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New Bitter-Masking Compounds: Hydroxylated Benzoic Acid Amides of Aromatic Amines as Structural Analogues of Homoeriodictyol

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Starting from the known bitter-masking flavanones eriodictyol and homoeriodictyol from herba santa some structurally related hydroxybenzoic acid amides of benzylamines were synthesized and evaluated as masking agents toward bitterness of caffeine by sensory methods. The closest structural relatives of homoeriodictyol, the hydroxybenzoic acid vanillylamides **5**−**9**, were the most active and were able to reduce the bitterness of a 500 mg L^{-1} caffeine solution by about 30% at a concentration of 100 mg L^{-1}. 2,4-Dihydroxybenzoic acid vanillylamide **7** showed a clear dose-dependent activity as inhibitor of the bitter taste of caffein between 5 and 500 mg L^{-1}. Additionally, it was possible to reduce the bitterness of quinine and salicine but not of the bitter peptide *N*-L-leucyl-L-tryptophan. Combinations of homoeriodictyol and amide **7** showed no synergistic or antagonistic changes in activity. The results for model compound **7** suggested that the hitherto unknown masking mechanism is probably the same for flavanones and the new amides. In the future, the new amides may be alternatives for the expensive flavanones to create flavor solutions to mask bitterness of pharmaceuticals or foodstuffs.

KEYWORDS: Bitter masking; taste modifiers; hydroxylated benzoic acid amides; homoeriodictyol; structure−activity relationship

INTRODUCTION

Pronounced bitter taste is mostly not tolerated in food and wanted only in exceptional cases, e.g., in coffee, dark chocolate, or tea. Frequently, the compounds that are responsible for the bitter, astringent, or harsh taste are either eliminated from raw materials and foodstuffs or masked by different techniques. In contrast, a lot of bitter-tasting phytonutrients, such as polyphenols, flavonoids, isoflavones, terpenes, and glucosinolates, are supposed to be healthy (*1*). Significant progress regarding the understanding of bitter taste reception on the molecular level was made during the past decade (*2*) and nowadays the agonist activity of several bitter molecules on bitter receptors is known (*3*−*6*).

Recently, some progress was made in the identification of molecules that are able to cover or mask some of the bad tastes caused by several bitter substances, e.g., Maillard reaction products related to alapyraidine (*7*), γ-aminobutyric acid (*8*), and nucleotides such as AMP (*9*). In our previous study we have shown that some flavanones, especially homoeriodicyol (**1**) and eriodictyol (**2**), are the main bitter-masking actives from herba santa (*Eriodictyon californicum*) (*10*); in addition, some of the first structure−activity relationships for several flavanones were discussed in this study. To get more information about the mechanism of inhibition, we have started a synthesis program to yield structural analogues of flavanones. Furthermore, the availability from nature of the most active flavanones eriodictyol and especially homoeriodictyol is limited. Some syntheses of these flavanones are known (*11*, *12*) but they mostly need multistep procedures using expensive or toxic raw materials. Therefore, as a first step we decided to investigate hydroxylated benzoic acid amides of benzylamines as structural analogues, which are much simpler to synthesize but contain the most important structural elements of the original flavanones (cf. **Figure 1**).

Some hydroxylated benzoic acid amides of benzylamines were known [e.g., 3,4-dihydroxybenzoic acid *N*-(3,4-dihydroxybenzyl)amide as an amyloid protection agent (*13*)], but amides showing a substitution pattern closest to the most active flavanones were not described yet. In the investigated structural class only a few naturally occurring amides of phenethylamines are known from the literature: aduncamide [**3**, 2-hydroxy-5-methoxybenzoic acid *N*-[2-(4-hydroxy-3-methoxyphenyl)ethyl]-amide, cf. **Figure 2**] from *Piper aduncum* (*14*) and some salicoyl tyramines such as 2-hydroxybenzoic acid *N*-[2-(4-hydroxyphenyl)ethyl]amide (**4**) from *Naiba riparia* (*15*, *16*). The closest known relative is 2,4,6-trihydroxybenzoic acid *N*-(3-hydroxy-

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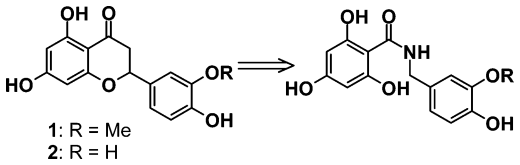

Figure 1. Structural relationship between homoeriodictyol (**1**), eriodictyol (**2**), and hydroxylated benzamides of benzylamines.

