# Chemistry of spices: Bornyl 4-methoxybenzoate from *Ferula ovina* (Boiss.) Boiss. induces hyperalgesia in mice

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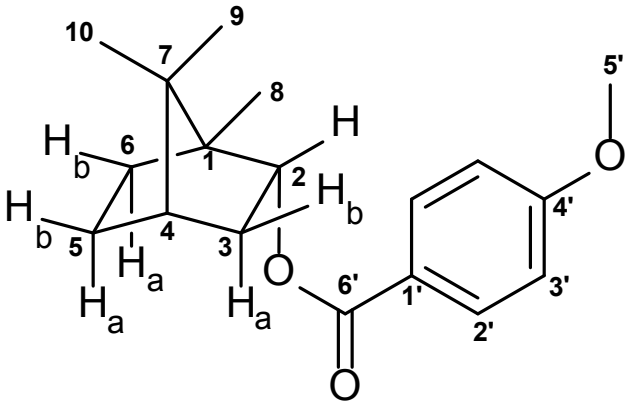


Table S1. $^{1}H$ and $^{13}C$ NMR chemical shifts of bornyl 4-methoxybenzoate

| $^{13}C$ Signals (δ/ppm) | Type of carbon | Assignment | $^{1}H$ Signals (δ/ppm, *J*/Hz) |
|---|---|---|---|
| 166.6 | C=O | C-6' | |
| 163.3 | quat. ArC | C-4' | |
| 131.5 | arCH | C-2' | 8.02 |
| 123.5 | quat. ArC | C-1' | |
| 113.6 | arCH | C-3' | 6.93 |
| 80.1 | CH | C-2 | 5.09, $^{3}J_{2,3b}$ = 9.9 Hz<br>$^{3}J_{2,3a}$ = 3.5 Hz<br>$^{4}J_{2,6b}$ = 2.2 Hz |
| 55.1 | $OCH_3$ | C-5' | 3.86 |
| 49.1 | $C_q$ | C-1 | |
| 47.9 | $C_q$ | C-7 | |
| 45.0 | CH | C-4 | $^{3}J_{3b,4}$ = 4.5 Hz |
| 36.9 | $CH_2$ | C-3 | a: 1.11, b: 2.46<br>$^{2}J_{3a,3b}$ = 13.8 Hz<br>$^{3}J_{2,3b}$ = 9.9 Hz<br>$^{3}J_{3b,4}$ = 4.5 Hz<br>$^{3}J_{2,3a}$ = 3.5 Hz |
| 28.1 | $CH_2$ | C-5 | a: 1.32. b: 1.83 |
| 27.4 | $CH_2$ | C-6 | a: 2.13. b: 1.41 |
| 19.7 | $CH_3$ | C-10 | 0.92 |
| 18.9 | $CH_3$ | C-9 | 0.97 |
| 13.6 | $CH_3$ | C-8 | 0.91 |

