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Butyl 2-(4-[1.1′-biphenyl]-4-yl-1H-imidazol-2-yl)ethylcarbamate, a potent sodium channel blocker for the treatment of neuropathic pain

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Abstract—A series of 4-arylimidazole carbamates was synthesized and their binding affinities to the site-2 sodium (Na^+) channel were determined. SAR studies led to the identification of compound **10**, a potent Na^+ channel blocker which was efficacious in pain models in vivo.

Neuropathic pain is initiated or caused by a primary lesion or dysfunction in the nervous system (e.g., diabetic neuropathy, post-herpetic neuralgia). This development of abnormal primary sensory neuron excitability may be explained by an upregulation of voltage-gated sodium channels (VGSCs) and a modification of sodium currents.[1–3] Anticonvulsant drugs such as carbamazepine, lamotrigine or lidocaine block VGSCs and show some efficacy in the treatment of neuropathic pain.[4] They have, however, low affinity for the Na channel[5] (IC_{50} in the 30–200 μM range) and poor side-effect profiles.[6]

The need for more potent and selective VGSC blockers has led to the identification of second generation compounds such as ralfinamide,[7] which is currently in phase II clinical trials and the preclinical candidate PPPA[8] (Fig. 1).

We report here the synthesis and structure–activity relationship (SAR) of a structurally novel series of VGSC blockers.

In a course of a pain research programme,[9] the imidazole **1** (Fig. 2) was identified as a hit by screening of our in-house sample collection (IC_{50} = 150 nM for the site 2 Na^+ channel[10]), and we present here the synthesis and structure–activity relationships (SARs) for a series of compounds. For the most potent analogue, in vivo activity is also reported.

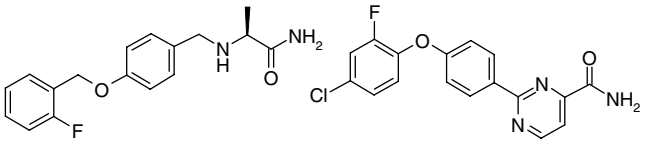
The compounds were prepared according to the reaction sequence[11] shown in Scheme 1.

The amino acid **3** was converted to the corresponding *N*-carbamate **4** using chloroformate derivatives under basic conditions.[12] When the halo-derivatives were not commercially available, chloroformylation was carried out using the appropriate alcohol and triphosgene under mild conditions.[13]

The imidazoles **5**–**24** were prepared by reacting the caesium salt of the acids **4** with an aryl bromoketone **2** to afford an intermediate keto-ester which on heating with an excess of ammonium acetate gave the required imidazoles.

Most of the required aryl bromoketones **2** are commercially available except those needed for **17**, **22**, **23** and **24** which were prepared using either poly(vinylpyridiniumhydrobromide perbromide) resin[14] or bromine[15] in ethanol as the brominating agent.

Initial SAR studies began with the modulation of the central linker.



ralfinamide PPPA

Figure 1. Structures of new drugs with Na^+ channel activity.

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The carbamate arylimidazole derivatives were tested for their ability to displace radiolabelled batrachotoxin ([^{3}H]BTX) from the sodium channel binding in synaptosomal preparations of rat cerebral cortex.[16†] The functional activity of the more potent compounds was assessed by their capacity to protect SH-SY5Y cells against veratridine-induced cytotoxicity.[17‡]

As shown in Table 1, the two-carbon linker **5** appears to be somewhat more active than **1** or **6**.