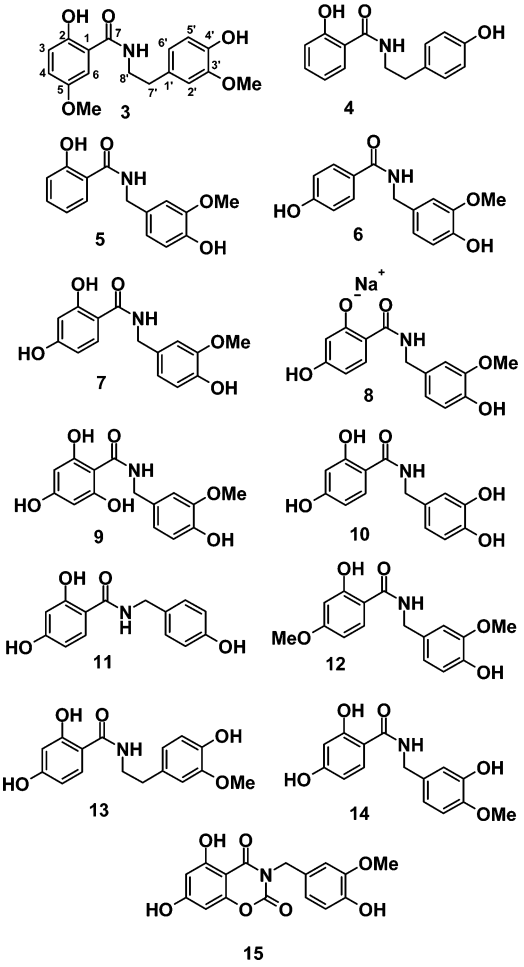

Figure 2. Structures of investigated hydroxylated benzamides.

4-methoxybenzyl)amide, which was synthesized in a study covering structural analogues of the intensely sweet neohesperedin dihydrochalcone, but the compound that is substituted analogously to hesperetin showed no sweetness at all (*17*).

MATERIALS AND METHODS

Dipeptide *N*-L-leucyl-L-tryptophan (H-Leu-Trp-OH) was obtained from Bachem (Weil am Rhein, Germany). 4-Hydroxybenzylamine hydrochloride and 3-hydroxy-4-methoxybenzylamine hydrochloride were synthesized analogously to the procedure described earlier starting from the corresponding aldehydes (*18*). All other chemicals were from Sigma-Aldrich (Deisenhofen, Germany), Acros (Schwerte, Germany), or Lancaster Synthesis (Frankfurt, Germany). NMR spectra were recorded using a Varian VXR400S (^{1}H, 400 MHz) spectrometer (Varian, Darmstadt, Germany) at 25 °C using tetramethylsilane as internal standard. LC−MS spectra were recorded using the LCQ HPLC system Finnigan MAT HP1100 (Finnigan MAT, Egelsbach, Germany; APCI, atmospheric pressure chemical ionization). Elemental analysis (combustion analysis for C, H, O and AAS for sodium) was performed by Bayer Technology Services, Germany. Sodium content was determined by capillary electrophoresis using inverse detection with imidazol on a Agilent 3DCE G1600AX equipment (Agilent, Waldbronn, Germany). Flash chromatography (FC) was performed on a Biotage Flash 40 system (Biotage AB, Uppsala, Sweden) using disposable prep-packed columns.