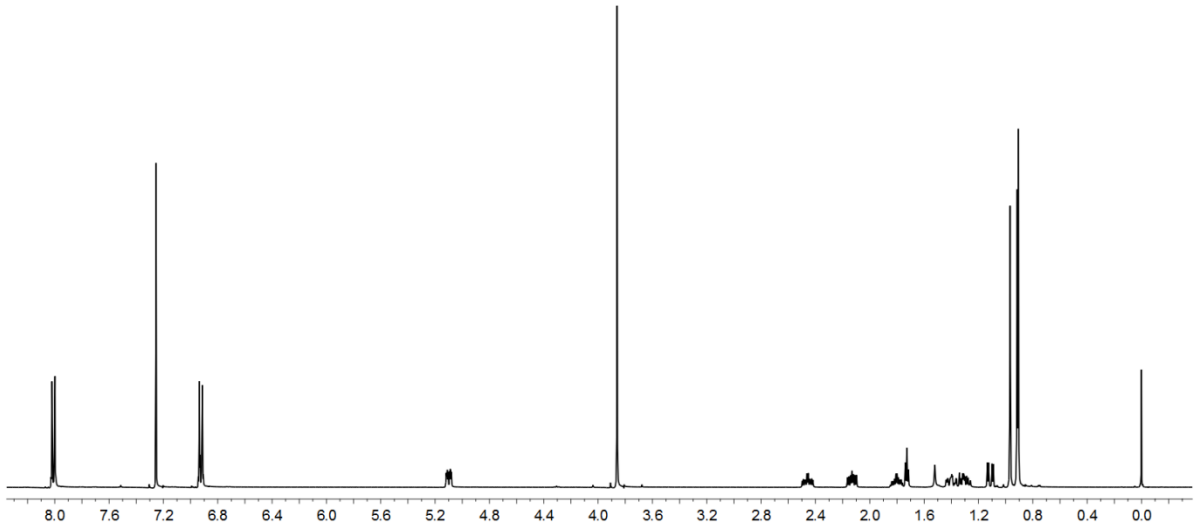


Fig. S1. $^{1}$H NMR spectrum

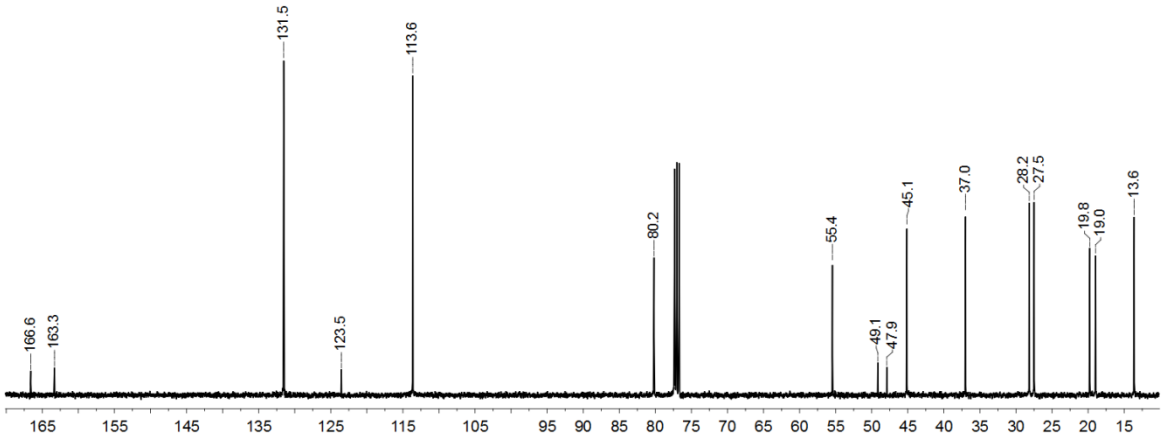


Fig. S2a. $^{13}$C NMR spectrum

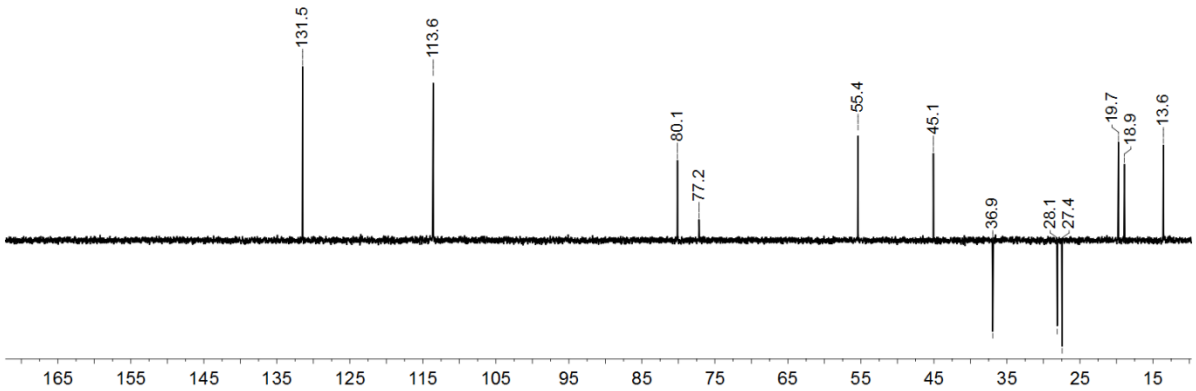


Fig. S2b. DEPT 135 spectrum. $CH_3$ and CH signals are above, $CH_2$ signals are below the baseline.

