University of Dundee

Identification of compounds acting as negative allosteric modulators of the LPA 1 receptor

Ellery, Jonathan; Dickson, Louise; Cheung, Toni; Ciuclan, Loredana; Bunyard, Peter; Mack, Stephen

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Fig. 1

The specific binding of TAK-615 (A), SAR-100842 (B) and BMS-896202 (C) to membranes

expressing the human LPA$_1$ receptor. Compounds were incubated with either parental

membranes or membranes expressing the LPA$_1$ receptor. The binding signal was expressed as

the phase shift in the interference fringe patterns measured in milliradians. The data from the

parental membrane fractions was subtracted from the LPA$_1$ receptor expressing fractions point

by point to obtain the specific binding interaction of the compounds with the LPA$_1$ receptor.

Data points are derived from at least three separate experiments and plotted as means with

S.E.M.. Compound affinity was calculated using a comparison fit of a one or two site binding

model.
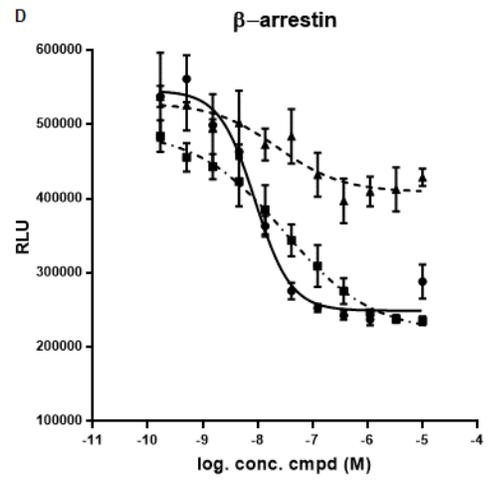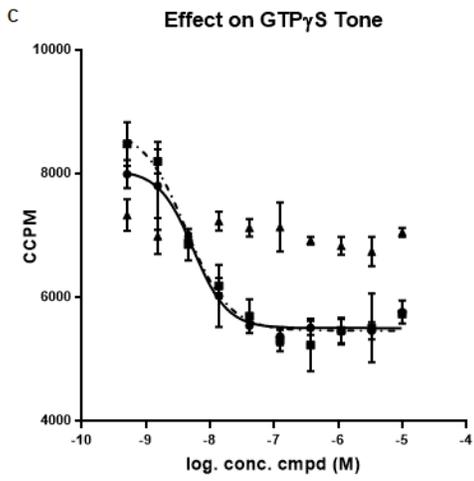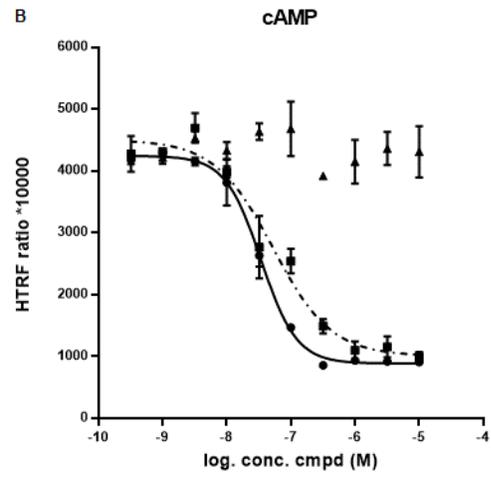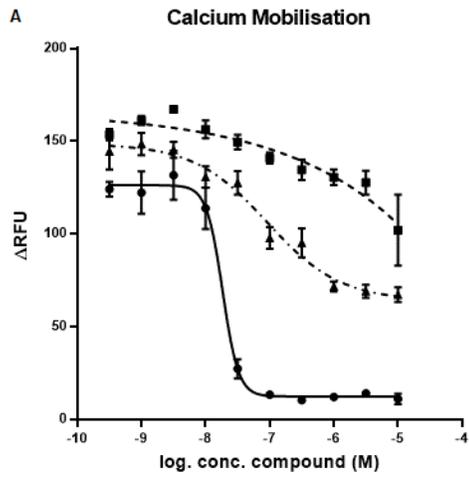
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Fig. 2