Syntheses of Hydroxylated Benzoic Acid Amides, General Procedure. The hydroxylated benzoic acid (10−20 mmol), *N*-hydroxysuccinimide (1 mol equiv), and *N*,*N*′-dicyclohexylcarbodiimide (1 mol equiv) were dissolved in 1,4-dioxane (2.0 mL/mmol) while stirring under nitrogen atmosphere. The turbid mixture was stirred at 20−25 °C for 16 h. The precipitate was filtered off and the filtrate directly added to a solution of the amine or its hydrochloride (1.1 mol equiv) and $NaHCO_3$ (1 mol equiv) in water (1 mL/mmol). The reaction mixture was heated to 50 °C under nitrogen and stirred for 1 h. After cooling to room temperature, dilute HCl (10%) was added and the mixture was extracted using ethyl acetate. The combined organic phases were washed with brine, dried with anhydrous Na_2SO_4, and filtered, and the filtrate was evaporated in vacuo. In some cases the aryl esters were found as minor byproducts. In such cases it was of advantage to saponify the crude product using 25% NaOH at 40 °C and subsequent treatment with 10% HCl and workup as described earlier. Final purification was done by FC on silica gel 60, using ethyl acetate/*n*-hexane mixtures and/or recrystallization when appropriate.

2-Hydroxy-5-methoxybenzoic Acid *N*-2-(4-Hydroxy-3-methoxyphenyl)ethylamide, Aduncamide (3). Starting from 10 mmol of 2-hydroxy-5-methoxybenzoic acid and 2-(4-hydroxy-3-methoxyphenyl)-ethylamine hydrochloride (11 mmol), the crude product (2.7 g) was purified by FC (eluant *n*-hexane/ethyl acetate 3:1 (v/v)) to yield 0.58 g (18%) of colorless crystals. HPLC−MS (RP-18, H_2O/acetonitrile 80:20 to 0:100 in 30 min, 15 min isocratic, APCI+): t_R 16.0 min, m/z = 318.14 (100%, $[M + H]^+$), >95%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.76 (1H, s, OH), 7.00 (1H, dd, J = 9.0 Hz, J = 2.9 Hz, H-4), 6.92 (1H, dd, J = 9.0 Hz, J = 0.4 Hz, H-3), 6.87 (1H, d, J = 8.5 Hz, H-5′), 6.74−6.70 (2H, m), 6.69−6.67 (1H, m), 6.33 (1H, bs, NH), 5.60 (1H, s, OH), 3.85 (3H, s, OCH_3), 3.73 (3 H, s, OCH_3), 3.66 (2H, dt, J = 7.0 Hz, J = 6.8 Hz, H-8′), 2.86 (2H, t, J = 6.87 Hz, H-7′) ppm. ^{13}C NMR (100 MHz; $CDCl_3$): δ 169.63 (C, C-7), 155.51 (C), 151.76 (C), 146.79 (C), 144.50 (C), 130.27 (C, C-1′), 121,39 (CH, C-6′), 120.89 (CH, C-4), 119.30 (CH, C-3), 114.58 (CH, C-5′), 114.19 (C, C-1), 111.22 (CH, C-2′), 109.62 (CH, C-6), 55.99 (CH_3, OCH_3), 55.91 (CH_3, OCH_3), 40.92 (CH_2, C-8′), 35.14 (CH_2, C-7′) ppm.