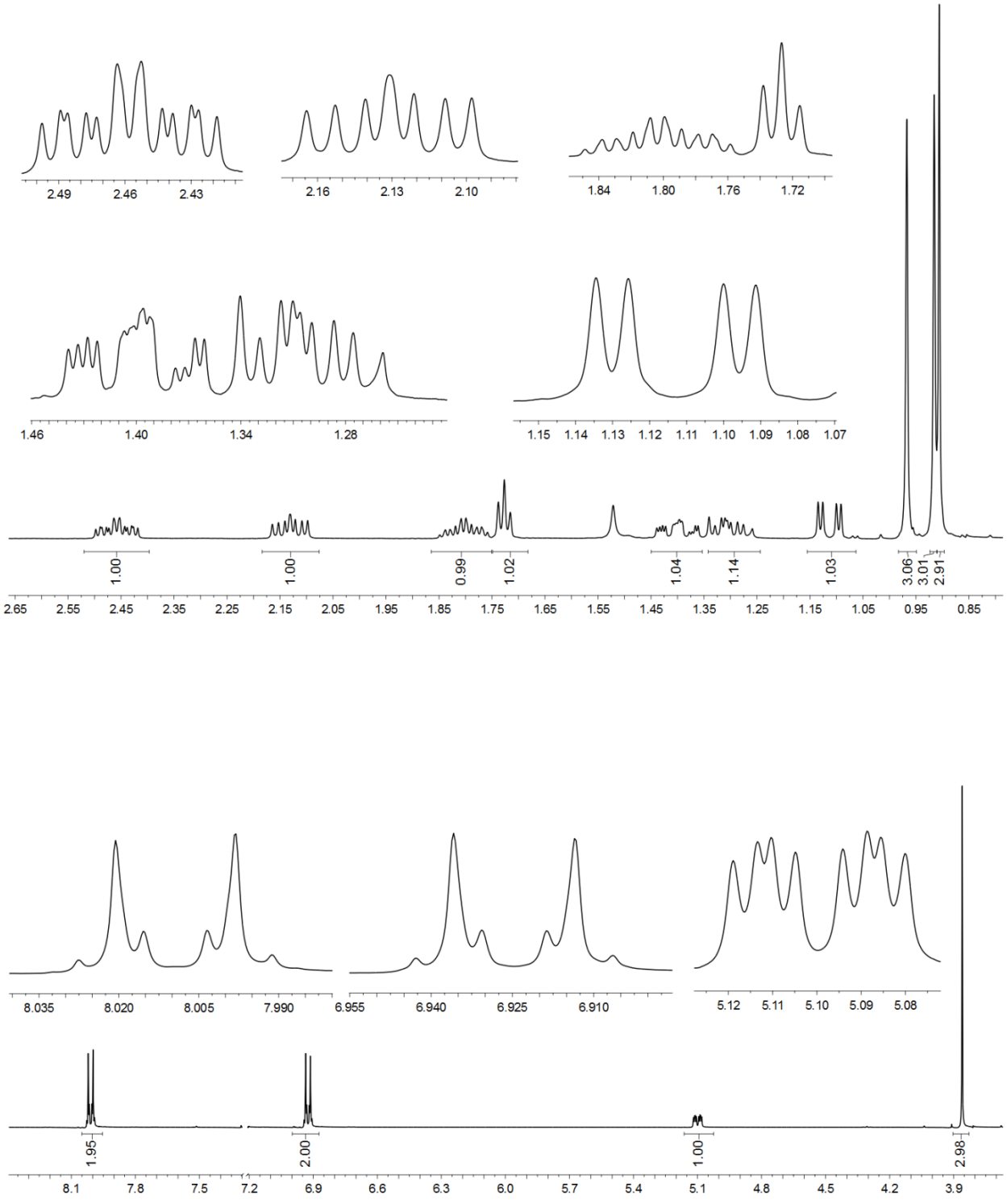


Fig. S3. Expanded regions of $^{1}$H NMR spectrum

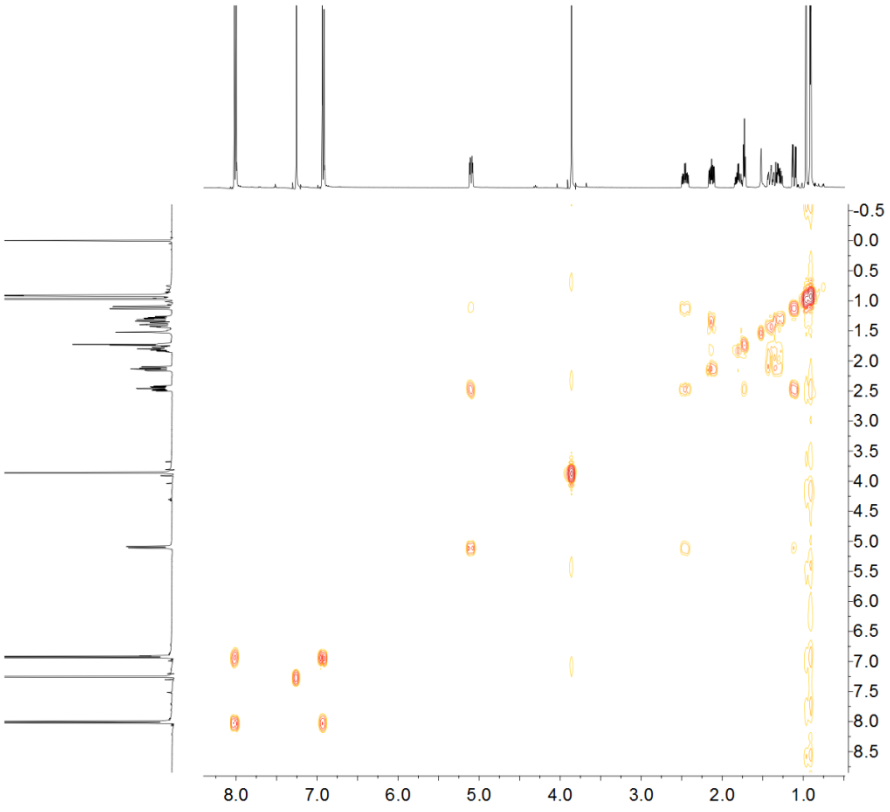


Fig. S4a. COSY spectrum

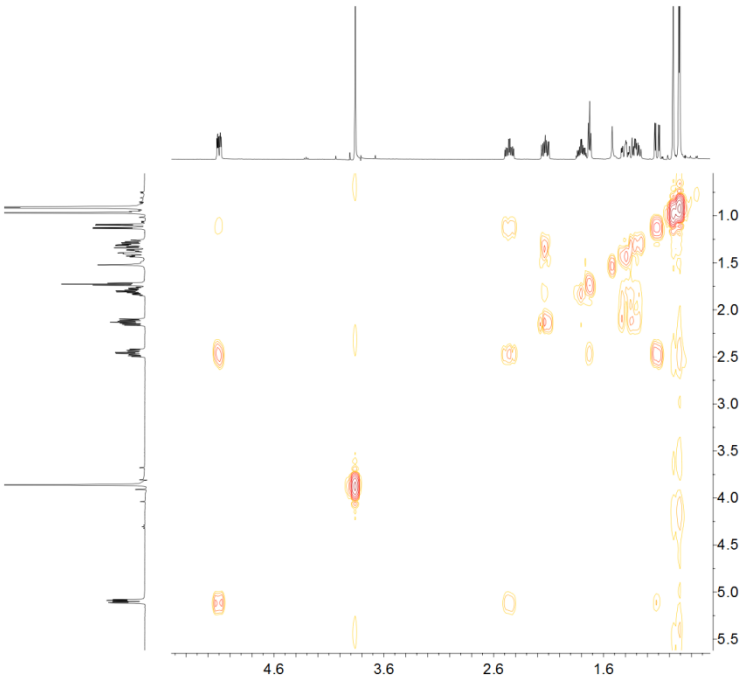


Fig. S4b. COSY spectrum expanded in the region from 0.5 to 5.5 ppm.

