Data and calculus on isobolographic analysis to determine the antinociceptive interaction between calcium channel blocker and a TRPV1 blocker in acute pain model in mice

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Abstract

Determining antinociceptive interaction between Phα1β toxin (a voltage gated calcium channel blocker) and SB366791 (selective TRPV1 antagonist) may have both clinical and mechanistic implications for the pain management. This data in brief article is associated to the research paper “Synergistic antinociceptive effect of a calcium channel blocker and a TRPV1 blocker in an acute pain model in mice”. This material supports the isobolographic analysis performed with the above drugs and shows: data of the dose response curves of the agents given as single drug or combination regimens. Mathematics and statistical processing of dose response curves, proportion of drugs dosage to be used in the combination, calculus of theoretical additive DE20 dose as well as experimentally obtained DE20 are provided. It is also presented details of statistical comparison between theoretical and experimentally obtained DE20.
### Specifications Table

| Subject area | Biology |
|--------------|---------|
| More specific subject area | Neuropharmacology, pain management |
| Type of data | Table, text file |
| How data was acquired | Experimental observation of nociceptive behavior in mice by using a chronometer. |
| Data format | Raw and analyzed |
| Experimental factors | Dose response curves for single drug administration of combined drugs, given at the same of at different sites |
| Experimental features | Isobolographic analysis using a fixed proportion ratio of two drugs. |
| Data source location | N.A. |
| Data accessibility | Data is with this article. |

### Value of the data

- Data tables and calculus from this article may serve as a practical guide on isobolographic analysis for testing other drug regimens or even using different pain models.
- Determining the interaction index by isobolographic analysis provides a measure of the in vivo degree of interaction of two drugs for a specified effect.
- Data analysis presented in this article can be systematically compared to other data from probit analysis with analgesic drugs given in combination.

### 1. Data

1.1. Summary of linear regression analyses for the agents administered alone

1.1.1. Intraplantar SB366791

See Table 1.

#### Table 1

Dose-effect data of SB366791 (i.p.) on capsaicin-induced nociception.

| Dose | \(x_i\) | \(y_i\) | \(x_i \cdot y_i\) | \((x_i - \bar{X})^2\) |
|------|--------|--------|-------------------|---------------------|
| 0.10 | -1.00  | 8.86   | -8.86            | 0.74                |
| 0.10 | -1.00  | 41.77  | 41.77            | 0.74                |
| 0.10 | -1.00  | 38.29  | -38.29           | 0.74                |
| 0.10 | -1.00  | 37.43  | -37.43           | 0.74                |
| 0.10 | -1.00  | 37.43  | -37.43           | 0.74                |
| 0.10 | -1.00  | 0.81   | -0.81            | 0.74                |
| 0.10 | -1.00  | 10.76  | 10.76            | 0.74                |
| 0.10 | -1.00  | 0.63   | 0.63             | 0.74                |
| 0.10 | -1.00  | 13.83  | 13.83            | 0.74                |
| 0.10 | 1.00   | 64.31  | -64.31           | 0.74                |
| 0.10 | -1.00  | 48.42  | -48.42           | 0.74                |
| 0.10 | -1.00  | 18.18  | -18.18           | 0.74                |
| 0.40 | -0.40  | 13.08  | 5.23             | 0.07                |
| 0.40 | -0.40  | -23.21 | 9.28             | 0.07                |
| 0.40 | -0.40  | 4.64   | -4.64            | 0.07                |
| 0.40 | -0.40  | 9.14   | -3.66            | 0.07                |
| 0.40 | -0.40  | 36.57  | -14.63           | 0.07                |
Table 1 (continued)

| Dose | $x_i$ | $y_i$ | $x_iy_i$ | $(x_i^2-X)^2$ |
|------|-------|-------|----------|---------------|
| 0.40 | -0.40 | 27.14 | -10.86   | 0.07          |
| 0.40 | -0.40 | 45.80 | -18.32   | 0.07          |
| 0.40 | -0.40 | 33.33 | -13.33   | 0.07          |
| 0.40 | -0.40 | 12.03 | -4.81    | 0.07          |
| 0.40 | -0.40 | 59.49 | -23.80   | 0.07          |
| 1.00 | 0.00  | -25.75| 0.00     | 0.02          |
| 1.00 | 0.00  | 18.16 | 0.00     | 0.02          |
| 1.00 | 0.00  | 12.20 | 0.00     | 0.02          |
| 1.00 | 0.00  | -1.90 | 0.00     | 0.02          |
| 1.00 | 0.00  | 17.72 | 0.00     | 0.02          |
| 1.00 | 0.00  | 25.32 | 0.00     | 0.02          |
| 1.00 | 0.00  | 2.53  | 0.00     | 0.02          |
| 1.00 | 0.00  | 5.06  | 0.00     | 0.02          |
| 1.00 | 0.00  | -19.61| 0.00     | 0.02          |
| 1.00 | 0.00  | 6.43  | 0.00     | 0.02          |
| 1.00 | 0.00  | -12.06| 0.00     | 0.02          |
| 1.00 | 0.00  | 50.79 | 0.00     | 0.02          |
| 1.00 | 0.00  | 29.37 | 0.00     | 0.02          |
| 1.00 | 0.00  | 15.08 | 0.00     | 0.02          |
| 1.00 | 0.00  | 44.44 | 0.00     | 0.02          |
| 2.00 | 0.30  | 26.58 | 7.97     | 0.19          |
| 2.00 | 0.30  | 70.46 | 21.14    | 0.19          |
| 2.00 | 0.30  | 53.59 | 16.08    | 0.19          |
| 2.00 | 0.30  | 22.86 | 6.86     | 0.19          |
| 2.00 | 0.30  | 40.86 | 12.26    | 0.19          |
| 2.00 | 0.30  | 13.43 | 4.03     | 0.19          |
| 2.00 | 0.30  | 13.28 | 3.98     | 0.19          |
| 2.00 | 0.30  | 22.49 | 6.75     | 0.19          |
| 2.00 | 0.30  | 52.85 | 15.85    | 0.19          |
| 2.00 | 0.30  | -10.13| -3.04    | 0.19          |
| 6.00 | 0.78  | 30.62 | 23.89    | 0.05          |
| 6.00 | 0.78  | 65.31 | 50.94    | 0.05          |
| 6.00 | 0.78  | 17.07 | 13.32    | 0.05          |
| 6.00 | 0.78  | 84.28 | 65.74    | 0.05          |
| 6.00 | 0.78  | 25.95 | 20.24    | 0.05          |
| 6.00 | 0.78  | 23.42 | 18.27    | 0.05          |
| 6.00 | 0.78  | 18.99 | 14.81    | 0.05          |
| 6.00 | 0.78  | 6.33  | 4.94     | 0.05          |
| 6.00 | 0.78  | 60.13 | 46.90    | 0.05          |

