

Supplementary Information

Antinociceptive activities of a novel diarylpentanoid analogue, 2-benzyol-6-(3-bromo-4-hydroxybenzylidene)cyclohexen-1-ol, and its possible mechanisms of action in mice

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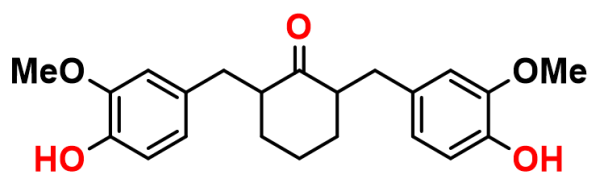
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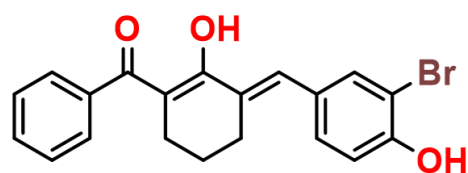
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Chemical structures of BHMC and BBHC



BHMC



BBHC

Sample size determination

In our pilot/exploratory study, 3 treatment groups (BBHC, vehicle and positive control) were carried out with the sample sizes per group using resource equation approach as stated below:

$$\begin{aligned}\text{Minimum } n &= DF/k + 1; DF = 10, k = 3 \text{ (number of treatment group)} \\ &= 10/3 + 1 \\ &= 3.3 + 1 \\ &= 4.3 \text{ (rounded up to 5 animals per group)}\end{aligned}$$

$$\begin{aligned}\text{Maximum } n &= DF/k + 1; DF = 20, k = 3 \\ &= 20/3 + 1 \\ &= 6.7 + 1 \\ &= 7.7 \text{ (rounded down to 7 animals per group)}\end{aligned}$$

The minimal and maximal numbers of animals per group are rounded up and down to ensure the DF of each sample size per group falls within the limit (DF limit: 10 – 20), respectively (e.g., DF = 12 for n = 5; DF = 18 for n = 7). Therefore, the number of animals per group, n=6 was applied for our pilot study.

Based on the calculation of sample size (n) in our pilot study and previous experiences in antinociceptive studies of our research group, the sample size per group was kept as n = 6 throughout the entire study to give us statistically significant and reliable data, with adequate controls.