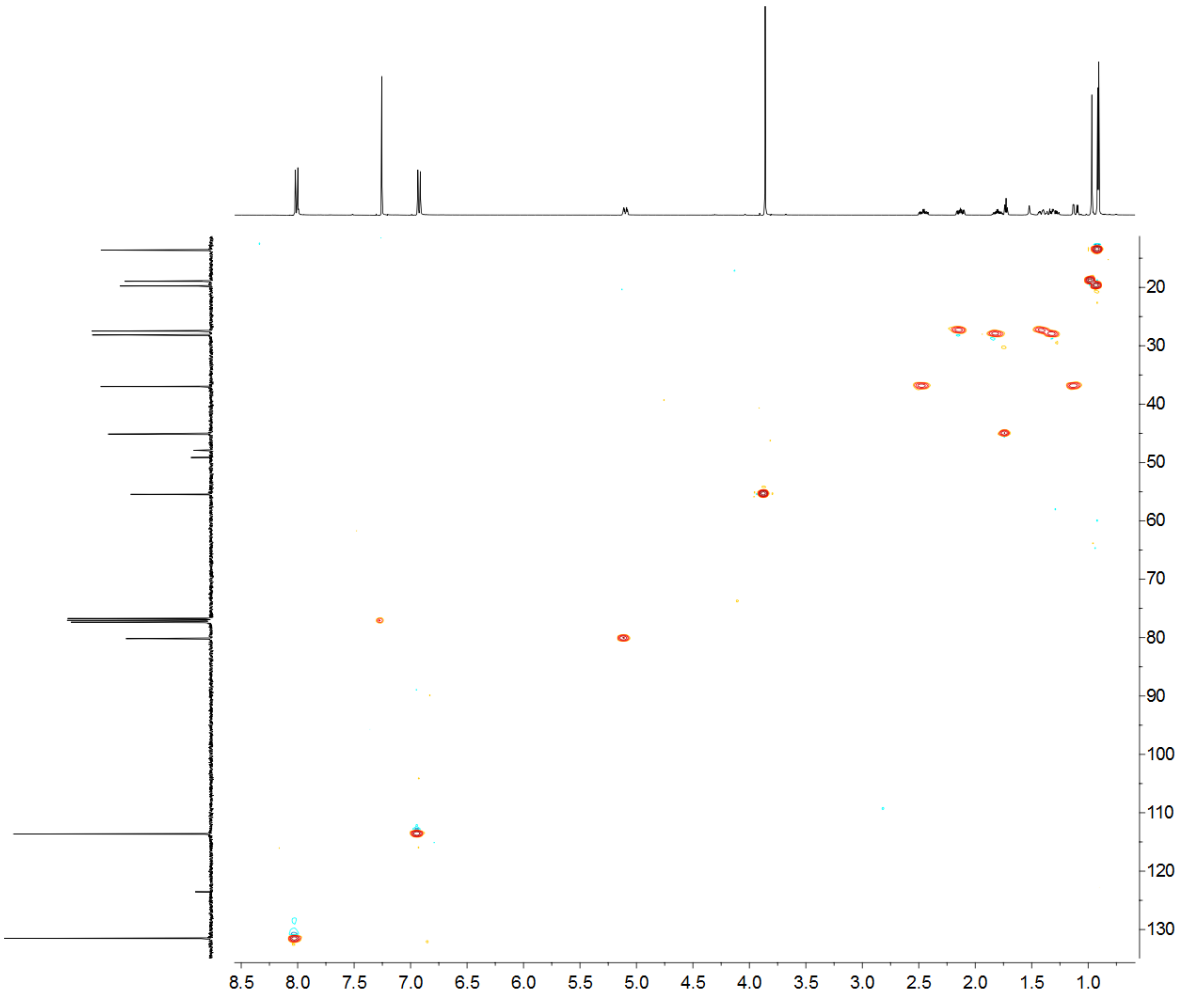


Fig. S5. HSQC spectrum

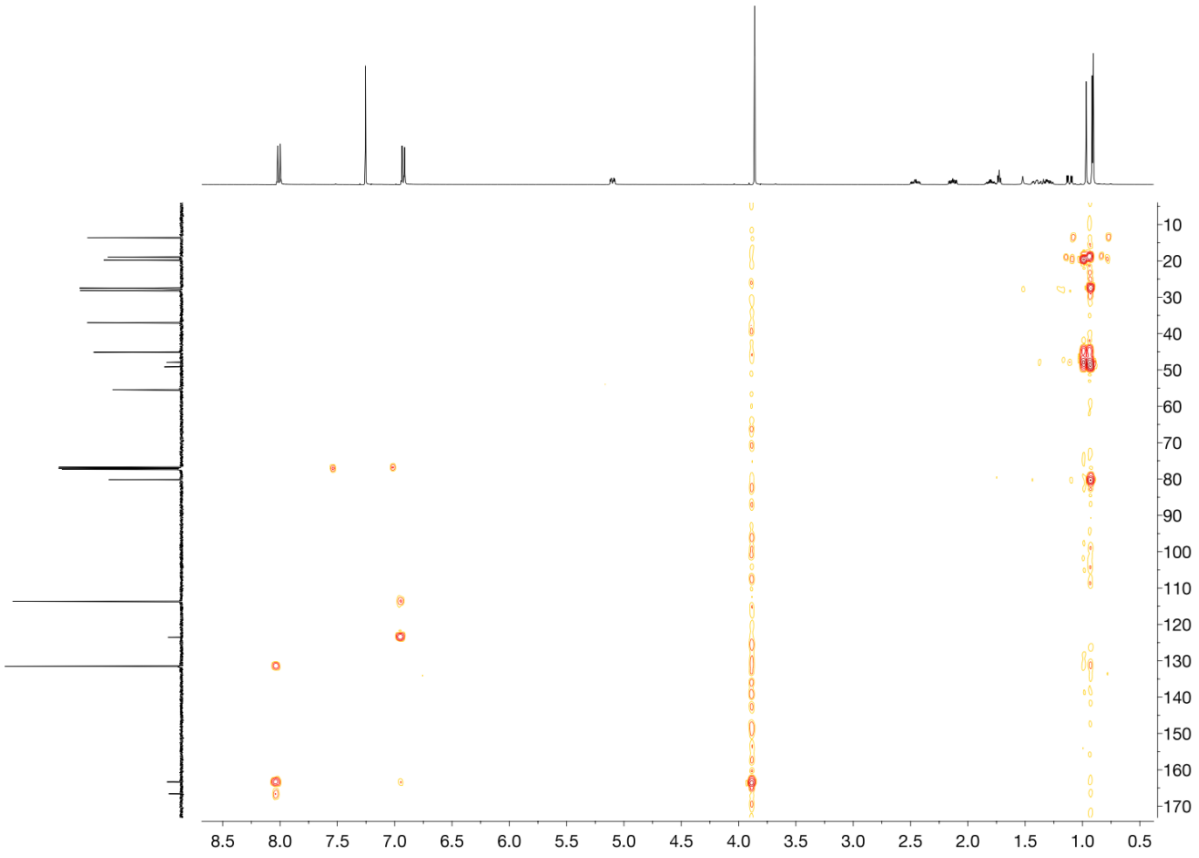


Fig. S6. HMBC spectrum

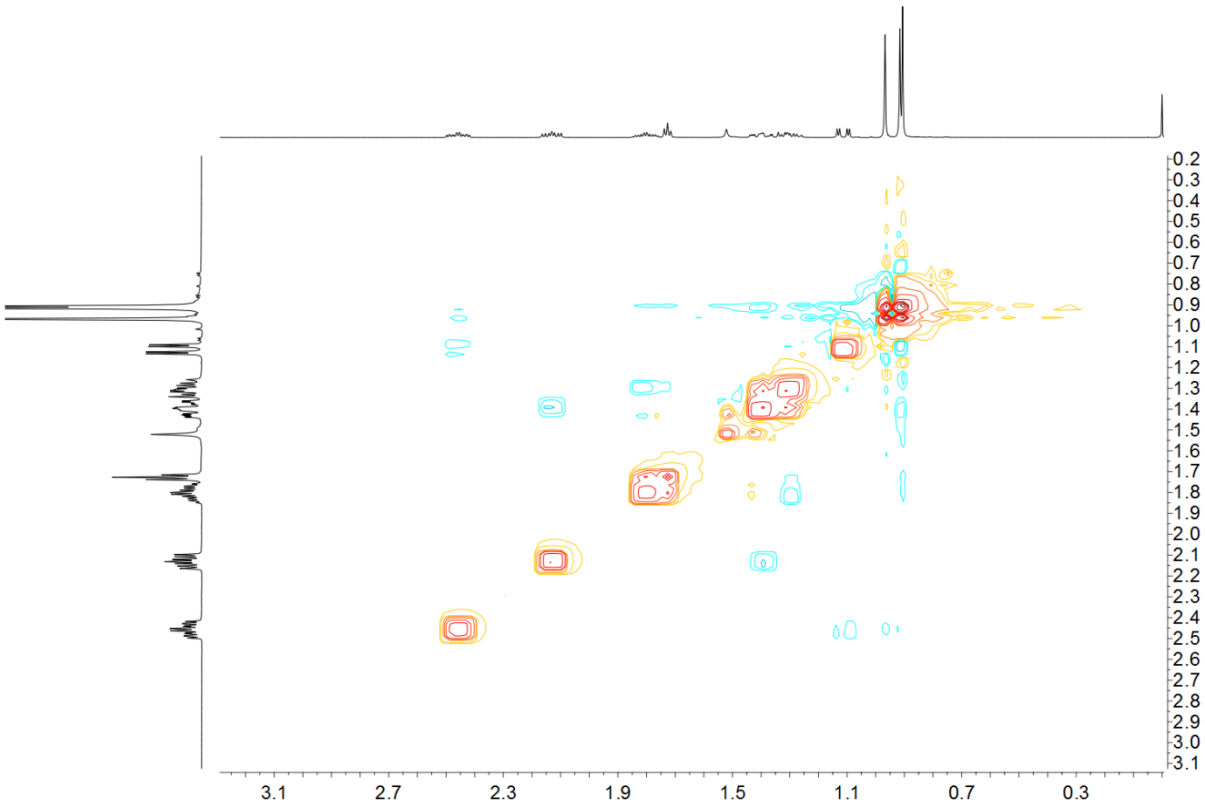


Fig. S7. NOESY spectrum. The correlation between the signals at 5.09 (H-2) and 0.97 ppm (H-9) allows us to differentiate between the signals at 0.97 and 0.92 ppm (the two methyl groups attached to the same carbon atom). Also, note the correlations between the signals at 1.41 and 1.83 ppm (*exo* hydrogens attached at C-5 and C-6) and the signal at 0.92 ppm.

