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SPECIAL REPORT

Interaction of biphenylimidazole and imidazoleacrylic acid nonpeptide antagonists with valine 108 in TM III of the AT_1 angiotensin receptor

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Interspecies amino acid exchange, based on pharmacological differences between mammalian AT_1 and amphibian xAT angiotensin II receptors, previously demonstrated that Val^{108} in transmembrane III (ValIII:08) is a critical structural requirement for binding the biphenylimidazole, losartan. Here, we investigated a series of biphenylimidazole and imidazoleacrylic acid nonpeptides to determine the general role of Val^{108} in nonpeptide recognition. Substitution of Val^{108} in the rAT_{1b} receptor with Ile, the corresponding residue in xAT_a, significantly reduced ligand affinities from both nonpeptide classes (F_{mut} values (mutant IC_{50}/$rAT_{1b}IC_{50}$): losartan > L-162,389 > L-162,313 > L-162,017 = L-163,491 > SB-203,220 > SK&F-108,566). While distinct molecular requirements exist for biphenylimidazole and imidazoleacrylic acid binding, these results suggest that Val^{108} is a common structural determinant of nonpeptide recognition on the AT_1 receptor.

Keywords: Angiotensin; angiotensin receptors; nonpeptide antagonists; biphenylimidazoles; imidazoleacrylic acids; site-directed mutagenesis; ligand binding

Introduction AT_1 receptor nonpeptide antagonists fall into two major categories, biphenylimidazole derivatives and imidazoleacrylic acids (Figure 1). Alignment of the imidazole from the lead compound, CV-2947, with the imidazole of His^6 in angiotensin II (AII) led to the development of biphenylimidazoles, while alignment with the aromatic component of Pro^7 led to imidazoleacrylic acids (Timmermans *et al.*, 1993). Mammalian AT_1 receptors bind the biphenylimidazole, losartan, with >25,000 fold greater affinity than amphibian AT receptors. Substitution of Val^{108} in the rAT_{1b} receptor with Ile which is the corresponding residue in the frog xAT_a receptor, markedly attenuated specific binding of losartan by 40 fold while AII peptide affinities remained identical to wild type receptors (Ji *et al.*, 1994). To investigate the general role of Val^{108} in nonpeptide recognition, various biphenylimidazole and imidazoleacrylic acid nonpeptides were studied.

Methods The rAT_{1b} mutants, Val^{108}→Ile (Ji *et al.*, 1994) and Val^{108}→Ala (Ji *et al.*, 1995) have been constructed previously. Radioligand binding assays were performed on membranes from COS-7 cells transfected by the calcium phosphate technique (Ji *et al.*, 1995). The IC_{50} and B_{max} values from the specific binding data were determined by computerized nonlinear regression analysis by use of the 'Kaleidagraph' programme. Monoiodinated [^{125}I]-[Sar^1, Ile^8] AII was obtained from Peptide Radioiodination Center. Angiotensin II peptides were purchased from Peninsula. Nonpeptides were provided as follows: SK&F 108,566 and SB 203,220 (SmithKline Beecham); L-163,017, L-162313, L-163491 and L-162,389 (Merck) and losartan (DuPont).

Results The rAT_{1b} mutant, Val^{108}→Ile, was evaluated for its affinity towards various biphenylimidazoles and imidazoleacrylic acids in transiently transfected COS-7 cell membranes. As previously described (Ji *et al.*, 1994), Val^{108}→Ile caused a 40 fold reduction in losartan affinity while peptide binding affinities and B_{max} values were indistinguishable from the wild type receptor (Table 1). Significant reductions in affinities for the Val^{108}→Ile mutant were observed for all the biphenylimidazoles. Although not as large, the imidazoleacrylic acid derivatives also exhibited reductions in ligand affinities for the mutant as evidenced by smaller F_{mut} values (= IC_{50} mutant/IC_{50} rAT_{1b}) compared with the biphenylimidazoles. The rank order of F_{mut} values was the following: losartan > L-162,389 > L-162,313 > L-162,017 = L-163,491 > SB-203,220 > SK&F 108,566. Comparison of biphenylimidazole affinities for the two mutants revealed 3-13 fold lower F_{mut} values for Ala^{108} compared to the Ile^{108} mutant. That is, the Ala^{108} variant had higher affinities for the biphenylimidazoles than the Ile^{108} mutant. In contrast, the imidazoleacrylic acids exhibited 12-14 fold increases in F_{mut} values for the Ala^{108} compared to the Ile^{108} mutant indicating that this class of nonpeptides had lower affinities for Ala^{108} compared to the Ile^{108} variant.