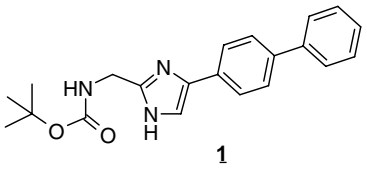

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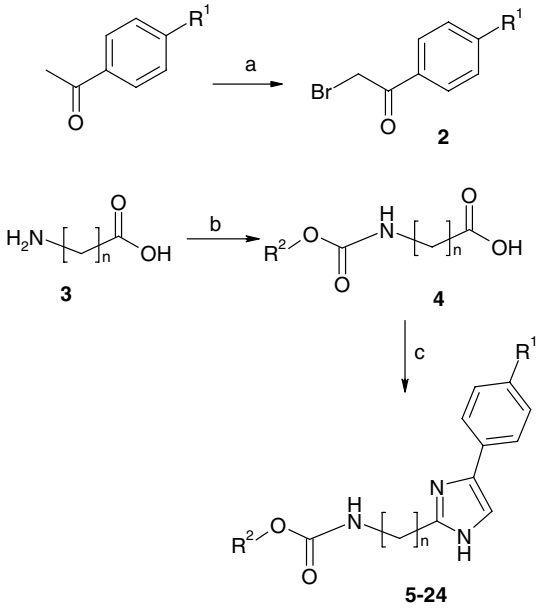
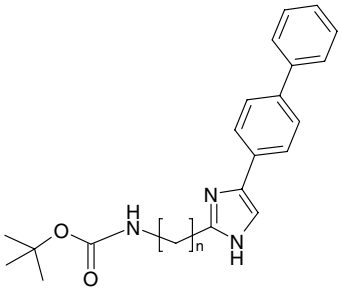
Figure 2. Structure of compound **1**.



Scheme 1. Formation of arylimidazole carbamates **5**–**24**. Reagents and condition: (a) Br_2, AcOH; (b) NaOH, H_2O, R^2OCOCl; (c) i—Cs_2CO_3, MeOH; ii—bromoketone **2**, DMF; iii—NH_4OAc, xylene, reflux.

Table 1. In vitro binding potencies



Compound	*n*	Na^+ binding IC_{50}[a] (nM)
1	1	144
5	2	64
6	3	184

[a] Data represent means of two experiments.

The two-carbon linker was conserved for studying the impact of the carbamate group on biological activity.

As shown in Table 2, most R^2 alkyl group analogues show potent binding affinity with the exception of methyl **7** and *tert*-butyl -CH_2 **12**.

† *Preparation of cortex membranes for Na^+ binding*. Cortex membranes were prepared as follows: rats were decapitated, the brains rapidly removed and the cortices dissected and weighed. The isolated cortices were homogenised by means of a Teflon-glass homogenizer in 10 volumes of ice-cold 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 albumin as a standard.

Na^+ binding protocol. Binding studies were carried out as follow: 100μl of the above membrane sample preparation (75 μg/ml) was added to buffer containing 1 μM tetrodotoxin, 50 μg/ml scorpion venom, 5 nM [^{3}H]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% poly-ethylene-imine. The filter was washed once with 2 mL of ice-cold washing buffer. Bound [^{3}H]BTX was determined by liquid scintillation spectrometry in Microscint scintillation cocktail (Packard). Non-specific 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}).

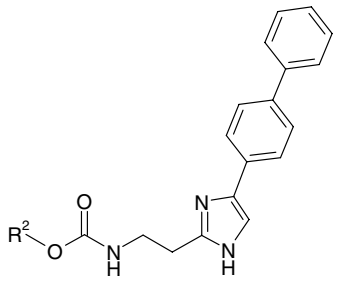
‡ *Veratridine-induced cytotoxicity on SH-SY5Y cell line*.SH-SY5Y neuroblastoma cells were seeded on 96-well plates 24 h prior to treatment. Cells were then pre-incubated with compounds for 20 min before addition of 100 μM veratridine and 1 mM ovabain mixture. After 3 h exposure, the medium was replaced by a culture medium containing 10% alamar blue and incubated for 18–24 h. Cytotoxicity was evaluated after plate reading on a spectrophotometer at double wavelength (570 and 620 nM). For each concentration of compound the percentage of cell injury was calculated as follows:

$$(\mathrm{OD}_{\text{values of veratridine-treated cells}} - \mathrm{OD}_{\text{values of control cells}})/(\mathrm{OD}_{\text{values of veratridine-untreated cells}} - \mathrm{OD}_{\text{values of control cells}}) \times 100$$

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 curves. Values used for calculations are the mean values of triplicates.