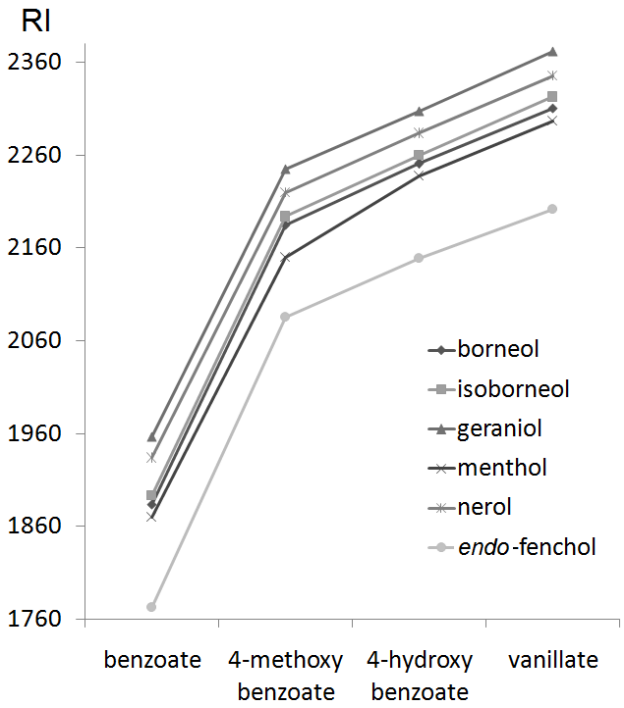


Figure S8. Retention indices of the synthesized aromatic esters

Details on the X-ray experiment and structure refinement

The diffraction data were collected from a weakly diffracting colorless crystal (0.21 x 0.19 x 0.04 $mm^3$) mounted on Agilent Gemini S diffractometer[1] equipped with CuK$\alpha$ radiation ($\lambda$ = 1.54184 Å). Data integration and scaling of the reflections were performed with CrysAlis software[1]. The data set was corrected empirically for absorption using SCALE3 ABSPACK scaling algorithm implemented in CrysAlis software. The crystal structure was solved by SHELXS and refined anisotropically by full matrix least-squares on $F^2$ using SHELXL program[2], 2012). The refinement of the crystal structure revealed high isotropic displacement parameters for the carbon atoms of the bornyl part of the molecule indicating a severe disorder of this fragment. The attempts to model the disorder remained unsuccessful.

