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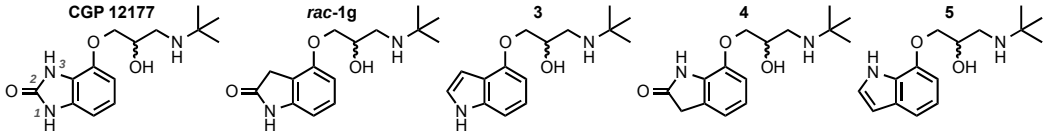
The composite influence of the imidazolone moiety of CGP 12177 on its affinity and efficacy at the two conformations of the human β1-adrenoceptor

There are at least two active conformations of the β1-adrenoceptor (AR): a high affinity conformation (HAC), where cimaterol is readily inhibited by antagonists (including CGP 12177); a secondary low affinity conformation (LAC) where CGP 12177 stimulates agonist responses that are relatively resistant to antagonism (1). Thus, CGP 12177 is a neutral antagonist of the HAC, but at higher concentrations, activates agonist responses via the secondary LAC. This study investigated the role of each component of the imidazolone moiety of CGP 12177 (carbonyl group at position 2 and NH groups in positions 1 and 3 of the aromatic core) towards this unusual pharmacological finding at the human β1-AR.

CGP 12177 analogues (***rac*-1g, 3-5**) were synthesised and ^{3}H-CGP 12177 whole cell binding and CRE-SPAP reporter gene assay were examined in cells stably expressing the human β1-AR as previously described (2).



The affinity at the HAC was investigated using ^{3}H-CGP 12177 binding. The affinity of ^{3}H-CGP 12177 was 0.27±0.02nM n=7 (log K_D = -9.48), determined from saturation binding. The analogue affinities (log K_D) were ***rac*-1g:** -9.47±0.08 n=10, **3:** -9.23±0.06 n=9, **4:** -8.08±0.06 n=6 and **5:** -7.75±0.11 n=6.

CRE-SPAP studies demonstrated that cimaterol responses (log EC_{50} -8.55±0.06, 69.9±2.5% isoprenaline maximum (isop max), n=11) were readily inhibited by CGP20712A (log K_D -9.27±0.07, n=13) whereas responses to CGP12177 (log EC_{50} -8.51±0.10, 84.7±6.0% isop max) required higher concentrations of CGP20712A (log K_D for CGP20712A = -7.14±0.10, n=12), thus demonstrating the presence of a HAC and LAC of the β1-AR. The agonist responses to ***rac*-1g** (log EC_{50}1 -8.94±0.10, log EC_{50}2 = -6.80±0.23, 57.8±4.7% site 1, 86.3±7.5% isop max, n=8) and **3** (log EC_{50}1 = -8.86±0.09, log EC_{50}2 -6.45±0.15, 51.0±3.2% site 1, 69.9±10.0% isop max, n=8) were best described by two-component responses, suggesting agonism at both conformations (2). Compounds **4** (log EC_{50} -6.40±0.10, 59.1±6.0% isop max n=9) and **5** (log EC_{50} -6.64±0.14, 73.7±5.7% isop max n=7) stimulated responses best described by a single component sigmoidal dose response. CGP12177, **4** and **5** inhibited the cimaterol responses as partial agonists, to yield log K_D values of -9.43±0.12, n=4, -8.45±0.15, n=5 and -8.09±0.12 n=5, respectively. Thus, the affinity (K_D) of CGP12177, **4** and **5** (measured in both binding and CRE-SPAP assays) is at odds with the concentration required to stimulate agonist responses (EC_{50}). Furthermore, responses to **4** and **5** were antagonised by CGP20712A to yield log K_D values of -7.35±0.10, n=10 and -7.31±0.10 n=6, in keeping with LAC interaction.

Thus the carbonyl moiety of CGP 12177 has little effect on its affinity or efficacy for the HAC. In contrast, each imidazolone NH group is important for either affinity or efficacy at the HAC. Molecules with the NH in corresponding 3-position of CGP 12177 have lower affinity, and low/no HAC efficacy but do stimulate agonist responses at the LAC of the human β1-AR.

(1) Kaumann and Molenaar (2008). *Pharmacol. Ther.* **118**: 303–336.
(2) Baker *et al*. (2003). *Mol. Pharmacol.* **63**: 1312–1321.