Representative graphs to demonstrate the different inhibitory functional profiles of TAK-615 (▲), SAR-100842 (■) & BMS-986202 (●) in a range of LPA driven functional assays using recombinant cell lines expressing the human LPA_1 receptor. In all cases a four-parameter curve fit algorithm was used to define compound potency. (A) Calcium mobilisation assay. Data points are derived from at least quadruplicate wells with S.E.M.. BMS-986202 showed full inhibition, SAR-100842 showed weak inhibition and TAK-615 showed partial inhibition. (B) cAMP accumulation assay. Data points are from duplicate wells. TAK-615 showed no inhibition, SAR-100842 & BMS-986202 showed full inhibition. (C) Millipore GTPγS assay. Data is derived from duplicate wells. SAR-100842 & BMS-986202 showed full inhibition. TAK-615 showed no inhibition. (D) DiscoverX PathHunter β-arrestin2 assay. TAK-615 gave partial inhibition. SAR-100842 & BMS-986202 showed full inhibition.
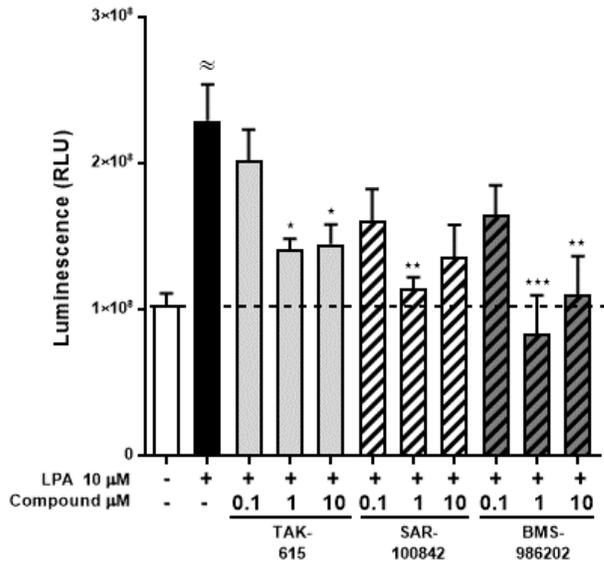
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Fig. 3

Representative data comparing the ability of TAK-615, SAR-100842 and BMS-986202 to inhibit 10 µM LPA stimulated RhoA activation in MeT-5A cells. Data points are derived from quadruplicate wells with S.E.M.. Data was analysed using a one-way ANOVA with a Dunnett's multiple comparisons test. $\approx$ P< 0.001 LPA vs Vehicle. * P< 0.05, ** P< 0.01, *** P<0.001 compound vs LPA.
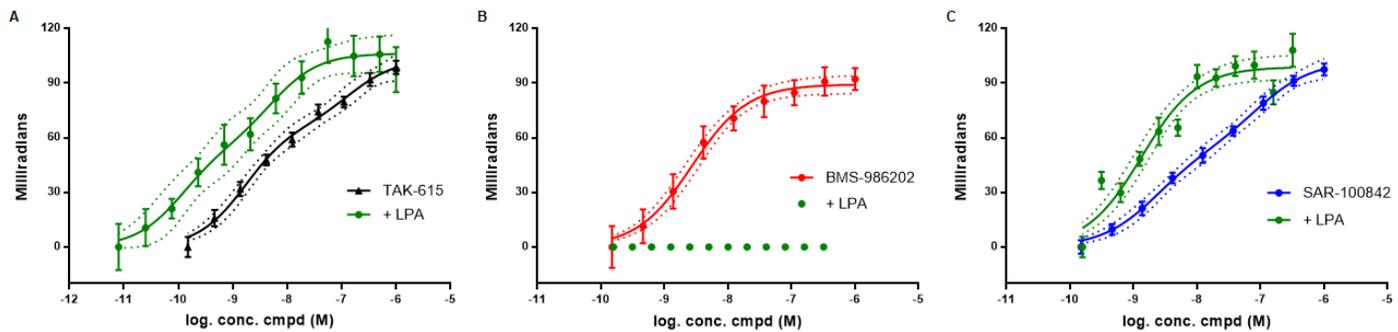
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Fig. 4