2-Hydroxybenzoic Acid *N*-2-(4-Hydroxyphenyl)ethylamide (4). Starting from 10 mmol of 2-hydroxybenzoic acid and 2-(4-hydroxyphenyl)ethylamine (11 mmol), the crude product was purified by FC (eluant *n*-hexane/ethyl acetate 1:1 (v/v)) to yield 0.91 g (35%) of colorless crystals. HPLC−MS (RP-18, H_2O/acetonitrile 80:20 to 0:100 in 30 min, 15 min isocratic, APCI+): t_R 14.5 min, m/z = 258.18 (100%, $[M + H]^+$), >95%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.18 (1H, s, OH), 8.85 (1H, bt, J = 5.7 Hz, NH), 7.82 (1H, dd, J = 7.9 Hz, J = 1.7 Hz, H-6), 7.39 (1H, ddd, J = 8.2 Hz, J = 7.3 Hz, J = 1.7 Hz, H-4), 7.04 (2H, m, H-2′ and H-6′), 6.89 (1H, dm, J = 7.6. Hz, H-3 or H-5), 6.88 (1H, dd, J = 7.2 Hz, J = 1.2 Hz, H-5 or H3) 6.68 (2H, m, H-3′ and H-5′), 3.46 (3H, ddm, J = 7.7 Hz, J = 5.9 Hz, H-8′), 2.74 (2H, t, J = 7.5 Hz, H-7′) ppm. ^{13}C NMR (100 MHz; DMSO-d_6): δ 168.69 (C, C-7), 159.93 (C-2), 155.61 (C-4′), 133.49 (CH, C-4), 129.42 (2 × CH, C-2′ and C-6′), 129.14 (C, C-1′), 127.56 (CH, C-6), 118.44 (CH, C-3 or C-5), 117.26 (CH, C-5 or C-3), 115.16 (C, C-1), 115.07 (2 × CH, C-3′ and C-5′), 40.80 (CH_2, C-8′), 33.95 (CH_2, C-7′) ppm.

2-Hydroxybenzoic Acid *N*-(4-Hydroxy-3-methoxybenzyl)amide (5). Starting from 10 mmol of 2-hydroxybenzoic acid and 4-hydroxy-3-methoxybenzylamine hydrochloride (11 mmol), the crude product was purified by FC (eluant *n*-hexane/ethyl acetate 1:1 to 1:2 (v/v)) to yield 1.87 g (68%) of colorless crystals. HPLC−MS (RP-18, H_2O/acetonitrile 100:0 to 0:100 in 60 min, APCI+): t_R 15.1 min, m/z = 273.90 (100%, $[M + H]^+$), >95%. HRMS: calcd for $C_{15}H_{15}NO_4$ 273.1001, found 273.0979. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.26 (1H, t, $J \approx 6$ Hz, NH), 8.89 (1H, bs, OH), 7.88 (1H, ddd, J = 7.9 Hz, J = 1.7 Hz, J = 0.4 Hz, H-6), 7.39 (1H, ddd, J = 8.3 Hz, J = 7.2 Hz, J = 1.7 Hz, H-4), 6.92 (1H, bs, H-2′), 6.89 (1H, dd, J = 8.3 Hz, J = 1.2 Hz, H-3), 6.88 (1H, ddd, J = 8.0 Hz, J = 7.3 Hz, J = 1.2 Hz, H-5), 6.73 (2H, m, H-5′, H-6′), 4.40 (2H, bd, J = 5.7 Hz, H-7′), 3.84 (3H, s, OCH_3) ppm. ^{13}C NMR (100 MHz; DMSO-d_6): δ 168.63 (C, C-7), 160.00 (C), 147.37 (C), 145.52 (C), 133.59 (C, C-4), 129.58 (C),

127.68 (CH, C-6), 119.83 (CH), 118.48 (CH), 117.30 (CH), 115.19 (CH, C-5′), 115.19 (C), 111.87 (CH, C-2′), 55.49 (CH_3, OCH_3), 42.13 (CH_2, C-7′) ppm.