Legend: Dose (nmol/site); $x_i$: Log of dose (nmol/site); $y_i$: (% of MEP); $x_iy_i$: product $x_iy_i$; $(x_i^2-X)^2$: $(x_i - \bar{x})^2$.

- Linear regression summary:

\[
\sum x = -8.38; \sum y = 1118.42; N = 60; \bar{x} = -0.1397; \bar{y} = 18.64; \sum x^2 = 22.13; \\
\sum xy = 185.67; N\bar{xy} = -156.20; \bar{x}^2 = 0.0195
\]

\[
b = \frac{\sum xy - \bar{x} \sum y}{\sum x^2 - N \bar{x}^2}.
\]

\[
a = \bar{y} - bx, \text{ thus } b = 16.3 ( \text{slope} ) \text{ and } a = 20.9 ( \text{intercept} )
\]

- Slope variance $= \frac{\sum y}{N-2 \cdot var(x)}$, wherein $s^2 = Q/(N-2)$ and $Q = \sum_{i=1}^{N}(y_i - a - bx_i)$

\[
V(b) = 31.3
\]

- Assessment if slope differs from 0:

\[
t_{slope} = b / \{V(b)\}^{1/2} = 2.91 \text{ which exceeds } t\text{-tabulated, thus slope differs from zero.}
\]

- $t_{table}$ (N-2 degrees of freedom; $P=0.05$; from $t$-distribution) = 1.96

- DE20 calculation: $DE20 = \left(20 - a\right)/b$ (in log scale); $DE20 = 0.056$ or 0.88 nmol/site

- Variance of DE20 ($V_{DE20}$): $= \left(1/\left[N + (\bar{x}^2 - \bar{x})/s_{xx}\right]\right)$; $V_{DE20} = 0.042$ (in log scale)
1.1.2. *Intraplantar Phαβ*

See Table 2.

| Dose | $\chi_i$ | $y_i$ | $\chi_i y_i$ | $(\chi_i - \bar{\chi})^2$ |
|------|----------|-------|--------------|----------------------------|
| 0.01 | -2.00    | 19.51 | -39.02       | 1.00                       |
| 0.01 | -2.00    | 27.44 | -54.88       | 1.00                       |
| 0.01 | -2.00    | 46.34 | -92.68       | 1.00                       |
| 0.01 | -2.00    | 0.33  | -0.66        | 1.00                       |
| 0.01 | -2.00    | 6.23  | -12.46       | 1.00                       |
| 0.01 | -2.00    | -36.30| 72.61        | 1.00                       |
| 0.01 | -2.00    | 20.49 | -40.98       | 1.00                       |
| 0.01 | -2.00    | 12.31 | -24.63       | 1.00                       |
| 0.03 | -1.52    | 18.90 | -28.73       | 0.27                       |
| 0.03 | -1.52    | 31.71 | -48.20       | 0.27                       |
| 0.03 | -1.52    | 18.69 | -28.41       | 0.27                       |
| 0.03 | -1.52    | 44.92 | -68.28       | 0.27                       |
| 0.03 | -1.52    | 7.54  | -11.46       | 0.27                       |
| 0.03 | -1.52    | 30.51 | -46.38       | 0.27                       |
| 0.03 | -1.52    | 30.51 | -46.38       | 0.27                       |
| 0.03 | -1.52    | -15.59| 23.70        | 0.27                       |
| 0.10 | -1.00    | 64.63 | -64.63       | 0.00                       |
| 0.10 | -1.00    | 26.22 | -26.22       | 0.00                       |
| 0.10 | -1.00    | 50.61 | -50.61       | 0.00                       |
| 0.10 | -1.00    | 48.17 | -48.17       | 0.00                       |
| 0.10 | -1.00    | 8.20  | -8.20        | 0.00                       |
| 0.10 | -1.00    | 14.10 | -14.10       | 0.00                       |
| 0.10 | -1.00    | 35.74 | -35.74       | 0.00                       |
| 0.10 | -1.00    | 37.05 | -37.05       | 0.00                       |
| 0.10 | -1.00    | 25.84 | -25.84       | 0.00                       |
| 0.10 | -1.00    | 29.18 | -29.18       | 0.00                       |
| 0.10 | -1.00    | 38.53 | -38.53       | 0.00                       |
| 0.30 | -0.52    | 54.27 | -28.22       | 0.23                       |
| 0.30 | -0.52    | 44.51 | -23.15       | 0.23                       |
| 0.30 | -0.52    | 43.61 | -22.68       | 0.23                       |
| 0.30 | -0.52    | 20.00 | -10.40       | 0.23                       |
| 0.30 | -0.52    | 30.49 | -15.86       | 0.23                       |
| 0.30 | -0.52    | 45.88 | -23.86       | 0.23                       |
| 0.30 | -0.52    | 55.23 | -28.72       | 0.23                       |
| 0.30 | -0.52    | 5.12  | -2.66        | 0.23                       |
| 0.50 | -0.30    | 67.68 | -20.30       | 0.49                       |
| 0.50 | -0.30    | 68.29 | -20.49       | 0.49                       |
| 0.50 | -0.30    | 62.20 | -18.66       | 0.49                       |
| 0.50 | -0.30    | 62.80 | -18.84       | 0.49                       |
| 0.50 | -0.30    | 65.85 | -19.76       | 0.49                       |
| 0.50 | -0.30    | 30.49 | -9.15        | 0.49                       |
| 0.50 | -0.30    | 35.08 | -10.52       | 0.49                       |
| 0.50 | -0.30    | 41.64 | -12.49       | 0.49                       |
1.1.3. Intrathecal Phα1β

