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Biarylcarboxybenzamide derivatives as potent vanilloid receptor (VR1) antagonistic ligands

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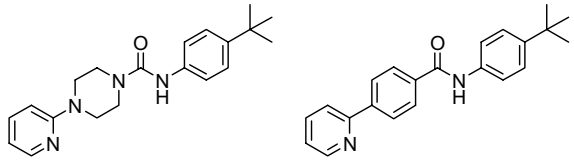
Abstract—Seventeen biarylcarboxybenzamide derivatives were prepared for the study of their agonistic/antagonistic activities to the vanilloid receptor (VR1) in rat DRG neurons. The replacement of the piperazine moiety of the lead compound **1** with phenyl ring showed quite enhanced antagonistic activity. Among the prepared derivatives, *N*-(4-*tert*-butylphenyl)-4-pyridine-2-yl-benzamide (**2**, IC_{50} = 31 nM) and *N*-(4-*tert*-butylphenyl)-4-(3-methylpyridine-2-yl)benzamide (**3g**, IC_{50} = 31 nM), showed 5-fold higher antagonistic activity than **1** in $^{45}Ca^{2+}$-influx assay.

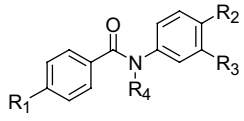
Vanilloid receptor (VR1) is a nonselective cation channel placed in the plasma membrane of peripheral sensory neurons,[1,2] which has been regarded as a new target for the treatment of pain.[3] The agonists desensitize the peripheral sensory neurons by influx of cations, especially Ca^{2+}, into neuronal cell, which leads to an analgesic effect.[4] However, their initial excitatory side effects, such as initial irritation, hypothermia, bronchoconstriction, and hypertension, derived from its inherent mechanism, make it hard to develop them as systemic analgesics.[5] In order to avoid the side effects from agonist, competitive antagonists have been pursued as novel analgesic drugs. So far, several synthetic and semi-synthetic antagonists were introduced and their pharmacological potential for pain treatment were evaluated.[6,7] Recently Purdue Pharma research group disclosed 4-(2-pyridyl)piperazine-1-carboxybenzamides (**1**) as potent VR1 antagonists (Fig. 1).[8] In this letter, we report the synthesis and functional assay on VR1 receptor of



1 **2**

Figure 1.

biarylcarboxybenzamide derivatives, based on molecular modeling studies.



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As part of our program to develop novel VR1 antagonists as potent analgesics, we attempted to replace the piperazine moiety of **1** with phenyl ring based on the

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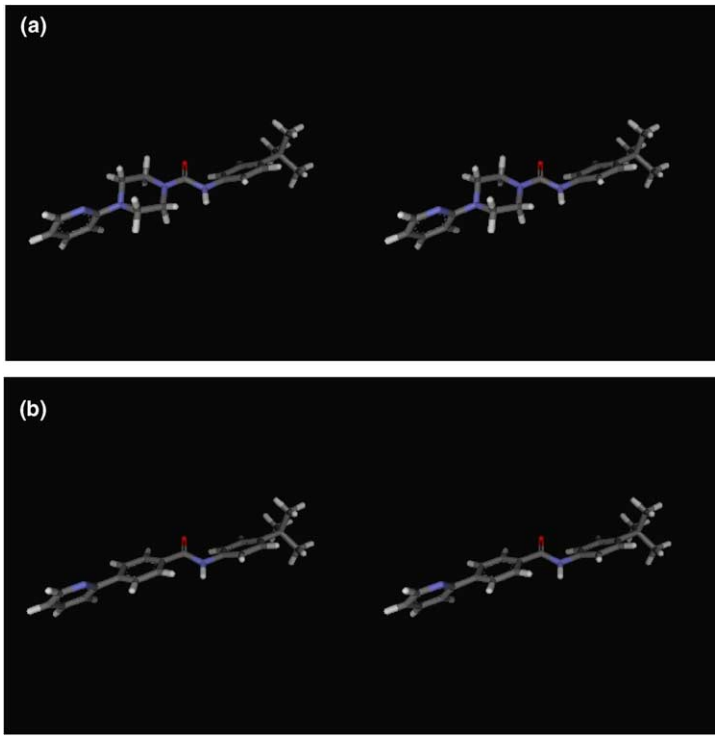
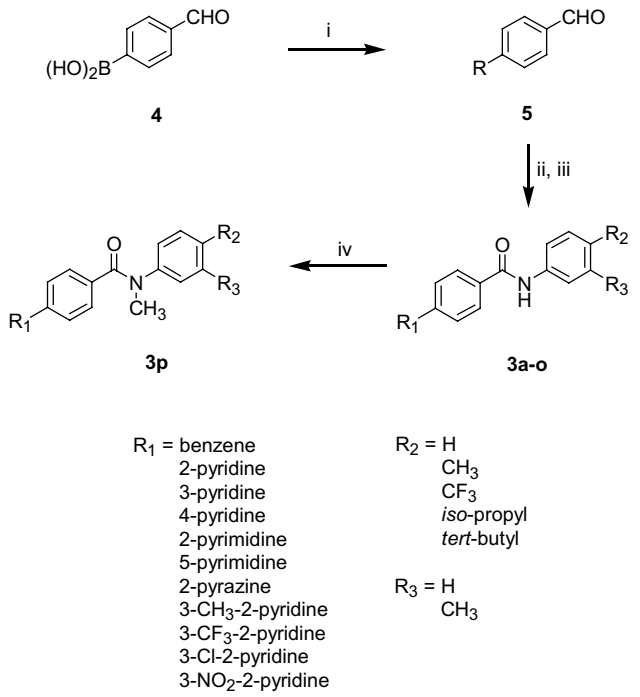