Discussion These results suggest that Val^{108} is a structural determinant of nonpeptide ligand recognition in the AT_1 receptor for both biphenylimidazoles and imidazoleacrylic acid nonpeptide classes. One interpretation is that Val^{108} is in close proximity to a general nonpeptide binding site on the AT_1 receptor and provides a hydrophobic interaction that stabilizes the ligand. If the hydrophobic side chains of Val^{108} are interacting more directly with imidazoleacrylic acid than biphenylimidazole ligands, then removal of the hydrophobic interaction by substitution of Val^{108} with Ala would cause larger reductions in imidazoleacrylic acid binding affinities compared with biphenylimidazoles. Furthermore, the difference in affinities towards the Ile^{108} mutant between nonpeptide classes could be explained by the nature of the amino acid-side chain interactions. The imidazoleacrylic acids may bind within the pocket such that the hydrophobic interaction between Val^{108} and nonpeptide could be mimicked by the similarly hydrophobic Ile^{108}. In comparison, the biphenylimidazoles may be unable to take full advantage of the hydrophobic interaction of Ile due to steric hindrance; there are 4 methyl groups in Ile compared with 3 in Val. This possibility could account for the larger F_{mut} values observed for biphenylimidazoles compared with imidazoleacrylic acid nonpeptides towards the Ile^{108} mutant.

If Val^{108} was influencing nonpeptide binding indirectly, substitution with Ala would be unlikely to reduce markedly F_{mut}

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Table 1 Binding affinities for angiotensin ligands

	Wild type and mutant angiotensin II receptors					
	rAT_{1b}		$Val^{108} \rightarrow Ile$		$Val^{108} \rightarrow Ala$	
Angiotensin II ligands	IC_{50} (nM)	F_{mut}	IC_{50} (nM)	F_{mu}	IC_{50} (nM)	F_{mut}
Angiotensin II	1.1±0.1	1.0	1.4±0.1	1.3	1.4±0.2	1.3
[Sar^1,Ile^8]AII	2.1±0.2	1.0	2.6±0.3	1.2	2.5±0.2	1.2
Biphenyllimidazoles						
Losartan	2.3±0.2	1.0	80±9	35	28±4	12
L-162389	1.3±0.2	1.0	24±9	18	6.6±6	5.1
L-162313	63±7	1.0	590±5	9.4	90±7	1.4
L-163017	4.2±0.9	1.0	32±10	7.6	5.3±3	1.3
L-163491	51±2	1.0	400±6	7.8	30±5	0.59
Imidazoleacrylic acids						
SB-203220	19±8	1.0	71±20	3.7	860±80	45
SK&F-108566	4.2±2	1.0	7.1±2	1.7	99±40	24

Data represent the mean of the IC_{50} values±s.e. obtained from 3–6 independent experiments each performed in triplicate with [^{125}I]-[Sar^1,Ile^8]AII as the radioligand. F_{mut} = mutant IC_{50}/rAT_{1b} IC_{50}, B_{max} values (mean±s.e.mean n=3–12) in fmol/10^5 cells: rAT_{1b}, 9.3±2; $Val^{108} \rightarrow Ile$ (rAT_{1b}), 11±2; $Val^{108} \rightarrow Ala$ (rAT_{1b}), 12±2.

