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Allosteric and Orthosteric Modulation of the Free Fatty Acid Receptor FFA2 by Bitopic Ligands

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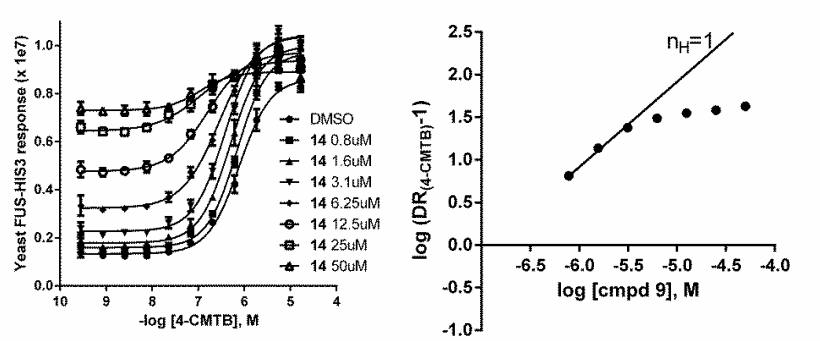
FFA2 is a G-protein-coupled receptor activated by propionate and other short-chain fatty acids. 4-CMTB is a synthetic agonist selective for FFA2 that lacks a carboxylate group and binds to a site different from propionate, acting allosterically (1). Here, we study bitopic FFA2 ligands containing both carboxylate and *N*-thiazolylamide, which is the characteristic chemical feature of 4-CMTB. By investigating the allosteric and orthosteric nature of interaction between ligand pairs, we characterise the mode of binding of bitopic ligands to FFA2. [^{35}S]GTPγS-incorporation and yeast reporter assays were used to measure FFA2 activation. Bitopic compounds showed system-dependent efficacy, in contrast to propionate and 4-CMTB (Table 1). Compound **14** ((R)-3-benzyl-4-((4-(2-chlorophenyl)thiazol-2-yl)(methyl)amino)-4-oxobutanoic acid) weakly activated FFA2 in yeast, and showed allosteric cooperativity with 4-CMTB (Fig.1) but not with propionate (not shown). Compound **9** ((*S*)-4-(4-(2-chlorophenyl)thiazol-2-ylamino)-4-oxo-3-phenylbutanoic acid) acted as an FFA2 inverse agonist in yeast (Table 1). Schild analysis indicated antagonism of propionate by **9** was competitive (n_H = 1; pA_2 = 6.64±0.17, n=2) but antagonism of 4-CMTB by **9** was allosteric (pK_B = 6.88±0.17 and cooperativity factor α= 0.01±0.004 (n=3) using an operational model; Fig.2). Thus, bitopic FFA2 ligands engage the orthosteric site, but appear not to compete at the site of 4-CMTB binding on an FFA2 receptor molecule. It seems paradoxical for two different FFA2 sites to bind the same chemical structure (*N*-thiazolylamide), and an alternative possibility is that FFA2 has a single *N*-thiazolylamide site but functions as a homodimer (or higher-order oligomer). This mode of action has recently been proposed to explain allosteric behaviour of a bitopic dopamine D_2 antagonist (2), and may be a common mechanism for allosteric interaction between GPCR ligands.

Table 1: Activity of FFA2 ligands (pEC_{50}, *pIC_{50} in italic*, mean±SD)

	[^{35}S]-GTPγS	**Yeast**
Propionate	4.5±0.08 (n=2)	3.4±0.11 (n=2)
4-CMTB	6.9±0.12 (n=3)	6.4±0.25 (n=45)
Cmpd **9**	7.0±0.08 (n=2)	*6.74±0.15 (n=3)*
Cmpd **14**	8.2±0.23 (n=4)	5.1±0.16 (n=4)

Fig.1 Allosterism of **14** and 4-CMTB

Fig.2 Allosterism of **9** and 4-CMTB



(1) Lee T *et al.* (2008) *Mol Pharmacol* **74**:1599-1609.

(2) Lane JR *et al.* (2014) *Nat Chem Biol* **10**:745-752