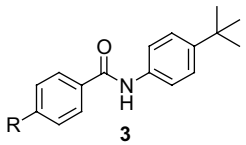
Figure 2. Stereoviews of the preferred three-dimensional conformations. (a) Energy-minimized conformation of **1** (5.113 kcal/mol); (b) energy-minimized conformation of **2** (6.786 kcal/mol).

molecular modeling studies of **1** and **2**. As shown in Figure 2, the energy-minimized conformation of **1** (5.113 kcal/mol) is very similar to that of biarylcarboxybenzamide **2** (6.876 kcal/mol).[9] The chair-like conformation of the piperazine in **1** maintains the molecular linear form, which is quite overlapped with that of **2**. Based on the similarity of the two conformations, we presumed that the biarylcarboxybenzamide analogues (**3**) could retain the antagonistic activity on VR1. Seventeen biarylcarboxybenzamide derivatives were easily prepared in three steps (**2**, **3a**–**o**) or four steps (**3p**) from **4** (Scheme 1). The Suzuki coupling of **4** with various aryl halides gave the corresponding 4-arylbenzaldehydes.[10] Oxidation of the aldehydes **5** with $NaClO_2$, followed by the coupling with the corresponding aniline derivatives in the presence of EDC afforded **2** and **3a**–**o**.[11] Compound **3p** was prepared by N-methylation of **2** in basic condition. The agonistic or antagonistic activities of the prepared derivatives[12] on VR1 receptor were evaluated by the $^{45}Ca^{2+}$-influx assay, previously reported by using neonatal rat cultured spinal sensory neurons.[13] As shown in Table 1, the phenyl-substituted analogue **3a** still retained antagonistic activity (IC_{50} = 3.6 μM), but less than **1** (IC_{50} = 0.15 μM). Among the pyridine derivatives, 2-pyridine analogue **2** (IC_{50} = 0.031 μM) showed the highest antagonistic activity, which is five times higher than that of the lead compound **1**. However, 3-pyridine analogue (**3b**, IC_{50} = 1.3 μM), and 4-pyridine analogue (**3c**, IC_{50} = 0.28 μM) showed lower activities, implying the *ortho*-N of pyridine is quite important for the binding with receptor, which is in accordance with the previous reports.[8] In the case of two-nitrogen possessing six-membered heterocycle series, 2-pyrimidine analogue (**3d**, IC_{50} = 0.06 μM), exhibited superior potency to 5-pyrim-



Scheme 1. Reagents and conditions: (a) (i) R^1X, $Pd(PPh_3)_4$, MeCN, 0.4 M $NaHCO_3$, reflux, 5 h, 40–70%; (ii) $NaClO_2$, $NaH_2PO_4{\cdot}2H_2O$, $CH_3CH{=}C(CH_3)_2$, *tert*-BuOH, H_2O, rt, 2 h, 80–90%; (iii) 4-R^2PhNH_2, EDC, DMAP, Et_3N, CH_2Cl_2, rt, 2 h, 70–80%; (iv) CH_3I, NaH, THF, 0 °C, 92%.