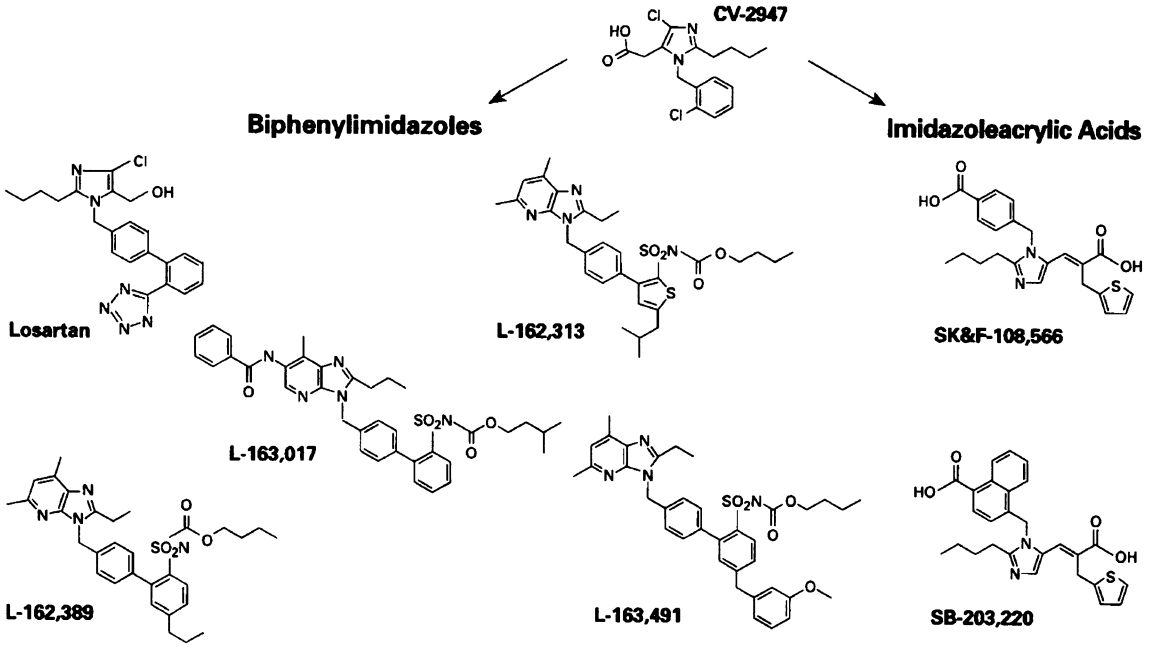

Figure 1 Biphenylimidazole derivatives and imidazoleacrylic acids used in this study.

values since disruption of local receptor conformation would be minimal due to the nature of the small uncharged Ala residue. However, these findings do not exclude the possibility that the Ala mutations do cause alterations in local conformation that indirectly influence nonpeptide binding. Future studies in which nonpeptide chemical moieties are systematically exchanged are likely to define specific ligand side chain-amino acid interaction points and thus a clearer picture of the exact nature of nonpeptide binding pockets on the AT_1 receptor.

These results suggest that significant overlap exists between the structural determinants of biphenylimidazole and imidazoleacrylic acid antagonist binding. Thus, these findings are consistent with previous mutagenesis study of the human AT_1 receptor suggesting that Asn^{295} in TMVII is a common point of interaction for biphenylimidazoles and imidazoleacrylic acids since both classes of nonpeptides were affected by site-directed mutagenesis of this residue (Schambye *et al.*, 1995). These findings are also consistent with a recent study in which structurally dissimilar series of NK_1 antagonists were shown to interact within the same binding site on the neurokinin NK_1 receptor (Cascieri *et al.*, 1995).

The observation that Val^{108} may be of general importance in nonpeptide binding in the AT_1 receptor supports the concept that a general nonpeptide binding site exists within the TM domain of all G-protein coupled receptors regardless of the nature of the native ligand (Cascieri *et al.*, 1995). When a common nomenclature (Schwartz, 1994) in which TM domains are numbered based on residues highly conserved across the whole superfamily is taken into account, Val^{108} (ValIII:08) is found to lie in the same position as residues shown to play crucial roles in ligand interactions in other G-protein coupled receptors including Glu^{113} in rhodopsin, Asp^{113} in monoamine receptors, His^{108} in the tachykinin NK_1 receptor and Lys^{182} in the endothelin ET_B receptor (Schwartz, 1994).

In conclusion, these findings indicate that biphenylimidazole and imidazoleacrylic acids share overlapping molecular determinants of ligand recognition on the AT_1 receptor. Additional investigations will be required to define fully the precise nature of the nonpeptide binding sites which will facilitate approaches to rational ligand design and thus potentially lead to improved therapeutics.

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