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2-Alkyl-4-arylimidazoles: structurally novel sodium channel modulators

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Abstract—A series of 2-alkyl-4-arylimidazoles were prepared and their binding affinities to the site-2 sodium (Na^+) channel were determined. SAR studies led to highly potent Na^+ channel blockers.

1. Introduction

Voltage gated Na^+ channels play a fundamental role in the propagation of action potentials in electrically excitable cells. They are widely distributed in the nervous system as well as in skeletal and cardiac muscle. Changes of Na^+ channel electrophysiological properties are implicated in numerous pathologies such as epilepsy, stroke, pain or cardiac arrhythmia.[1] Currently used drugs for neurological disorders, for example, lidocaine, carbamazepine or lamotrigine (Fig. 1) show affinity for the rat brain site 2 Na^+ channel in the micromolar range and their clinical efficacy is limited by their side effects.

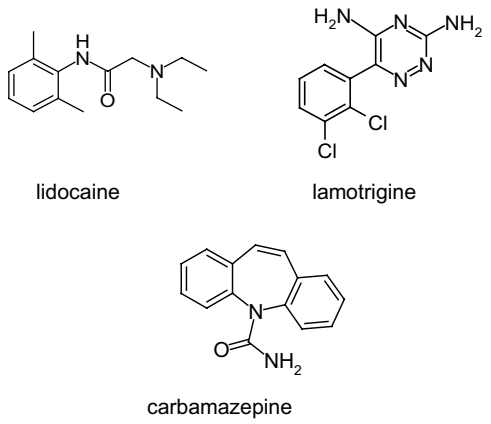

Figure 1. Structure of marketed drugs with Na^+ channel activity.

The development of more potent and selective drugs acting on Na^+ channels is therefore of considerable interest for the treatment of the above-mentioned pathologies.

In the course of a program involving the preparation of β-carboline derivatives as $SSTR_3$ antagonists,[2] we found that certain compounds, for example **1** (Fig. 2), exhibited affinity for the site 2 Na^+ channel. In the search for more active compounds and in order to simplify the stereochemical problems inherent to the β-carboline series, we envisaged the synthesis of simplified compounds of general formula **2**, where the R2 group could mimic the high lipophilicity of the indole and phenyl groups of **1**.

We report here the synthesis and the in vitro activity of these novel sodium channel modulators.

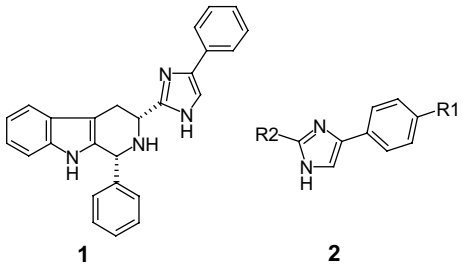

Figure 2. Arylimidazoles **2** derived from β-carboline **1**.

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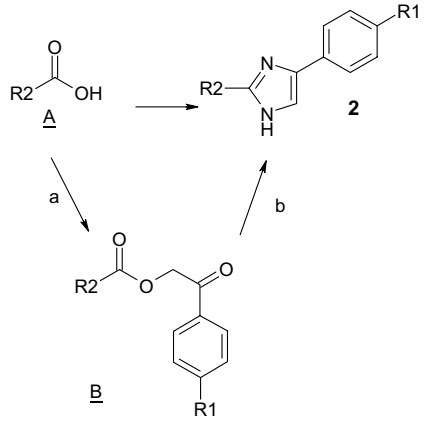
2. Chemistry

The imidazoles **2** were prepared by a one-pot method[3] according to the method shown in Scheme 1.

The formation of the imidazole can be divided into a 2-step sequence. The first step using the caesium salt of the corresponding acid **A** and the appropriate aryl bromoketone provided the keto ester **B**, which on heating with an excess of ammonium acetate afforded the arylimidazole **2**.

Most of the required aryl bromoketones are commercially available except those needed for **2b**, **2e** and **2f**, which were prepared using either poly(vinylpyridiniumhydrobromide perbromide) resin[4] or bromine[5] in acetic acid as the brominating agent.