Table 1. In vitro biological activity of the derivatives by $^{45}Ca^{2+}$-influx assay in rat DRG (Dorsal root ganglion) neurons



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No.	R	$^{45}Ca^{2+}$-influx activity (μM)[a]	
		Agonist (IC_{50})	Antagonist (IC_{50})
1	—	NE	0.15
3a		NE	3.6
2		NE	0.031
3b		NE	1.3
3c		NE	0.28
3d		NE	0.06
3e		NE	1.0
3f		NE	10>
3g		NE	0.031
3h		NE	0.14
3i		NE	0.055
3j		NE	0.06

NE: not effective at 30 μM.

[a] EC_{50} (the concentration of derivative necessary to produce 50% of the maximal response) and IC_{50} values (the concentration of derivative necessary to reduce the response to 0.5 μM capsaicin by 50%) were estimated with at least three replicates at each concentration. Each compound was tested in two independent experiments. Antagonist data were fitted with a sigmoidal function.

idine analogue (**3f**, IC_{50} > 10 μM) and 2-pyrazine analogue (**3e**, IC_{50} = 1.0 μM). Most of 3-substituted-2-pyridine analogues (**3g–j**) showed higher antagonistic activities than **1** except **3h**. The electron-donating group (CH_3, **3g**, IC_{50} = 0.031 μM) exhibited higher antagonistic activity than that of the electron-withdrawing groups (**3h**, CF_3, IC_{50} = 0.14 μM; **3i**, Cl, IC_{50} = 0.055 μM; **3j**, NO_2, IC_{50} = 0.06 μM). Based on the cumulative in vitro antagonistic activity results, the general tendency could be summarized as follows: (1) The *ortho*-N plays an integral role in antagonistic activity, but di-*ortho*-N_2 cannot give additive effect; (2) the antagonistic potency is in the order of *ortho*-N > *para*-N > *meta*-N; (3) There is some space at the 3-position of 2-pyridine group around the

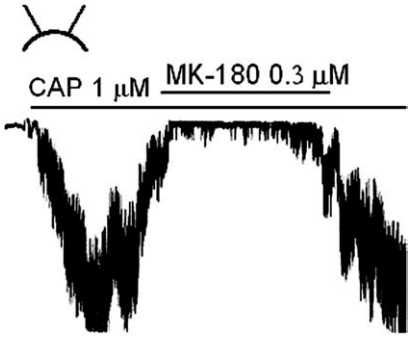


Figure 3. Reversible antagonistic effects of **2** (MK-180) on the capsaicin receptor.

binding site, and the electron-donating groups seem more favorable for binding, compared to electron-withdrawing groups. In a series of aniline amide analogues (**2**, **3k–o**) in Table 2, the bulky and hydrophobic substituted analogues, **2** (*tert*-butyl, IC_{50} = 0.031 μM) and **3n** (*iso*-propyl, IC_{50} = 0.038 μM) gave higher antagonistic activities than relatively smaller or polar group analogues, **3k** (H, IC_{50} > 10 μM), **3l** (4-CH_3, IC_{50} = 2.3 μM), **3m** (3,4-di-CH_3, IC_{50} = 2.0 μM), and **3n** (4-CF_3, IC_{50} = 7.9 μM), implying that the hydrophobic interaction contributes to efficient binding. In particular, N–CH_3 analogue **3p** (IC_{50} > 10 μM), exhibited dramatic loss of activity. This might support the hypothesis that the hydrogen bonding of N–H is essential for the favorable binding with VR1. One of the best antagonistic analogues, **2** (MK-180) was chosen for the confirmation of its VR1 antagonism via the inhibition of the capsaicin-induced agonist action on patch-clamped rat DRG neurons. As shown in Figure 3, the application of capsaicin (1 μM) greatly activated the capsaicin receptors in inside-out membrane patches. However, **2** (0.3 μM) almost inhibited the channel activity, evoked by capsaicin (1 μM). After **2** was removed by washing, capsaicin (1 μM) reactivated the channel activity, suggesting that **2** clearly antagonizes the capsaicin at the VR1 in a reversible manner. Figure 4 shows the magnitude of inhibition by **2** (0.3 μM) in the presence of capsaicin (1 μM).