Table 2. In vitro binding potencies and cytoprotection against veratridine-induced cell cytotoxicity



Compound	R^2	Na^+ binding IC_{50}[a] (nM)	Cytoprotection IC_{50}[a] (nM)
5	*tert*-Butyl	64	—
7	Methyl	282	—
8	Ethyl	71	—
9	Propyl	86	—
10	*n*-Butyl	32	230
11	*iso*-Butyl	26	610
12	*tert*-Butyl–CH_2	372	—
13	Hexyl	15	670
14	Cyclohexyl	26	650
15	Cyclopentyl	52	870
16	Cyclohexylmethyl	21	700

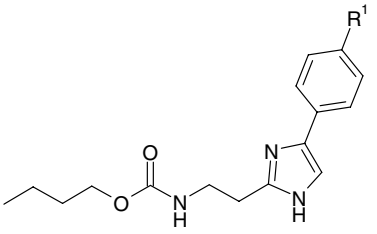
[a] Data represent means of two experiments.

In the functional (cell-based) assay, the compounds tested show little difference in activity, with the exception of compound **10**.

The left-hand-side compound of **10**[18] was retained as a template for optimization studies on the R^1 group.

As shown in Table 3, a selection of aryl substituents failed to improve the cytoprotective activity, although certain compounds (**17** and **21**) showed good affinity for the Na^+ channel. Compound **17** was not tested for its cytoprotective activity since it proved to be rapidly metabolized by human microsomes.[19]

Table 3. In vitro binding potencies and cytoprotection against veratridine-induced cell cytotoxicity



Compound	R^1	Na^+ binding IC_{50}[a] (nM)	Cytoprotection IC_{50}[a] (nM)
10	Phenyl	32	230
17	Cyclohexyl	7	—
18	F	1000	—
19	Br	210	900
20	CF_3	453	—
21	Pyrolidine	14	1120
22	*iso*-Butyl	41	750
23	Propyl	62	3990
24	Ethyl	96	3220

[a] Data represent means of two experiments.

Compound **10** was selected for in vivo evaluation in two rat models of pain: in the carrageenan hyperalgesia model,[20] compound **10** elicits an anti-hyperalgesic effect at 10 and 30 mg/kg, intra-peritoneally (ip) (Fig. 3A). In the chronic constriction injury (CCI)-induced neuropathic pain model,[21] compound **10** affords significant protection at 30 mg/kg, ip (Fig. 3B). Little, or no-effect was seen on the contralateral paw in either model, suggesting an anti-hyperalgesic rather than an analgesic effect. Furthermore, no significant drug-related effect was observed on motor performance in the rotarod test at 30 mg/kg (ip) (data not shown), suggesting a lack of