Scheme 1. One-pot formation of arylimidazoles **2**. (a) (i) Cs_2CO_3, MeOH, rt, (ii) $ArCOCH_2Br$, DMF, rt; (b) NH_4OAc, xylene, reflux with Dean–Stark.

3. Pharmacology

3.1. Results and discussion

The compounds were tested in vitro for their inhibition of the binding of radiolabelled batrachotoxin ([^{3}H] BTX) to the rat brain site 2 Na^+ channel[6] and their functional activity assessed by their capacity to protect SH-SY5Y cells against veratridine-induced cytotoxicity.[7]

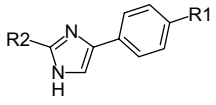
As shown in Table 1, all the 2-substitued-4-arylimidazoles compounds tested (**2a**–**n**) were remarkably potent in the Na^+ channel binding assay compared to lidocaine, lamotrigine, or carbamazepine, and showed markedly higher affinity than the β-carboline **1**.

A comparison of **2l** and **2m** illustrates the importance of a lipophilic R_2 group. Branched aliphatic chains also gave highly potent compounds, with dipentyl being superior to dipropyl (**2b** vs **2d**, and **2g** vs **2k**). Highest affinities were obtained with R_1 = phenyl, for example **2g**[8] (8 nM).

Screening of the more potent compounds in a cellular assay (Table 1) confirmed their functional activity as Na^+ channel blockers.

In conclusion, the 2-alkyl-4-arylimidazoles **2** derived from the more complex β-carboline **1** are found to be more potent than the latter, and show remarkably high affinities compared to lidocaine, lamotrigine or car-

Table 1. In vitro binding potencies of aryl imidazole derivatives **2** and cytoprotection against veratridine-induced cell cytotoxicity



2a-n

Compounds	R1	R2	Na^+ binding IC_{50} (nM)	Cytoprotection IC_{50} (nM)[a]
1			1460	—
2a	H	$(Pentyl)_2$–CH	86	—
2b	F	$(Pentyl)_2$–CH	25	770
2c	F	Cyclohexyl–CH_2	146	—
2d	F	$(Propyl)_2$–CH	518	—
2e	Terbutyl	$(Propyl)_2$–CH	30	430
2f	Isobutyl	Hexyl	28	380
2g	Phenyl	$(Pentyl)_2$–CH	8	810
2h	Phenyl	Cyclohexyl–CH_2	70	—
2i	Phenyl	Cyclohexyl–C_2H_4	26	760
2j	Phenyl	Cyclohexyl–C_3H_9	19	1000
2k	Phenyl	$(Propyl)_2$–CH	100	—
2l	Phenyl	Hexyl	16	450
2m	Phenyl	Methyl	1460	—
2n	4Br-phenyl	$(Propyl)_2$–CH	322	—
Lidocaine			56,000	—
Lamotrigine			31,000	—
Carbamazepine			167,000	—

[a] Data represent the mean of two experiments.

bamazepine. In addition, the compounds tested behave as functional Na^+ channel blockers in a cellular assay.

Imidazoles **2** merit further study in terms of electrophysiological use-dependency and in in vivo models.

Acknowledgements

We would like to thank José Camara and his team for mass spectral and NMR ^1H analysis.