All H atoms were placed at geometrically calculated positions with the D–H distances fixed to 0.98, 0.97 and 0.96 Å from phenyl, methylene and methyl C atoms respectively. The corresponding $U_{iso}$ values of the H atoms were set at $1.5U_{eq}$ for the methyl group and at $1.2U_{eq}$ otherwise.

Geometrical calculations were performed with PARST95[3] and molecular graphics with ORTEP[4] and Mercury[5]. A summary of the crystallographic data is given in Table S1.

References:

1 Agilent Technologies, CrysAlis PRO. Yarnton, Oxfordshire, England, 2013.

2 G. M. Sheldrick, *Acta Crystallogr.*, 2008, **A64**, 112-122.

3 M. Nardelli, *J. Appl. Cryst.*, 1995, **28**, 659.

4 L. J. Farrugia, *J. Appl. Cryst.*, 2012, **45**, 849–854.

5 C. F. Macrae, P. R. Edgington, P. McCabe, E. Pidcock, G. P. Shields, R. Taylor, M. Towler and J. van de Streek, *J. Appl. Cryst.*, 2006, **39**, 453.

Table S2. Crystallographic data

| | |
|---|---|
| Empirical formula | $C_{18}H_{24}O_3$ |
| Formula weight | 288.4 |
| Color, crystal shape | colorless, plate |
| Crystal size ($mm^3$) | 0.21 x 0.19 x 0.04 |
| Temperature (K) | 293(2) |
| Wavelength (Å) | 1.5418 |
| Crystal system | Orthorhombic |
| Space group | $P2_12_12_1$ |
| Unit cell dimensions | |
| $a$ (Å) | 11.004(2) |
| $b$ (Å) | 11.212(3) |
| $c$ (Å) | 13.436(2) |
| $V$ ($Å^3$) | 4425.7(2) |
| $Z$ | 4 |
| $D_{calc}$ ($Mg/m^3$) | 1.155 |
| $\mu$ ($mm^{-1}$) | 0.614 |
| $\theta$ range for data collection (°) | 3.28 to 72.20 |
| Reflections collected | 3937 |
| Independent reflections, $R_{int}$ | 1933, 0.0240 |
| Completeness (%) to $\theta = 67°$ | 98.1 |
| Refinement method | Full-matrix least-squares on $F^2$ |
| Refined parameters | 191 |
| Goodness-of-fit on $F^2$ | 1.286 |
| Final $R_1/wR_2$ indices $[I>2\sigma(I)]$ | 0.1060/ 0.1264 |
| Largest diff. peak and hole (e $Å^{-3}$) | 0.438 /−0.317 |

Table S3. Selected bond lengths (Å) and angles (º) within the well ordered part of bornyl 4-methoxybenzoate molecule

| | | | |
|---|---|---|---|
| C1–O1 | 1.415(9) | C1–O1–C2 | 118.5(5) |
| C2–O1 | 1.352(7) | C8–O3–C9 | 118.0(4) |
| C8–O2 | 1.210(6) | C5–C8–O2 | 123.2(5) |
| C8–O3 | 1.315(6) | O2–C8–O3 | 123.9(5) |
| C9–O3 | 1.449(7) | C2–C3–C4 | 119.6(5) |
| C2–C3 | 1.400(7) | C3–C4–C5 | 120.5(4) |
| C3–C4 | 1.374(7) | C4–C5–C6 | 119.1(5) |
| C4–C5 | 1.400(7 | C5–C6–C7 | 120.2(5) |
| C5–C6 | 1.375(7) | C6–C7–C2 | 121.1(5) |
| C6–C7 | 1.381(9) | C4–C5–C8 | 122.4(4) |
| C2–C7 | 1.363(9) | C6–C5–C8 | 118.5(4) |

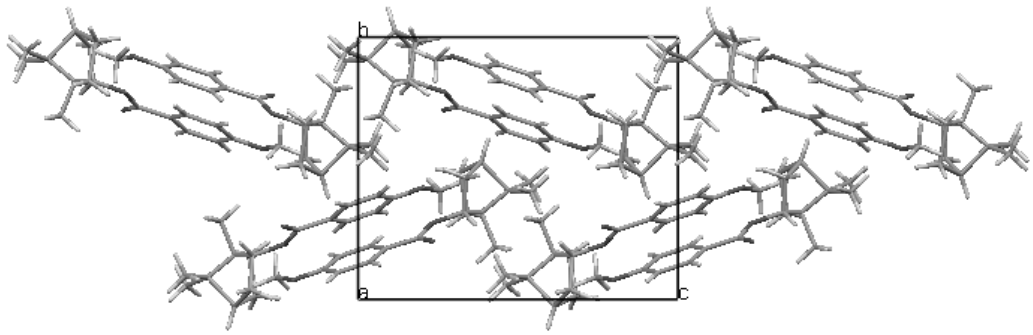


Figure S9. Crystal packing of bornyl 4-methoxybenzoate, viewed along the *a* axis.