4-Hydroxybenzoic Acid *N*-(4-Hydroxy-3-methoxybenzyl)amide (6). Starting from 10 mmol of 4-hydroxybenzoic acid and 4-hydroxy-3-methoxybenzylamine hydrochloride (11 mmol), the crude product was purified by FC (eluant *n*-hexane/ethyl acetate 1:1 (v/v)) to yield 1.0 g (37%) of colorless crystals. HPLC−MS (RP-18, H_2O/acetonitrile 100:0 to 0:100 in 60 min, APCI+): t_R 15.1 min, m/z = 273.92 (66%, $[M + H]^+$), 546.54 ($[2M + H]^+$) >95%. HRMS: calcd for $C_{15}H_{15}NO_4$ 273.1001, found 273.273.0996. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.63 (1H, bd, J = 5.8 Hz, NH), 7.74 (2H, dm, J = 8.5 Hz, H-2, H-6), 6.88 (1H, m, H-2′), 6.78 (2H, dm, J = 8.8 Hz, H-3, H-5), 6.69 (2H, m, H-5′, H-6′), 4.32 (2H, bd, J = 5.9 Hz, H-7′), 3.73 (3H, s, OCH_3) ppm. ^{13}C NMR (100 MHz; DMSO-d_6): δ 165.67 (C, C-7), 159.99 (C), 147.27 (C), 145.25 (C), 130.72 (C), 129.04 (2×CH, C-2, C-6), 125.11 (C), 119.67 (CH, C-6′), 115.07 (CH, C-5′), 114.67 (2×CH, C-3, C-5), 111.75 (CH, C-2′), 55.49 (CH_3, OCH_3), 42.13 (CH_2, C-7′) ppm.

2,4-Dihydroxybenzoic Acid *N*-(4-Hydroxy-3-methoxybenzyl)amide (7). Starting from 20 mmol of 2,4-dihydroxybenzoic acid and 4-hydroxy-3-methoxybenzylamine hydrochloride (22 mmol), the crude product was saponified as described earlier; after addition of dilute HCl the product precipitated and was filtered to yield 1.49 g (64%) of colorless crystals. HPLC−MS (RP-18, H_2O/acetonitrile 95:5 to 0:100 in 30 min and then 15 min isocratic, APCI+): t_R 16.6 min, m/z = 289.87 (100%, $[M + H]^+$), >95%. HRMS: calcd for $C_{15}H_{15}NO_5$ 289.0950, found 289.0927. ^{1}H NMR (400 MHz, CD_3OD, internal standard TMS): δ 7.62 (1H, d, J = 8.7 Hz, H-6), 6.93 (1H, d, J = 1.8 Hz, H-2′), 6.78 (1H, dd, J = 8.1 Hz, J = 1.9 Hz, H-6′), 6.75 (1H, d, J = 8.1 Hz, H-5′), 6.32 (1H, dd, J = 8.7 Hz, J = 2.4 Hz, H-5), 6.29 (1H, d, J = 2.4 Hz, H-3), 4.45 (2H, bs, H-7′), 3.83 (3H, s, OCH_3) ppm. ^{13}C NMR (100 MHz; CD_3OD): δ 171.06 (C, C-7), 163.82 (C, C-2), 163.52 (C, C-4), 149.05 (C, C-3′), 146.82 (C, C-4′), 131.70 (C, C-1′), 130.27 (CH, C-6), 121.38 (CH, C-6′), 116.16 (CH, C-5′), 112.47 (CH, C-2′), 108.82 (C, C-1), 108.48 (CH, C-5), 103.94 (CH, C-3), 56.38 (CH_3, OCH_3), 43.80 (CH_2, C-7′) ppm.

2,4-Dihydroxybenzoic Acid *N*-(4-Hydroxy-3-methoxybenzyl)amide, Sodium Salt (8). 2,4-Dihydroxybenzoic acid *N*-(4-hydroxy-3-methoxybenzyl)amide (**7**) (260 mg, 0.9 mmol) was dissolved in NaOH (1 mol L^{-1}, 0.9 mL), water (1 mL), and ethanol (2 mL) and stirred at 50 °C for 1 h. The reaction mixture was evaporated to dryness in vacuo at 40 °C, the residue triturated with ethyl acetate (10 mL), and the product filtered and dried to yield 0.246 g of pale yellow crystals. Sodium content (CE, inverse detection at 350/380 nm, polarity positive, U = 28 kV, fused silica 50 μm × 5.6 cm): calcd for $C_{15}H_{14}NNaO_5$ 7.4%, found 7.65%. ^{1}H NMR (400 MHz, CD_3OD): δ 7.62 (1H, d, J = 8.7 Hz, H-6), 6.95 (1H, d, J = 1.8 Hz, H-2′), 6.79 (1H, dd, J = 8.1 Hz, J = 1.9 Hz, H-6′), 6.73 (1H, d, J = 8.1 Hz, H-5′), 6.10 (1H, dd, J = 8.7 Hz, J = 2.4 Hz, H-5), 6.03 (1H, d, J = 2.4 Hz, H-3), 4.48 (2H, bs, H-7′), 3.83 (3H, s, OCH_3) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ 171.88 (C, C-7), 170.39 (C, C-2), 166.03 (C, C-4), 149.37 (C, C-3′), 147.45 (C, C-4′), 131.71 (C, C-1′), 131.35 (CH, C-6), 121.30 (CH, C-6′), 116.34 (CH, C-5′), 112.33 (CH, C-2′), 110.62 (C, C-1), 107.51 (CH, C-5), 106.16 (CH, C-3), 56.37 (CH_3, OCH_3), 43.82 (CH_2, C-7′) ppm.

