

Financial support from Italian MIUR and Siena Biotech S.p.A. is gratefully acknowledged. We are grateful to the Centro Interdipartimentale Misure of the University of Parma for providing the NMR instrumentation.

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