See Table 3.

### Table 2 (continued)

| Dose | xi | yi | xiyi | (xi−X)² |
|------|----|----|------|--------|
| 0.50 | -0.30 | 41.64 | -12.49 | 0.49 |
| 0.50 | -0.30 | 41.87 | -12.56 | 0.49 |
| 0.50 | -0.30 | 58.57 | -17.57 | 0.49 |
| 0.50 | -0.30 | 56.57 | -16.97 | 0.49 |

**Legend:** Dose (nmol/site); xi: Log of dose (nmol/site); yi: (% of MEP); xiyi: product xi.yi; (xi−X̅)²: (xi − xi averaged)².

Linear regression summary:
- $\sum x = -46.92$; $\sum y = 1589.6$; $N = 47$; $X̅ = -0.99$; $Y̅ = 33.82$; $\sum x^2 = 64.72$; $\sum x.y = -1175.24$; $N.x.y = -1586.9$; $X̅^2 = 0.99$
- $b = \frac{\sum xyi - Nxy}{\sum xi^2 - Nx^2}$
- $a = \frac{\sum yi - N \cdot \bar{y}}{N} - b \cdot \bar{x}$
- $s^2 = \frac{Q}{N-2}$ and $Q = \sum_{i=1}^{N} (y_i - a - bx_i)$
- $V(b) = \frac{s^2}{b^2} \left[ \frac{1}{N} + \frac{(\bar{x} - x)^2}{S_{xx}} \right]$; $V_{DE20} = 0.025$ (in log scale)
- $t_{slope} = b / \sqrt{V(b)}$; $t_{table} = 1.96$ (N-2 degrees of freedom; P=0.05; from t-distribution)
- DE20 calculation: $DE20 = (20 - a)/b$ (in log scale); DE20 = −160 or 0.025 nmol/site
- **Variance of DE20** ($V_{DE20}$) = $s^2 / b^2$ (in log scale)

### Table 3

Dose-effect data of Phα1β (i.t.) on capsaicin-induced nociception.

| Dose | xi | yi | xiyi | (xi−X)² |
|------|----|----|------|--------|
| 0.0003 | -3.52 | 39.54 | -139.20 | 2.47 |
| 0.0003 | -3.52 | -25.58 | 90.05 | 2.47 |
| 0.0003 | -3.52 | 31.40 | -110.50 | 2.47 |
| 0.0003 | -3.52 | -4.65 | 16.37 | 2.47 |
| 0.0003 | -3.52 | 41.29 | -145.30 | 2.47 |
| 0.0003 | -3.52 | 13.11 | -46.15 | 2.47 |
| 0.0003 | -3.52 | 4.50 | -15.84 | 2.47 |
| 0.0003 | -3.52 | -0.98 | 3.44 | 2.47 |
| 0.0003 | -3.52 | 13.82 | -48.64 | 2.47 |
| 0.0003 | -3.52 | 12.88 | -45.34 | 2.47 |
| 0.0003 | -3.52 | -1.17 | 4.12 | 2.47 |
| 0.0003 | -3.52 | 20.38 | -71.72 | 2.47 |
| 0.0003 | -2.52 | 50.00 | -126.00 | 0.33 |
| 0.0003 | -2.52 | 24.63 | -62.06 | 0.33 |
| 0.0003 | -2.52 | 39.55 | -99.67 | 0.33 |
| 0.0003 | -2.52 | 34.18 | -86.13 | 0.33 |
| 0.0003 | -2.52 | 20.38 | -68.55 | 0.33 |
| 0.0003 | -2.52 | 24.07 | -108.00 | 0.33 |
| 0.0003 | -2.52 | 17.44 | -43.95 | 0.33 |
| 0.0003 | -2.52 | 23.26 | -58.60 | 0.33 |
| 0.0003 | -2.52 | 20.93 | -52.74 | 0.33 |
| 0.0003 | -2.52 | 27.20 | -68.55 | 0.33 |
| 0.0003 | -2.52 | 63.47 | -159.90 | 0.33 |
| 0.0003 | -2.52 | 42.86 | -108.00 | 0.33 |
| 0.0003 | -2.52 | 37.24 | -93.84 | 0.33 |
| 0.0003 | -2.00 | 19.84 | -39.68 | 0.00 |
| 0.0003 | -2.00 | 30.95 | -61.91 | 0.00 |
| 0.0003 | -2.00 | 47.76 | -95.52 | 0.00 |
| 0.0003 | -2.00 | 50.00 | -100.00 | 0.00 |
| 0.0003 | -2.00 | 96.27 | -192.50 | 0.00 |
### Table 3 (continued)