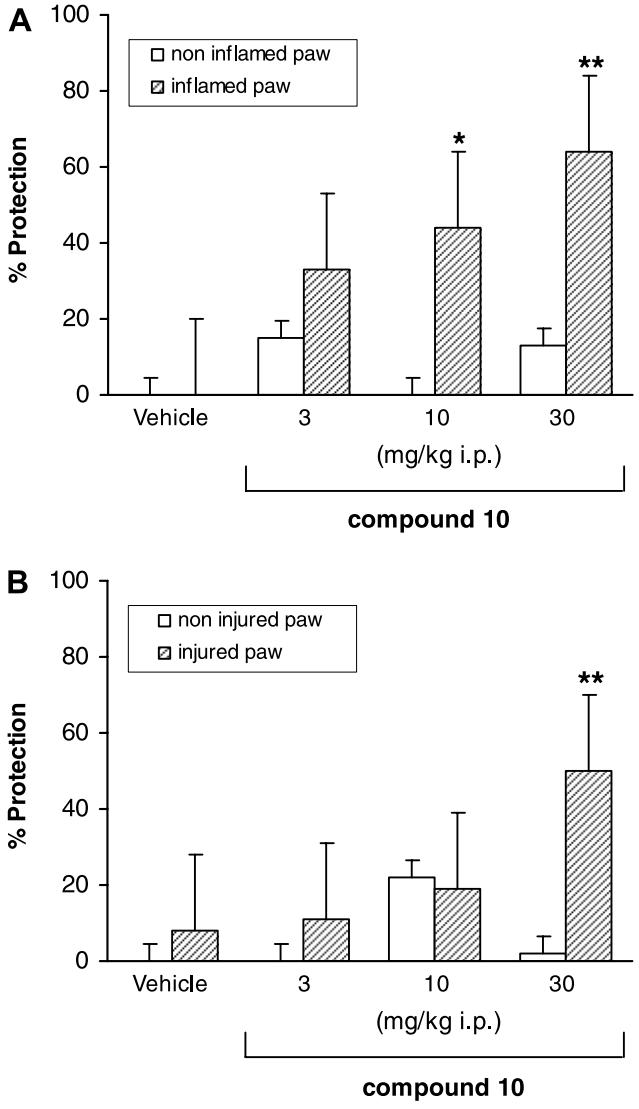


Figure 3. In vivo activity of compound **10** in two rat models: carrageenan-induced hyperalgesia (A) and chronic constriction injury (B). Compound or vehicle was administered intraperitoneally 30 min prior to measurement of paw withdrawal thresholds after mechanical stimulus (Randall and Selitto method)[22] on carrageenan-inflamed paw and on contralateral (non-inflamed paw) (A) or on injured paw and on non-injured paw (B) comparison between vehicle and treated groups was made using a two-sided Student/Dunnet test means ± SEM ($n = 4$–6).[23] $^*p < 0.05$; $^{**}p < 0.01$.

myorelaxant or sedative effect; an observation that could be of relevance in terms of the side-effect profile of the compound.

In conclusion, in the SAR studies of the 4-arylimidazole carbamate series derived from **1**, a lead compound **10** has been identified which shows high affinity for the sodium channel with functional and in vivo activity in two animal models of pain.