Table S4. Antinociceptive effect (percent of inhibition) of *i.p.* administration of the essential oil of *Ferula ovina*, bornyl 4-methoxybenzoate (BMB) and the positive controls (ASA* for abdominal writhings test and morphine** for the hot plate and tail immersion tests).

| Group | Dose (mg/kg) | % of inhibition in | | |
|---|---|---|---|---|
| | | abdominal writhings test* | hot plate test** | tail immersion test** |
| FO | 50 | 71.7 | 13.2 | 38.5 |
| | 100 | 77.1 | 21.1 | 42.8 |
| | 200 | 92.4 | 28.4 | 55.8 |
| BMB | 50 | 52.2 | -7.1# | 32.6 |
| | 100 | 64.1 | -7.4# | 42.8 |
| | 200 | 82.6 | -1.7# | 49.9 |
| Positive control | 200*/5** | 90.2 | 72.7 | 80.6 |

# - reaction times of mice were shorter than the base line

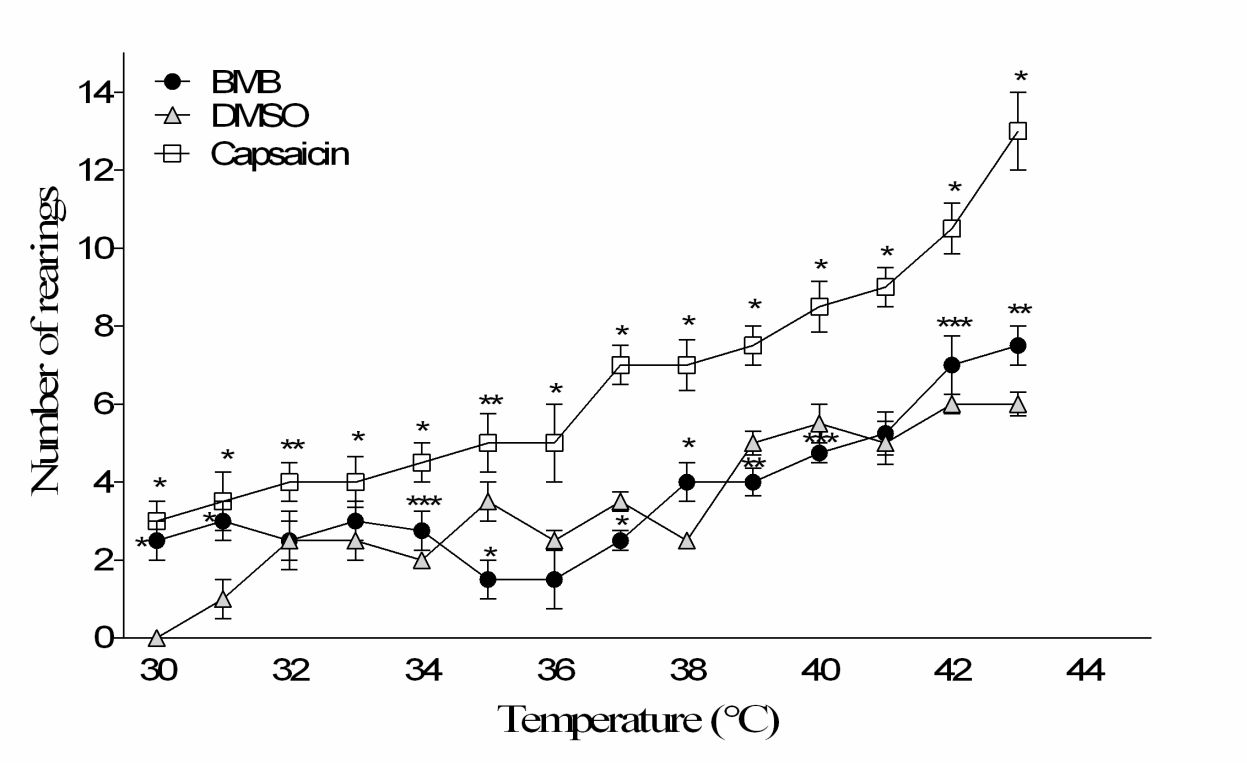


Figure S10. Effect of the topically applied DMSO, capsaicin and bornyl 4-methoxybenzoate (BMB) on mice performance in the DHP test. It represents the number of rearings noted. The results are presented as mean ± S.D. ($n$ = 6), statistical significance was calculated by one way ANOVA followed by Tukey test. *p<0.001, **p<0.01, *** p<0.05 vs. DMSO.