| Dose | $x_i$ | $y_i$ | $x_i y_i$ | $(x_i - \bar{X})^2$ |
|------|-------|-------|-----------|-------------------|
| 0.01 | -2.00 | 40.08 | -80.17 | 0.00 |
| 0.01 | -2.00 | 33.33 | -66.67 | 0.00 |
| 0.01 | -2.00 | 26.58 | -53.16 | 0.00 |
| 0.01 | -2.00 | 26.19 | -52.38 | 0.00 |
| 0.01 | -2.00 | 43.52 | -83.33 | 0.00 |
| 0.01 | -2.00 | 37.96 | -75.93 | 0.00 |
| 0.01 | -2.00 | 35.19 | -70.37 | 0.00 |
| 0.01 | -2.00 | 43.52 | -87.04 | 0.00 |
| 0.01 | -2.00 | 45.37 | -90.74 | 0.00 |
| 0.01 | -2.00 | 50.00 | -100.00 | 0.00 |
| 0.10 | -1.00 | 50.00 | -50.00 | 0.90 |
| 0.10 | -1.00 | 36.51 | -36.51 | 0.90 |
| 0.10 | -1.00 | 39.55 | -39.55 | 0.90 |
| 0.10 | -1.00 | 69.40 | -69.40 | 0.90 |
| 0.10 | -1.00 | 68.66 | -68.66 | 0.90 |
| 0.10 | -1.00 | 29.11 | -29.11 | 0.90 |
| 0.10 | -1.00 | 40.93 | -40.93 | 0.90 |
| 0.10 | -1.00 | 28.27 | -28.27 | 0.90 |
| 0.10 | -1.00 | 33.33 | -33.33 | 0.90 |
| 0.10 | -1.00 | 58.33 | -58.33 | 0.90 |
| 0.10 | -1.00 | 42.59 | -42.59 | 0.90 |
| 0.30 | -0.52 | 57.14 | -29.71 | 2.04 |
| 0.30 | -0.52 | -3.97 | 2.06 | 2.04 |
| 0.30 | -0.52 | 58.21 | -30.27 | 2.04 |
| 0.30 | -0.52 | 71.64 | -37.25 | 2.04 |
| 0.30 | -0.52 | 69.44 | -69.44 | 2.04 |
| 0.30 | -0.52 | 75.93 | -39.48 | 2.04 |
| 0.30 | -0.52 | 51.85 | -26.96 | 2.04 |
| 0.30 | -0.52 | 62.96 | -32.74 | 2.04 |
| 0.30 | -0.52 | 77.78 | -40.44 | 2.04 |

Legend: Dose (nmol/site); $x_i$: Log of dose (nmol/site); $y_i$: (% of MEP); $x_i y_i$: product $x_i y_i$; $(x_i - \bar{X})^2$: ($x_i - \bar{x}_i$ averaged)$^2$.

- Linear regression summary:
  \[ \sum x = -132.32; \sum y = 2787; N = 72; \bar{X} = -1.84; \bar{Y} = 38.72; \sum x^2 = 320.64; \sum x y = -4120; N \bar{x} \bar{y} = -5123; \bar{x}^2 = 3.38 \]

\[
b = \frac{\sum x y - N \bar{x} \bar{y}}{\sum x^2 - N \bar{x}^2},
\]

\[a = -\bar{y} - b \bar{x}, \text{ thus } b = 12.9 \text{ (slope) and } a = 62.5 \text{ (intercept)}\]

- Slope variance \[V(b) = \frac{\sum y - a \sum x - b}{N - 2}^2\]

\[t_{slope} = b / \sqrt{V(b)} ; t_{slope} = 5.84 \text{ which exceeds } t\text{-tabulated, thus slope differs from zero.}\]

\[t_{table} (N-2 \text{ degrees of freedom}; P = 0.05; \text{ from } t\text{-distribution}) = 1.96\]

- DE20 calculation: \[DE20 = (20 - a)/b \text{ (in log scale); } DE20 = -3.28 \text{ or 0.5 pmol/site}\]

- Variance of DE20 \[V(\text{DE20}) = s^2 / b^2 \text{ (in log scale)}\]

- $\bar{x}^2$, $\bar{x}$, and $\bar{y}$, averaged, for calculations.
1.2. Calculation of the proportion of constituents in the mixture

\[ a + b = c \] wherein:
- \( a \) = the quantity (in nmol) of \( \text{SB366791} \)
- \( b \) = quantity (nmol) of \( \text{Ph\alpha_1\beta} \)
- \( c \) = sum (in nmol) of quantities of \( \text{Ph\alpha_1\beta} \) and \( \text{SB366791} \) in the mixture

The proportion of \( a \) and \( b \) was fixed and calculated according to the formulae below:

\[ a = A \times f \]
\[ b = (1-f) \times B \] wherein:
- \( A \) = \( \text{DE}_{20} \) of \( \text{SB366791} \)
- \( B \) = \( \text{DE}_{20} \) of \( \text{Ph\alpha_1\beta} \)
- \( f \) = proportion factor.

