

Identification of a Small-Molecule GPR43 Agonist that increases Glucose Uptake and inhibits Lipolysis in Adipocytes

Jérôme Bernard, Anne-France Hartiel, Cyrille Brantis, Hamid Hoveyda and Graeme Fraser

Contact: jbernard@euroscreen.com, Euroscreen SA, 6041 Gosselies, Belgium; tel: +32 71 348500; fax: +32 71 348519, www.euroscreen.com

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Introduction

The Free Fatty Acid family of G-protein coupled receptors (i.e. GPR40, GPR41, GPR43, GPR120) has been recently characterized and implicated as targets of interest in the field of metabolic diseases. Specifically, GPR43 (also known as FFA2) is activated by short-chain fatty acids (SCFA) including propionate and acetate. GPR43 is expressed in adipocytes and therefore this receptor has been investigated in models pertaining to the treatment of type 2 diabetes and dyslipidemia⁽¹⁾. A synthetic, small-molecule Phenylacetamide (Phacetamide) has recently been published to activate GPR43 in an allosteric manner⁽²⁾. In comparison, we present here the *in vitro* pharmacological profile of ESN-282, a first-in-class, orthosteric agonist of GPR43. ESN-282 is a synthetic, small-molecule identified following a campaign of high-throughput screening and subsequent medicinal chemistry optimization. This report provides the first, pharmacological evidence that a synthetic, orthosteric GPR43 agonist modulates glucose uptake and lipolysis in adipocytes and thereby provides additional evidence for the physiological role of GPR43 in metabolic regulation.

(1)Ge H et al., *Endocrinol* 2008, 149:4519; (2) Lee T et al, *Mol Pharmacol* 2008, 74 (6): 1599.

Methods

GTPγ³⁵S binding and cAMP assays were performed to measure the agonist activity of test compounds at human (*hGPR43*), mouse (*mGPR43*) and rat (*rGPR43*). Compounds are discriminated as agonists by the maximal efficacy (E_{max} , relative to propionate) and potency (EC_{50}) of receptor activation.

Aequorin assays were performed to assess the agonist selectivity of test compounds at *hGPR40*, *hGPR41* and *hGPR120*.

Radioligand binding assays measuring the ligand-receptor affinity of test compounds were performed using CHO-cells expressing human, recombinant GPR43 ('*hGPR43*') with a tritiated (³H), proprietary radioligand.

RT-PCR were performed to assess GPR43 mRNA expression in 3T3-L1 cell line as well as in rat mature adipocytes.

Glucose Uptake assays were performed using mouse 3T3-L1 cell line. Cells were treated with compound throughout the differentiation process (14 days). The effect of compounds on insulin responsiveness was evaluated by incorporation of 2-Deoxy-³H-Glucose. Glucose uptake was measured with a liquid scintillation analyzer (Tri-Carb, Packard). Results are reported as a percentage of the insulin response.

Lipolysis assays were used to measure ex-vivo efficacy of the compounds. Adipocytes recovered from lean Sprague-Dawley rat were incubated with test compounds (or vehicle) for 3h prior to stimulation with isoprenaline to induce non-esterified fatty acid release (NEFA). Cell supernatants were harvested and NEFA levels were measured using a colorimetric assay (WAKO). Results are reported as a percentage of the isoprenaline response.

Table 1: Characterization of ESN-282 in *hGPR43*, *mGPR43*, *rGPR43* GTPγ³⁵S binding and cAMP assays

	GTPγ ³⁵ S			cAMP	
	<i>hGPR43</i> (EC_{50} , nM)	<i>mGPR43</i> (EC_{50} , nM)	<i>rGPR43</i> (EC_{50} , nM)	<i>hGPR43</i> (EC_{50} , nM)	<i>mGPR43</i> (EC_{50} , nM)
Propionate	100,000	125,000	125,000	61,800	41,250
ESN-282*	63	316	316	398	1,580
Phacetamide	125	158	100	398	316

*: ESN-282 does not cross react with either *hGPR40*, *hGPR41* or *hGPR120* in aequorin assay.

Fig 1 A-B: ESN-282 is an orthosteric agonist of *hGPR43*

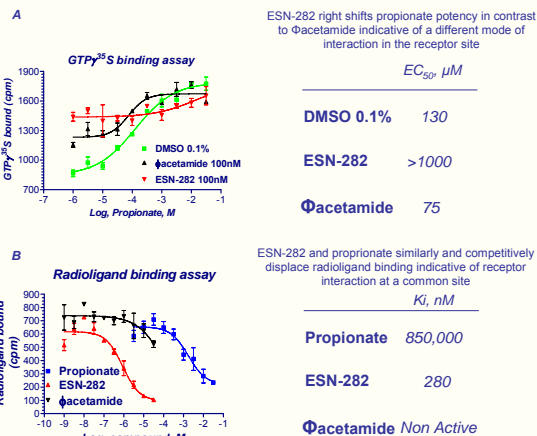


Fig 2 A-B: ESN-282 increases Glucose Uptake in response to insulin in the mouse 3T3-L1 cell line.

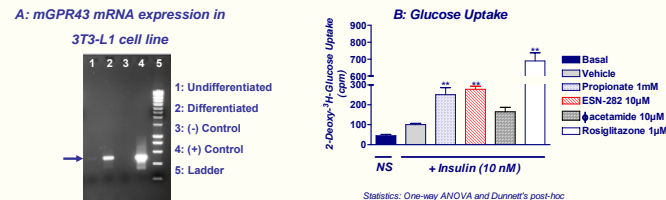
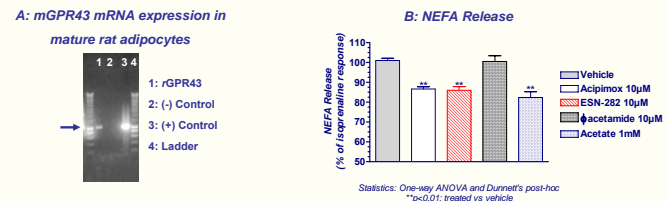


Fig 3 A-B: ESN-282 inhibits NEFA release in primary rat adipocytes



Conclusions

- ESN-282 is a proprietary, small-molecule GPR43 orthosteric agonist in preclinical development as a first-in-class approach for the treatment of Type 2 Diabetes and Dyslipidemia
- ESN-282 treatment during adipocyte differentiation leads to a significant increase in insulin-induced glucose uptake similar to that of Rosiglitazone®
- ESN-282 treatment inhibits isoprenaline-induced lipolysis of primary adipocytes with equivalent maximal efficacy to that of Acipimox®