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   Preparation of cortex membranes for Na^+ binding: Cortex membranes were prepared as follow. Rats were decapitated, the brains rapidly removed and the cortices dissected and weighed. The isolated cortex was homogenised by means of a Teflon-glass homogeniser in 10 volumes of ice-cooled 0.32 M sucrose-5 mM potassium hydrogen phosphate (pH 7.4, 4 °C.) solution. The resulting homogenate was centrifuged at 1000*g* (4 °C) for 10 min and the supernatant was further centrifuged at 20,000*g* (4 °C) for 15 min. The pellet was suspended and washed in 0.32 M sucrose buffer and centrifuged again at 20,000*g* (4 °C) for 15 min. The residue was recovered. The membrane sample thus obtained was suspended in Na-free assay buffer (50 mM HEPES, 5.4 mM KCl, 0.8 mM $MgSO_4$, 5.5 mM glucose, 130 mM choline chloride (pH 7.4) to give a final concentration of about 4 mg protein/mL and stored at −80 °C until use. Protein concentration was determined by the Bradford method using bovine serum albumine as a standard.
   Na^+ binding protocol: Binding studies were carried out as follow. 100 μL of the above membrane sample preparation (75 μg/mL) was added to buffer containing 1 μM tetrodotoxin, 50 μg/mL scorpion venom, 5 nM [^3H] BTX (34.0 Ci/mmol) and the compound at different concentrations to make a final volume of 0.5 mL. The reaction was carried out at 25 °C and was terminated after 90 min by the addition of 2 mL of ice-cold washing buffer (5 mM HEPES, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 130 mM choline chloride, (pH 7.4). The mixture was immediately vacuum filtered on a unifilter GF/C (Packard) presoaked with 0.1% polyethylene-imine. The filter was washed once with 2 mL of ice-cold washing buffer. Bound [^3H] BTX was determined by liquid scintillation spectrometry in Microscint 0 scintillation cocktail (Packard). Nonspecific binding (NSB) was determined in the presence of 100 μM flunarizine, and the total binding was obtained without inhibitor.
   For each concentration of compound, percent of control is calculated as follows:

$$((\mathrm{cpm}_{\mathrm{compound}} - \mathrm{cpm}_{\mathrm{NSB}}) * 100)/(\mathrm{cpm}_{\mathrm{control}} - \mathrm{cpm}_{\mathrm{NSB}}).$$

   Points are performed in duplicate.
   For each compound the values included in the linear part of each experiment's sigmoid were retained in a linear regression analysis and were used to estimate the 50% inhibitory concentration (IC_{50}).
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$$(\mathrm{OD}_{\text{values of veratridine treated cells}} - \mathrm{OD}_{\text{values of control cells}})/(\mathrm{OD}_{\text{values of veratridine untreated cells}} - \mathrm{OD}_{\text{values of control cells}}) \times 100$$

   Each OD was initial OD−blank OD. The IC_{50} value was the concentration, which decreased the % of cell injury by 50% and was derived by extrapolation from graphs of dose–response relations. Values used for calculations are the mean values of triplicates.
8. Selected data for compound **2g**: Typical experimental procedure. Synthesis of **2g**: *4-(1,1′-biphenyl-4-yl)-2-(1-pentylhexyl)-1H-imidazole* (**2g**). Caesium carbonate (2.03 g, 6.25 mmol) was added to a solution of dipentylacetic acid (2.5 g, 12.5 mmol) in methanol (50 mL). The reaction mixture was stirred for 1 h, the solvent was evaporated, and 4′-phenyl bromoacetophenone (3.43 g, 12.5 mmol) and dimethylformamide (40 mL) were added and the mixture stirred overnight. The reaction mixture was evaporated to dryness under reduced pressure and xylene (60 mL) added to the residue. The caesium bromide salt was filtered, ammonium acetate (20 g) added to the filtrate and the reaction mixture refluxed for 1.5 h using a Dean–Stark apparatus. After cooling, the reaction was diluted with ice water and ethyl acetate (200 mL). The organic phase was washed with a saturated solution of sodium bicarbonate (2 × 100 mL) followed by brine (100 mL), dried over sodium sulfate, filtered and evaporated to dryness under reduced pressure. Purification was carried out on a silica gel column (mobile phase: 5% methanol in dichloromethane) The evaporated fractions were suspended in diethyl ether (50 mL), filtered and rinsed with the same volume of ether to afford 0.46 g (10%) of **2g** as a light cream powder.
   Melting point: 177–178 °C.
   ^1H NMR (400 MHz, DMSO-d_6, δ): 11.84 (br s, 1H), 7.83–7.33 (m, 10H), 2.70 (br s, 1H), 1.66–1.58 (m, 4H), 1.22–1.11 (m, 12H), 0.8–0.83 (m, 6H). Elemental analysis for $C_{26}H_{24}N_2$: Theoretical: C 83.37%, N 7.48%, H 9.15%. Found: C 83.42%, N 7.64%, H 8.85%.