The factor \( f \) was calculated based on the variances of the \( \text{DE}_{20} \) values from \( \text{SB366791} \) (A) and \( \text{Ph\alpha_1\beta} \) (B) according to the formula:

\[ f = \frac{V(B)}{V(A) + V(B)} \] wherein:
- \( V(A) \) = variance of \( \text{DE}_{20} \) of \( \text{SB366791} \) and
- \( V(B) \) = variance of \( \text{DE}_{20} \) of \( \text{Ph\alpha_1\beta} \);

- For combination of intraplantar \( \text{Ph\alpha_1\beta} \) with \( \text{SB366791} \), \( f = 0.38 \) (Table 4 and 5).
- For combination of intraplantar \( \text{SB366791} \) with intrathecal \( \text{Ph\alpha_1\beta} \), \( f = 0.69 \) (Table 4 and 5).

### Table 4
Doses used in the dose-response curve for intraplantar combination of \( \text{SB366791} \) and \( \text{Ph\alpha_1\beta} \).

| Drug pair | SB366791 (nmol/site) | Ph\alpha_1\beta (nmol/site) | Composed drug pair (nmol/site) |
|-----------|----------------------|-----------------------------|------------------------------|
| 1         | 0.012                | 0.0006                      | 0.013                        |
| 2         | 0.037                | 0.001                       | 0.038                        |
| 3         | 0.11                 | 0.005                       | 0.115                        |
| 4         | 0.33                 | 0.016                       | 0.346                        |
| 5         | 0.99                 | 0.048                       | 1.038                        |
| 6         | 2.97                 | 0.144                       | 3.114                        |

### Table 5
Doses used in the dose-response curve for concurrent treatment with \( \text{SB366791} \) (i.p.) and \( \text{Ph\alpha_1\beta} \) (i.t.).

| Drug pair | SB366791 (nmol/site) | Ph\alpha_1\beta (nmol/site) | Composed drug pair (nmol/site) |
|-----------|----------------------|-----------------------------|------------------------------|
| 1         | 0.022                | 0.000000048                 | 0.022000048                  |
| 2         | 0.066                | 0.00000014                  | 0.06600014                   |
| 3         | 0.2                  | 0.0000043                   | 0.2000043                    |
| 4         | 0.60                 | 0.00013                     | 0.60013                      |
| 5         | 1.8                  | 0.00039                     | 1.80039                      |
| 6         | 5.4                  | 0.00117                     | 5.40117                      |
1.3. Summary of linear regression analyses for the agents administered in combination

1.3.1. Phα1β (i.p.) combined with SB366791 (i.p.)

See Table 6.

Table 6

Dose-effect data of Phα1β (i.p.) combined with SB366791 (i.p.) on capsaicin-induced nociception.

| Dose  | \( x_i \) | \( y_i \) | \( x_i y_i \) | \((x_i - \bar{x})^2\) |
|-------|-----------|-----------|---------------|------------------|
| 0.013 | -1.92     | 34.00     | -64.79        | 1.57             |
| 0.013 | -1.92     | 24.85     | -47.71        | 1.57             |
| 0.013 | -1.92     | 3.93      | -7.54         | 1.57             |
| 0.013 | -1.92     | 27.50     | -52.81        | 1.57             |
| 0.013 | -1.92     | -19.89    | 38.19         | 1.57             |
| 0.013 | -1.92     | -2.37     | 4.55          | 1.57             |
| 0.013 | -1.92     | 32.66     | -62.72        | 1.57             |
| 0.013 | -1.92     | -63.64    | 122.18        | 1.57             |
| 0.013 | -1.92     | 25.00     | -48.00        | 1.57             |
| 0.013 | -1.92     | 29.09     | -55.85        | 1.57             |
| 0.013 | -1.92     | -2.00     | 38.40         | 1.57             |
| 0.013 | -1.92     | 25.68     | -49.31        | 1.57             |
| 0.038 | -1.42     | 36.81     | -52.27        | 0.57             |
| 0.038 | -1.42     | 43.56     | -61.85        | 0.57             |
| 0.038 | -1.42     | 26.38     | -37.46        | 0.57             |
| 0.038 | -1.42     | 30.86     | -43.82        | 0.57             |
| 0.038 | -1.42     | 29.27     | -41.57        | 0.57             |
| 0.038 | -1.42     | 44.01     | -62.49        | 0.57             |
| 0.038 | -1.42     | 26.09     | -37.05        | 0.57             |
| 0.038 | -1.42     | 39.23     | -55.71        | 0.57             |
| 0.038 | -1.42     | 38.14     | -54.16        | 0.57             |
| 0.038 | -1.42     | 27.05     | -38.40        | 0.57             |
| 0.038 | -1.42     | 20.23     | -28.72        | 0.57             |
| 0.038 | -1.42     | 16.82     | -23.88        | 0.57             |
| 0.115 | -0.93     | 15.95     | -14.83        | 0.07             |
| 0.115 | -0.93     | 46.01     | -42.79        | 0.07             |
| 0.115 | -0.93     | 27.61     | -25.67        | 0.07             |
| 0.115 | -0.93     | 50.10     | -46.59        | 0.07             |
| 0.115 | -0.93     | 44.09     | -41.00        | 0.07             |
| 0.115 | -0.93     | 28.09     | -26.13        | 0.07             |
| 0.115 | -0.93     | 32.22     | -29.96        | 0.07             |
| 0.115 | -0.93     | 46.37     | -43.12        | 0.07             |
| 0.115 | -0.93     | 26.09     | -24.27        | 0.07             |
| 0.115 | -0.93     | 41.42     | -38.52        | 0.07             |
| 0.115 | -0.93     | 37.59     | -34.96        | 0.07             |
| 0.115 | -0.93     | 31.82     | -29.59        | 0.07             |
| 0.115 | -0.93     | 25.00     | -23.25        | 0.07             |
| 0.115 | -0.93     | 16.00     | -15.01        | 0.07             |
| 0.115 | -0.93     | 16.82     | -15.64        | 0.07             |
| 0.346 | -0.46     | 51.53     | -23.71        | 0.04             |
| 0.346 | -0.46     | 52.15     | -23.99        | 0.04             |
| 0.346 | -0.46     | 31.90     | -14.67        | 0.04             |
| 0.346 | -0.46     | 54.31     | -24.98        | 0.04             |
| 0.346 | -0.46     | 51.30     | -23.60        | 0.04             |
| 0.346 | -0.46     | 59.12     | -27.19        | 0.04             |
| 0.346 | -0.46     | 40.01     | -18.40        | 0.04             |
| 0.346 | -0.46     | 33.99     | -15.63        | 0.04             |
| 0.346 | -0.46     | 46.90     | -21.57        | 0.04             |
| 0.346 | -0.46     | 60.04     | -27.62        | 0.04             |
| 0.346 | -0.46     | 74.27     | -34.16        | 0.04             |
| 0.346 | -0.46     | 35.91     | -16.52        | 0.04             |
| 0.346 | -0.46     | 30.45     | -14.01        | 0.04             |
Table 6 (continued)