2,4,6-Trihydroxybenzoic Acid *N*-(4-Hydroxy-3-methoxybenzyl)amide (9). Starting from 20 mmol of 2,4,6-trihydroxybenzoic acid and 4-hydroxy-3-methoxybenzylamine hydrochloride (22 mmol), the crude product was purified as described for compound **7** to yield 2.74 g (45%) of colorless crystals. HPLC−MS (RP-18, H_2O/MeOH 50:50 to 5:95 in 15 min and then 15 min isocratic, APCI+): t_R 17.07 min, m/z = 305.91 (100%, $[M + H]^+$), >95%. HRMS: calcd for $C_{15}H_{15}NO_6$ 305.0899, found 305.0903. ^{1}H NMR (400 MHz, CD_3OD): δ 6.93 (1H, d, J = 1.6 Hz, H-2′), 6.79 (1H, dd, J = 8.0 Hz, J = 1.7 Hz, H6′), 6.76 (1H, dd, J = 8.0 Hz, J = 0.5 Hz, H-5′), 5.85 (2H, s, H-3, H-5), 4.45 (2H, bs, H-7′), 3.84 (3H, s, OCH_3) ppm. ^{13}C NMR (100 MHz; CD_3OD): δ 171.85 (C, C-7), 163.48 (C, C-3, C-6), 163.20 (C, C-4), 149.14 (C, C-3′), 146.92 (C, C-4′), 131.31 (C, C-1′), 121.31 (CH, C-6′), 116.28 (CH, C-5′), 112.35 (CH, C-2′), 96.99 (C, C-1), 96.02 (CH, C-3, C-5), 56.38 (CH_3, OCH_3), 43.67 (CH_2, C-7′) ppm.

2,4-Dihydroxybenzoic Acid *N*-(3,4-Dihydroxybenzyl)amide (10). Starting from 20 mmol of 2,4-dihydroxybenzoic acid and 3,4-dihydroxybenzylamine hydrobromide (22 mmol), the crude product was purified by FC (eluant *n*-hexane/ethyl acetate 3:1 (v/v)) to yield 1.3 g (29%) colorless crystals. HPLC−MS (RP-18, H_2O/acetonitrile 80:20 to 0:100 in 30 min and then 15 min isocratic, APCI+): t_R 10.34 min, m/z = 276.0 (100%, $[M + H]^+$). HRMS: calcd for $C_{14}H_{13}NO_5$ 275.0794, found 275.0785. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.05 (1H, s, OH), 8.93 (1H, t, J = 6.0 Hz, NH), 8.85 (1H, s, OH), 8.72 (1H, s, OH), 7.72 (1H, d, J = 8.8 Hz, H-6), 6.71 (1H, d, J = 2.1 Hz, H-2′), 6.66 (1H, d, J = 8.1 Hz, H-5′), 6.56 (1H, dd, J = 8.1 Hz, J = 2.1 Hz, H-6′), 6.28 (1H, dd, J = 8.7 Hz, J = 2.4 Hz, H-5), 6.23 (1H, d, J = 2.4 Hz, H-3), 4.29 (2H, d, J = 5.9 Hz, H-7′) ppm. ^{13}C NMR (100 MHz; DMSO-d_6): δ 169.04 (C, C-7), 162.52 (C, C-4), 162.13 (C-2), 145.00 (C, C-3′), 144.12 (C, C-4′), 129.90 (C, C-1′), 128.83 (CH, C-6), 118.15 (CH, C-6′), 115.23 (CH, C-5′), 114.73 (CH, C-2′), 106.89 (CH, C-5), 102.61 (CH, C-3), 41.62 (CH_2, C-7′) ppm.