LPA increases the apparent affinity of TAK-615, shifts SAR-100842 binding to a single high

affinity site but blocks binding of BMS-986202 to the LPA$_1$ receptor. Parental membranes or

membranes expressing the LPA$_1$ receptor were incubated with a single concentration of LPA

(10μM) for 30 mins before addition of TAK-615 (A), SAR-100842 (B) or BMS-986202 (C).

The binding signal was expressed as the phase shift in the interference fringe patterns measured

in milliradians. The data from the parental membrane fractions was subtracted from the LPA$_1$

receptor expressing fractions point by point to obtain the specific binding interaction of the

compounds with the LPA$_1$ receptor in the presence or absence of LPA. Data points are derived

from at least three separate experiments and plotted as means with S.E.M.. Compound affinity

was calculated using a comparison fit of a one or two site binding model.
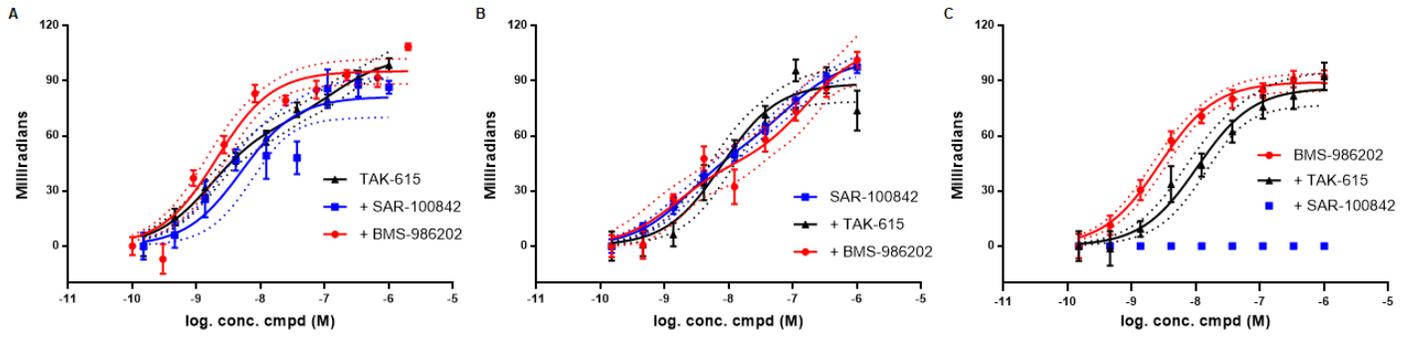
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Fig. 5

Pre-incubating membranes expressing the LPA$_1$ receptor with SAR-100842 prevents binding of BMS-986202. Parental membranes or membranes expressing the LPA$_1$ receptor were pre-incubated for 30 mins with 10 µM of a compound before titration with TAK-615 (A), SAR-100842 (B) or BMS-986202 (C). The binding signal was expressed as the phase shift in the interference fringe patterns measured in milliradians. The data from the parental membrane fractions was subtracted from the LPA$_1$ receptor expressing fractions point by point to obtain the specific binding interaction of the compounds with the LPA$_1$ receptor in the presence of another compound. Data points are derived from at least three separate experiments and plotted as means with S.E.M.. Compound affinity was calculated using a best fit one or two-site binding model. (A) TAK-615 binding is best described by a one-site model in the presence of SAR-100842 and BMS-986202. (C) TAK-615 decreased the apparent affinity of BMS-986202. SAR-100842 oblates BMS-986202 binding but (B) BMS-986202 has no effect upon SAR-100842 binding. The binding of SAR-100842 in the presence of TAK-615 best fits a one-site model.