| Dose   | x<sub>i</sub> | y<sub>i</sub> | x<sub>i</sub>y<sub>i</sub> | (x<sub>i</sub>−X̅)<sup>2</sup> |
|--------|--------------|--------------|---------------------|------------------|
| 0.346  | -0.46        | 36.59        | -16.83              | 0.04             |
| 0.346  | -0.46        | 15.45        | -7.11               | 0.04             |
| 1.038  | 0.02         | 52.76        | 0.84                | 0.47             |
| 1.038  | 0.02         | 60.12        | 0.96                | 0.47             |
| 1.038  | 0.02         | 53.99        | 0.86                | 0.47             |
| 1.038  | 0.02         | 86.77        | 1.39                | 0.47             |
| 1.038  | 0.02         | 49.50        | 0.79                | 0.47             |
| 1.038  | 0.02         | 63.93        | 1.02                | 0.47             |
| 1.038  | 0.02         | 54.03        | 0.86                | 0.47             |
| 1.038  | 0.02         | 45.78        | 0.73                | 0.47             |
| 1.038  | 0.02         | 49.90        | 0.80                | 0.47             |
| 1.038  | 0.02         | 49.64        | 0.79                | 0.47             |
| 1.038  | 0.02         | 65.51        | 1.05                | 0.47             |
| 1.038  | 0.02         | 59.49        | 0.95                | 0.47             |
| 1.038  | 0.02         | 45.45        | 0.73                | 0.47             |
| 1.038  | 0.02         | 45.45        | 0.73                | 0.47             |
| 1.038  | 0.02         | 16.14        | 0.26                | 0.47             |
| 1.038  | 0.02         | 50.23        | 0.80                | 0.47             |
| 3.114  | 0.49         | 54.60        | 26.75               | 1.34             |
| 3.114  | 0.49         | 63.80        | 31.26               | 1.34             |
| 3.114  | 0.49         | 66.26        | 32.47               | 1.34             |
| 3.114  | 0.49         | 69.94        | 34.27               | 1.34             |
| 3.114  | 0.49         | 61.52        | 30.15               | 1.34             |
| 3.114  | 0.49         | 65.13        | 31.91               | 1.34             |
| 3.114  | 0.49         | 61.10        | 29.94               | 1.34             |
| 3.114  | 0.49         | 58.74        | 28.78               | 1.34             |
| 3.114  | 0.49         | 68.17        | 33.40               | 1.34             |
| 3.114  | 0.49         | 67.70        | 33.17               | 1.34             |
| 3.114  | 0.49         | 68.25        | 33.44               | 1.34             |
| 3.114  | 0.49         | 72.08        | 35.32               | 1.34             |

Legend: Dose (nmol/site); x<sub>i</sub>: Log of dose (nmol/site); y<sub>i</sub>: (% of MEP); x<sub>i</sub>y<sub>i</sub>: product x<sub>i</sub>y<sub>i</sub>; (x<sub>i</sub>−X̅)<sup>2</sup>: (x<sub>i</sub> – x<sub>i</sub> averaged)<sup>2</sup>.