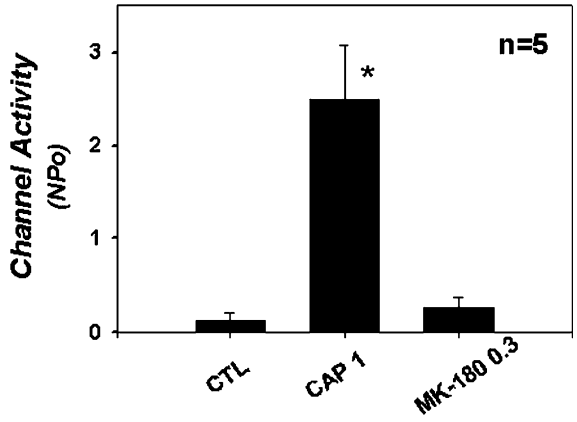
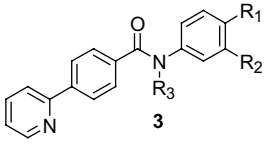


Figure 4. Comparison of the channel activity of capsaicin (1 μM) to **2** (0.3 μM) in the presence of capsaicin (1 μM). CTL is control activity before the application of capsaicin.

Table 2. In vitro biological activity of the derivatives by $^{45}Ca^{2+}$-influx assay in rat DRG neurons



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No.	R_1	R_2	R_3	$^{45}Ca^{2+}$-influx activity (μM)[a]	
				Agonist (IC_{50})	Antagonist (IC_{50})
1	—	—	—	NE	0.15
3k	H	H	H	NE	10>
3l	CH_3	H	H	NE	2.3
3m	CH_3	CH_3	H	NE	2.0
3n	CF_3	H	H	NE	7.9
3o	*iso*-propyl	H	H	NE	0.038
2	*tert*-butyl	H	H	NE	0.031
3p	*tert*-butyl	H	CH_3	NE	10>

NE: not effective at 30 μM.

[a] EC_{50} and IC_{50} values were estimated by the same method described in Table 1.

In conclusion, 17 diarylcarboxybenzamides were prepared and their biological activities were evaluated. Quite highly enhanced antagonistic activities were observed by the replacement of piperazine moiety of the lead compound **1** with phenyl ring. Among them, *N*-(4-*tert*-butylphenyl)-4-pyridine-2-yl-benzamide (**2**, MK-180) and *N*-(4-*tert*-butylphenyl)-4-(3-methylpyridine-2-yl)benzamide (**3g**) showed the best antagonistic activities. We believe this pharmacophore information would be very useful to design more potent antagonistic scaffolds for the development of potential analgesics.

Acknowledgements

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9. The calculations were done using the program SYBYL 6.5 from Tripos Software Inc. St. Louis, MO, USA. **1** and **2** were minimized using Tripos forcefield parameters and the conjugate gradient algorithm with a gradient convergence value of 0.005 kcal/mol Å. Partial atomic charges were calculated using the Gasteiger–Hückel method. Low energy conformation was searched by grid search method.
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12. All compounds gave satisfactory spectroscopic data consistent with the proposed structures. Selected spectral data for **2**: mp 177 °C. ^1H NMR ($CDCl_3$, 300 MHz), δ 8.72 (d, J = 4.77 Hz, 1H), 8.11 (d, J = 8.43 Hz, 2H), 7.96 (d, J = 8.43 Hz, 2H), 7.84 (br, 1H), 7.78 (m, 2H), 7.57 (d, J = 8.61 Hz, 2H), 7.38 (d, J = 8.79 Hz, 2H), 7.28 (m, 1H), 1.31 (s, 9H). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 165.36, 156.05, 149.77, 147.54, 142.29, 136.96, 135.25, 135.15, 127.51, 127.10, 125.86, 122.80, 120.90, 120.08, 34.38, 31.32. IR (KBr) 3291, 2960, 1650, 1593, 1526 cm^{-1}. MS (ESI) m/z, 331 $[M+H]^+$. HRMS (EI) calcd for $[C_{22}H_{22}N_2O]$: 330.4230, found: 330.1709 $[M]^+$. Anal. Calcd for $C_{22}H_{22}N_2O$: C, 79.97; H, 6.71; N, 8.48. Found: C, 79.39; H, 6.69; N, 8.35.
13. The uptake and the accumulation of $^{45}Ca^{2+}$ by the biarylcarboxybenzamide derivatives were studied in neonatal rat cultured spinal sensory neurons, by the method described in detail in Ref. 3b.