Selective Inhibition of Human Type-5 17β-Hydroxysteroid Dehydrogenase (AKR1C3) by Baccharin, a Component of Brazilian Propolis

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Table S1. Primers used for amplification of 17β-HSD isoenzyme genes in A549 cells.

17β-HSD	Sequences of forward (f) and reverse (r) primers (5'-……-3')	anneal-ing (˚C)	PCR product (bp)
type-1	f: TTTTCATATGATGGCCCGCACCGTGGT r: TTTTGTCGACTTACTGCGGGGCGGCCG	55	987
type-3	f: CCCCGGATCCATGGGGGACGTCCTGGAA r: TTTTGTCGACCTACCTGACCTTGGTGTTG	55	933
AKR1C3	f: AGCCAGGTGAGGAACTTTC r: ACTGGGTCCTCCAAGAGC	57	352
type-6	f: TTTTCATATGATGTGGCTCTACCTGGCG r: TTTTGTCGACTTAGACTGCCTGGGCTGG	55	954
type-7	f: CCCGGATCCATGCGAAAGGTGGTTTTGATCA r: TTTTGTCGACTTATAGGCATGAGCCACTGAG	55	1026

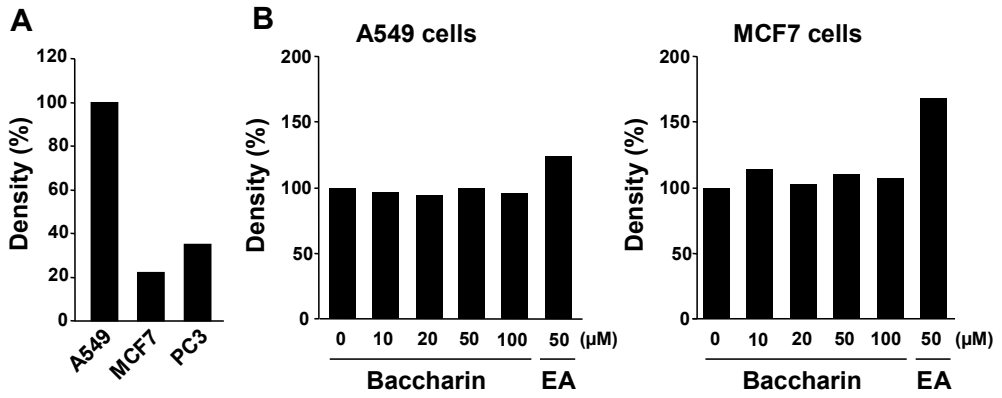

Figure S1. Effect of baccharin (**1**) on AKR1C3 expression in carcinoma cells.

(A) Expression level of AKR1C3 in A549, MCF7 and PC3 cells. The cell extracts (40 μg) were applied to Western blotting using the antibody against AKR1C3, and the band density was normalized to that in A549 cells. Data are represented as means of duplicate experiments. (B) Effect of **1** on AKR1C3 expression in A549 and MCF7 cells. The cells were treated for 24 h with the indicated concentrations of **1** or ethacrynic acid (EA), a potent inducer of human AKR1C isoforms including AKR1C3, and the cell extracts (40 μg) were applied to Western blotting using the antibody against AKR1C3. The band density was normalized to that treated with a vehicle dimethysulfoxide alone (0 μM), and data are represented as means of duplicated experiments.