- Linear regression summary:
  \[ \sum x = -54.8; \sum y = 3260; N = 82; X = 0.66822; Y = 40; \sum x^2 = 87,4664; \]
  \[ \sum x.y = -1089.7; N.x.y = -2178.55; x^2 = 0.446517 \]

\[ b = \frac{\sum x_i y_i - N \bar{x} \bar{y}}{\sum x_i^2 - N \bar{x}^2} ; \]

\[ a = \bar{y} - bx, \text{ thus } b = 21.4 (\text{slope}) \text{ and } a = 54.1 (\text{intercept}) \]

- Slope variance = \[ \text{V(b)} = \frac{s^2}{\sum x_i^2 - N \bar{x}^2} \]
  wherein \( s^2 = Q/(N-2) \) and \( Q = \sum_{i=1}^{N} (y_i - a - bx_i) \)

\[ \text{V(b)} = 4.9 \]

- Assessment if slope differs from 0:

\[ t_{\text{slope}} = \frac{b}{\text{V(b)}}^{1/2}; t_{\text{slope}} = 9.64 \text{ which exceeds t-table, thus slope differs from zero} \]

\[ t_{\text{table}} (N-2 \text{ degrees of freedom}; P = 0.05; \text{ from t-distribution}) = 1.96 \]

- DE20 calculation: \[ DE20 = (20 - a)b \] (in log scale); DE20 = −1.59 or 0.025 nmol/site

- Variance of DE20 \[ \text{V(DE20)} = s^2 / b^2 \mid 1/N + (x^2 - \bar{x}^2) / S_{xx} \]; \[ \text{V(DE20)} = 0.016 \] (in log scale)
1.3.2. Phα1β (i.t.) combined with SB366791 (i.p.)

See Table 7.

Table 7
Dose-effect data Phα1β (i.t.) and SB366791 (i.p.) on capsaicin-induced nociception.

| Dose  | xi   | yi   | xiyi  | (xi−X̄)² |
|-------|------|------|-------|----------|
| 0.022 | -1.66| 2.00 | -3.07 | 1.50     |
| 0.022 | -1.66| -33.77| 56.06 | 1.50     |
| 0.022 | -1.66| 32.95| -54.70| 1.50     |
| 0.022 | -1.66| -34.79| 57.76 | 1.50     |
| 0.022 | -1.66| 29.24| -48.54| 1.50     |
| 0.022 | -1.66| 32.19| -53.43| 1.50     |
| 0.022 | -1.66| 49.14| -81.57| 1.50     |
| 0.022 | -1.66| 5.42 | -9.00 | 1.50     |
| 0.022 | -1.66| 23.73| -39.39| 1.50     |
| 0.022 | -1.66| 37.13| -61.64| 1.50     |
| 0.066 | -1.18| 34.40| -57.10| 1.50     |
| 0.066 | -1.18| 15.30| -18.06| 0.56     |
| 0.066 | -1.18| 51.72| -60.09| 0.56     |
| 0.066 | -1.18| 50.92| -60.09| 0.56     |
| 0.066 | -1.18| 25.35| -29.91| 0.56     |
| 0.066 | -1.18| -29.95| 35.35 | 0.56     |
| 0.066 | -1.18| 34.40| -40.59| 0.56     |
| 0.066 | -1.18| 39.56| -46.68| 0.56     |
| 0.066 | -1.18| 49.14| -57.99| 0.56     |
| 0.066 | -1.18| 24.75| -29.20| 0.56     |
| 0.066 | -1.18| 32.88| -38.80| 0.56     |
| 0.066 | -1.18| 34.40| -40.59| 0.56     |
| 0.066 | -1.18| 26.88| -31.72| 0.56     |
| 0.200 | -0.69| 27.18| -18.75| 0.07     |
| 0.200 | -0.69| 5.01 | -3.46 | 0.07     |
| 0.200 | -0.69| 54.88| -37.87| 0.07     |
| 0.200 | -0.69| 14.98| -10.33| 0.07     |
| 0.200 | -0.69| -2.30| 1.59  | 0.07     |
| 0.200 | -0.69| 50.23| -34.66| 0.07     |
| 0.200 | -0.69| 52.09| -35.94| 0.07     |
| 0.200 | -0.69| 46.93| -32.38| 0.07     |
| 0.200 | -0.69| 68.30| -47.13| 0.07     |
| 0.200 | -0.69| 31.86| -21.99| 0.07     |
| 0.200 | -0.69| 40.00| -27.60| 0.07     |
| 0.200 | -0.69| 59.32| -40.93| 0.07     |
| 0.200 | -0.69| 35.08| -24.21| 0.07     |
| 0.200 | -0.69| 34.00| -23.73| 0.07     |
| 0.200 | -0.69| 55.58| -38.35| 0.07     |
| 0.600 | -0.22| 62.01| -13.64| 0.05     |
| 0.600 | -0.22| 37.47| -8.24 | 0.05     |
| 0.600 | -0.22| 35.88| -7.89 | 0.05     |
| 0.600 | -0.22| 70.28| -15.46| 0.05     |
| 0.600 | -0.22| 54.38| -11.96| 0.05     |
| 0.600 | -0.22| 53.69| -11.81| 0.05     |
| 0.600 | -0.22| 54.30| -11.95| 0.05     |
| 0.600 | -0.22| 53.56| -11.78| 0.05     |
| 0.600 | -0.22| 65.36| -14.38| 0.05     |
| 0.600 | -0.22| 41.02| -9.02 | 0.05     |
| 0.600 | -0.22| 36.95| -8.13 | 0.05     |
| 0.600 | -0.22| 59.32| -13.05| 0.05     |
| 0.600 | -0.22| 52.16| -11.48| 0.05     |
| 0.600 | -0.22| 41.23| -9.07 | 0.05     |
| 0.600 | -0.22| 56.95| -12.53| 0.05     |
1.4. Statistical comparisons between experimentally obtained DE20 (Zmix) and Theoretical additive DE20 (Zadd)