Acknowledgment

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References and notes

1. Ochoa, J.; Torebjork, H. E. *Brain* **1980**, *103*, 835.
2. Nordin, M.; Nystrom, B.; Wallin, U.; Hagbarth, K-E. *Pain* **1984**, *20*, 231.
3. Devor, M.; Wall, P. D.; Melzack, R. *Textbook of Pain*, 2nd ed.; Churchill Livingstone: Edinburgh, 1994, pp. 79–100.
4. Hunter, J. C.; Gogas, K. R.; Hedley, L. R.; Jacobson, L. O.; Kassotakis, L.; Thompson, J.; Fontana, D. J. *Eur. J. Pain* **1997**, *18*, 153.
5. Deffois, A.; Fage, D.; Carter, C. *Neurosci. Lett.* **1996**, *220*, 117.
6. Mao, J.; Chen, LL. *Pain* **2000**, *87*, 7; Jensen, T. S. *Eur. J. Pain* **2002**, *6*, 61; Petersen, K. L.; Maloney, A.; Hoke, F.; Dahl, J. B.; Rowbotham, M. C. *J. Pain* **2003**, *4*, 400.
7. Stummann, T. C.; Salvati, P.; Fariello, R. G.; Faravelli, L. *Eur. J. Pharmacol.* **2005**, *510*, 197.
8. Ilyin, Victor I.; Pomonis, James D.; Whiteside, Garth T.; Harrison, James E.; Pearson, Michelle S.; Mark, Lilly; Turchin, Paul I.; Gottshall, Susan; Carter, Richard B.; Nguyen, Phong; Hogenkamp, Derk J.; Olanrewaju, Shakira; Benjamin, Elfrida; Woodward, Richard M. *J. Pharmacol. Exp. Therapeutics* **2006**, *318*, 1083.
9. Liberatore, AM.; Schulz, J.; Pommier, J.; Barthelemy, MA.; Huchet, M.; Chabrier, PE.; Bigg, D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3521.
10. Mc Neal, E. T.; Lewandowski, G. A.; Daly, J. W.; Creveling, C. R. *J. Med. Chem.* **1985**, *28*, 381.
11. Gordon, T. D.; Singh, J.; Hansen, P. E.; Morgan, B. A. *Tetrahed. Lett.* **1993**, *34*, 1901.
12. Aitken, R. Alan; Karodia, Nazira; Massil, Tracy; Young, Robert J. *J. Chem. Soc., Perkin Trans. 1* **2002**, *4*, 533.
13. Berry, Jane M.; Howard, Philip W.; Kelland, Lloyd R.; Thurston, David E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1413.
14. Frechet, J. M.; Farall, MJ. *J. Macromol. Sci. Chem.* **1997**, 507.
15. Rather, J. B.; Reid, E. E. *J. Am. Chem. Soc.* **1919**, *41*, 75.
16. Biological method described by McKinnon, A. C.; Wyatt, K. M.; McGivern, J. G.; Sheridan, R. D.; Brown, C. M. *Br. J. Pharmacol.* **1995**, *115*, 1103.
17. Biological method described by Pauwels, P. J.; Van Assouw, H. P.; Leysen, J. E.; Janssen, P. A. *Mol. Pharmacol.* **1989**, *36*, 525.
18. Selected data for compound **10** Typical experimental procedure:Synthesis of **10**: **butyl 2-(4-[1.1′-biphenyl]-4-yl-1H-imidazol-2-yl)ethylcarbamate** (**10**). *N-(butoxycarbonyl)-β-alanine.* A solution containing β-alanine (8.9 g, 0.1 mol) and 100 ml of a 1-N solution of sodium hydroxide were cooled to 10 °C. *n*-Butyl chloroformate (13.66 g, 0.1 mol) and 50 ml of a 2-N solution of sodium hydroxide was added simultaneously. After stirring for 16 h at 23 °C, approximately 10 ml of a solution of concentrated hydrochloric acid (approximately 11 N) was added in order to adjust the pH to 4–5. The oil obtained was extracted with ethyl acetate (2 × 50 ml), washed with water then dried over magnesium sulfate. The product crystallized from isopentane in the form of a white powder (68% yield). Mp: 50.5 °C. *Butyl 2-(4-[1.1′-biphenyl]-4-yl-1H-imidazol-2-yl)ethylcarbamate.* A mixture of *N*-(butoxycarbonyl)-β-alanine (5.67 g, 0.03 mol) and caesium carbonate (4.89 g, 0.015 mol) in 100 ml of ethanol was stirred at 23 °C for 1 h. The ethanol was eliminated by evaporation under reduced pressure. The mixture obtained was dissolved in 100 ml of dimethylformamide and 4-phenyl-bromoacetophenone (8.26 g, 0.03 mol) was added. After stirring for 16 h, the solvent was evaporated under reduced pressure. The mixture obtained was taken up in ethyl acetate and the caesium bromide removed by filtration. The filtrate was evaporated and the reaction oil was taken up in a mixture of xylenes (100 ml) and ammonium acetate (46.2 g, 0.6 mol). The reaction medium was heated at reflux and, after cooling, a mixture of ice-cold water and ethyl acetate was poured into the reaction medium. After decantation, the organic phase was washed with a saturated solution of sodium bicarbonate, dried over magnesium sulfate, and evaporated under reduced pressure; The solid obtained was filtered and washed with ether to afford a pale beige-coloured powder (50% yield). Mp: 136–137 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 12.01 (br s, 1H), 7.83–7.19 (m, 11H), 3.94 (m, 2H); 3.33 (m, 2H), 2.8 (m, 2H), 1.51 (m, 2H), 1.32 (m, 2H); 0.8–0.83 (m, 3H). Elemental analysis for $C_{26}H_{24}N_2$: Theoretical: C 72.70%, H 6.93%, N 11.56%. Found: C 72.72%, H 6.85%, N 11.54%.
19. Eighty-nine percent after 30 min of incubation on human microsomes using the method described by Chesné, C.; Guyomard, C.; Guillouzo, A.; Schmid, J.; Ludwig, E.; Sauter, T. *Xenobiotica* **1988**, *28*, 1.
20. Souter, A. J.; Garry, M. G. *Pain* **2000**, *86*, 63.
21. Bennett, G. J.; Xie, Y. K. *Pain* **1988**, *33*, 87.
22. Randall, L. O.; Selitto, J. *Arch. Int. Pharmacodyn.* **1957**, *4*, 409.
23. For technical reasons, two animals have been excluded from the carrageenan-induced hyperalgesia experiment (A) at 30 mg/kg.