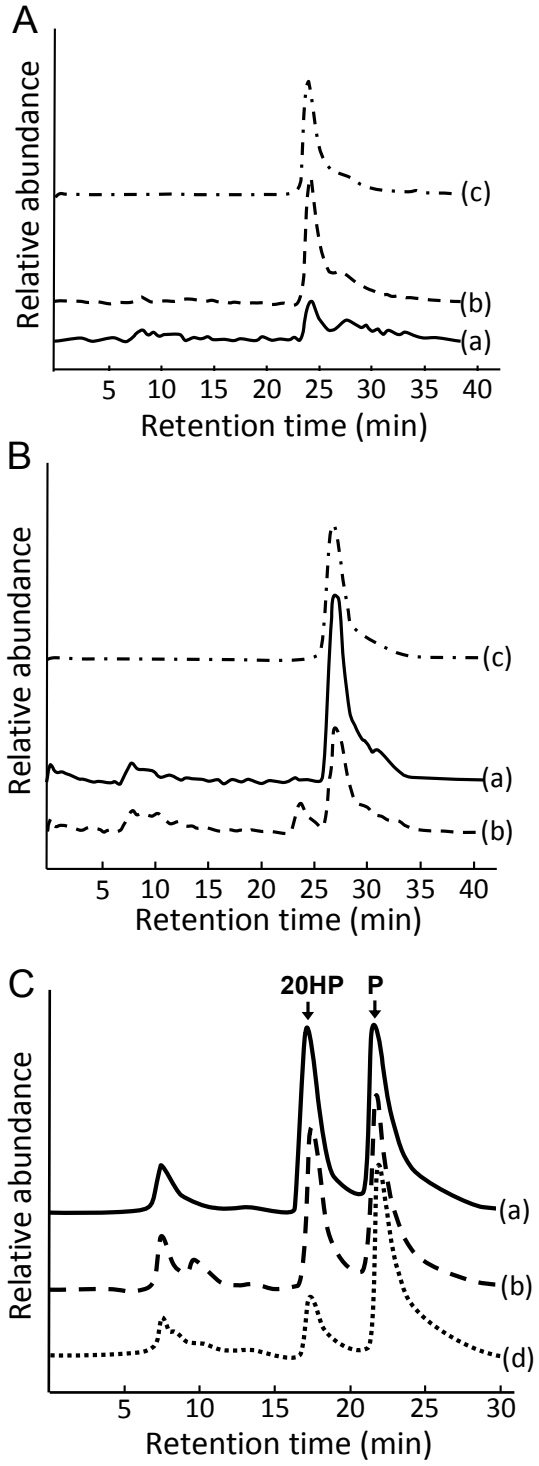

Figure S2. LC/MS chromatograms of androsterone (A), its metabolite 5α-androstane-3α,17β-diol (B), and progesterone and 20α-hydroxyprogesterone (C).

In panels A and B, A549 cells were incubated with 50 μM androsterone for 24 h in the absence (a) or presence (b) of 20 μM baccharin (**1**). The extract of the medium was subjected to the LC/MS analysis. Androsterone and 5α-androstane-3α,17β-diol in the samples were detected in the positive ion mode by monitoring their fragment ions (m/z = 256.5 and 258.5, respectively), as the major peaks in panels A and B were identical to those of their authentic steroids (c). The amounts of androsterone and 5α-androstane-3α,17β-diol were calculated from standard curves constructed using the authentic steroids. The chromatograms without **1** also showed that approximately 80% of androsterone was reduced into 5α-androstane-3α,17β-diol during a 24 h-incubation period with the cells.

In panel C, the cells were incubated with 20 μM progesterone for 6 h in the absence (a) or presence (b) of 100 μM **1** (b) and 5 μM 3-bromo-5-phenylsalicylic acid (d). Progesterone (P) and its metabolite 20α-hydroxyprogesterone (20HP) were detected by monitoring their total ions.[32]

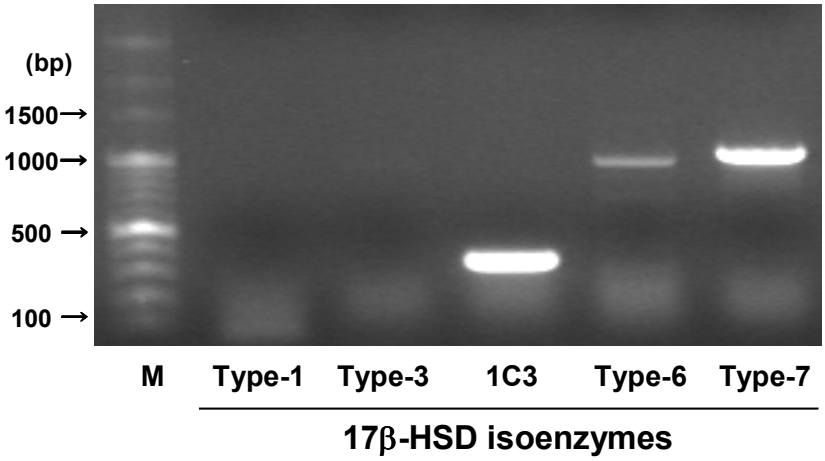

Figure S3. Reverse transcription-PCR analysis of expression of mRNAs for NADPH-dependent 17β-HSD isoenzymes in A549 cells.

The preparation of total RNA and reverse transcription-PCR were performed as described previously.[28] The primers, annealing temperatures, and amplified fragment sizes of the transcription-PCR are summarized in Table S1. The PCR products were subjected to electrophoresis on a 1% agarose gel using DNA size markers (M), and revealed after staining with ethidium bromide. Among human 17β-HSD isoenzymes, the above five isoenzymes act as 17-ketosteroid reductases.[4] The expression of mRNAs for type-6 and type-7 17β-HSDs belonging to the SDR superfamily were detected, as well as that for AKR1C3 (1C3).