- Calculation of the Zadd:
  \[ Z_{add} = fA + (1-f)B \]

  - \( f \) is the proportion factor (obtained from topic B);
  - \( A = DE20 \) of SB366791;
  - \( B = DE20 \) of Phα1β.
\[ \text{Variance of } Z_{\text{add}} = f^2 \cdot \text{V}(A) + (1-f)^2 \cdot \text{V}(B). \]

wherein

\[ f = \text{proportion factor}; \]

\[ \text{V}(A) \text{ and } \text{V}(B) = \text{Variances of the DE}_{20} \text{ of SB366791 and Ph}_{\alpha}1\beta, \text{ respectively.} \]

### Comparison test

\[ t_{\text{critical}} = \frac{(x-y)}{\sqrt{(SE_x)^2 + (SE_y)^2}}; \]

wherein:

\[ x = \log \text{ of } Z_{\text{add}} \]

\[ y = \log \text{ of } Z_{\text{mix}} \]

\[ (SE_x)^2 = \text{V}(Z_{\text{add}}) \]

\[ (SE_y)^2 = \text{V}(Z_{\text{mix}}) \]

\[ T_{\text{tabulated}} = \frac{(t_{\text{add}}(SE_x)^2 + t_{\text{mix}}(SE_y)^2)}{[(SE_x)^2 + (SE_y)^2]}; \]

where in:

\[ t_{\text{add}} = \text{tabular value of } t \text{ based on } N^-2 \text{ degrees of freedom for 95% of significance} \]

\[ t_{\text{mix}} = \text{tabular value of } t \text{ based on } N^-2 \text{ degrees of freedom for 95% of significance} \]

\[ (SE_y)^2 = \text{Variance of } Z_{\text{mix}} \]

Interpretation: If \( t_{\text{critical}} < T_{\text{tabulated}} \), then the difference is not significant, which means an additive effect. When \( t_{\text{critical}} > T_{\text{tabulated}} \), \( Z_{\text{mix}} \) is significantly smaller (95% of confidence) than the \( Z_{\text{add}} \), which implies a synergistic effect of combined drugs (Table 8).

### 2. Experimental design, materials and methods

#### 2.1. Capsaicin-induced nociceptive responses

This test was conducted essentially as described previously [1]. Briefly, individual animals were placed in transparent acrylic square boxes (20 cm per side) immediately after the capsaicin injection (1 nmol/paw i.p.), and nociceptive behaviours were recorded continuously for 300 s. Behaviours were quantified by recording the time spent licking, biting or flinching (nociceptive time) the paw injected with capsaicin. Ph\(\alpha1\beta\) (i.t. or i.p.), SB366791 (i.p.), combined Ph\(\alpha1\beta + SB366791\) or their respective vehicles were injected 10 min before the capsaicin injection. The initial dose ranges of Ph\(\alpha1\beta\) and SB366791 were selected based on previous data [1]. Antinociceptive effects were measured as a percentage of the maximum possible (% MPE) effect according to the formula: % MPE = 100\(^*(A - B) / A\), where A is the averaged nociceptive time of the vehicle group and B is the nociceptive time of each animal in the treated group (drugs alone or in combination) [2].

#### 2.2. Experimental design and statistical isobolographic analysis

Experimental design and statistical analyses were conducted essentially as previously described [3–5]. Briefly, dose-response curves were first obtained for Ph\(\alpha1\beta\) and SB366791 administered alone. Line equations, slope values and respective variances were obtained using linear regression [3]. \(DE_{20}\) values and 95% confidence limits were calculated using probit analysis. \(DE_{20}\) values (doses that
exhibit 20% MPE) were used to assess whether the dose-effect of these drugs alone exhibited a constant potency ratio, which is necessary to perform fixed dose-pair combination of drugs [4].

Drug doses in association studies were determined as a proportion of their DE20 values. This proportion was constant and estimated based on a factor derived from the individual variances of the DE20 values. This fixed proportion of agents was necessary to assess whether the combination displayed enhanced potency indicative of synergism. Dose-response curves of associated drugs were constructed to obtain the doses that achieved the same effect level (20% MPE) compared to drugs given alone. This experimentally obtained DE20 (here called $Z_{mix}$) was compared to theoretically calculated DE20 value for additive interactions ($Z_{add}$). The criterion for establishing a statistical significance was $P < 0.05$. Graphical assessments of synergy are also presented using isobolographic analyses. Measurement of the interaction index ($\alpha$) was obtained by dividing experimentally obtained DE20 of the drug pair by the theoretical additive DE20 of the drug pair. The $\alpha$ interaction index provides a measure of the degree of synergism.

Acknowledgements

We thank Dr. Ronald J. Tallarida (in memorian) for the support on the isobolographic experimental design. We also thank for the funding agencies for supporting this work: Fapemig CBB-RED-00006-14, CNPq Universal 456048/2014, FAPEMIG Universal APQ-01553-14, Capes Toxinology 1444/2011, Capes Decit 2865/10, and INCT Medicina Molecular, CNPq 471070-2012. We thank those funds agencies for fellowship support.

Transparency document. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dib.2017.07.059.

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