2,4-Dihydroxybenzoic Acid *N*-(4-Hydroxybenzyl)amide (11). Starting from 20 mmol of 2,4-dihydroxybenzoic acid and 4-hydroxybenzylamine hydrochloride (22 mmol), the crude product was purified as described for compound **7** to yield 1.39 g (54%) of colorless crystals. HPLC−MS (RP-18, H_2O/acetonitrile 90:10 to 0:100 in 30 min and then 15 min isocratic, APCI+): t_R 15.05 min, m/z = 259.89 (100%, $[M + H]^+$). HRMS: calcd for $C_{14}H_{13}NO_4$ 259.0845, found 259.0827. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.05 (1H, s, OH), 9.29 (1H, s, OH), 8.96 (1H, bt, J = 6.0 Hz, NH), 7.71 (1H, d, J = 8.8 Hz, H-6), 7.12 (2H, m, H-2′ and H-6′), 6.71 (2H, m, H-3′ and H-5′), 6.28 (1H, ddd, J = 8.7 Hz, J = 2.4 Hz, J = 0.8 Hz, H-5), 6.23 (1H, dd, J = 2.4 Hz, J = 0.84 Hz, H-3), 4.35 (1H, d, J = 5.8 Hz, H-7′) ppm. ^{13}C NMR (100 MHz; DMSO-d_6): δ 169.07 (C, C-7), 162.50 (C, C-4 or C-2), 162.15 (C, C-2 or C-4), 156.22 (C, C-4′), 129.28 (C, C-1′), 128.86 (CH, C-6), 128.57 (2×CH, C-2′ and C-6′), 114.95 (2×CH, C-3′ and C-5′), 106.91 (CH, C-5), 106.49 (C, C-1), 102.62 (CH, C-3), 41.58 (CH_2, C-7′) ppm.

2-Hydroxy-4-methoxybenzoic Acid *N*-(4-Hydroxy-3-methoxybenzyl)amide (12). Starting from 13.3 mmol of 2-hydroxy-4-methoxybenzoic acid and 4-hydroxy-3-methoxybenzylamine hydrochloride (14.6 mmol), the crude product was purified by recrystallization from ethyl acetate to yield 1.45 g (33%) of colorless crystals. HPLC−MS (RP-18, H_2O/acetonitrile 80:20 to 0:100 in 30 min and then 15 min isocratic, APCI+): t_R 15.91 min, m/z = 304.11 (100%, $[M + H]^+$). HRMS: calcd for $C_{16}H_{17}NO_5$ 303.1107, found 303.1078. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.07 (1H, t, J = 5.93 Hz, NH), 8.87 (1H, s, OH), 7.83 (1H, d, J = 8.9 Hz, H-6), 6.91 (1H, s, H-2′), 6.725 (1H, s, H-5′ or H-6′), 6.720 (1H, s, H-6′ or H-5′), 6.47 (1H, dd, J = 8.9 Hz, J = 2.5 Hz, H-5), 6.42 (1H, d, J = 2.5 Hz, H-3), 4.38 (1H, d, J = 5.9 Hz, H-7′), 3.79 (3H, s, OCH_3), 3.75 (3H, s, OCH_3) ppm. ^{13}C NMR (100 MHz; DMSO-d_6): δ 168.93 (C, C-7), 163.47 (C, C-4), 162.55 (C, C-2), 147.34 (C, C-3′), 145.48 (C, C-4′), 129.71 (C, C-1′), 128.71 (CH, C-6), 119.80 (CH, C-6′), 115.17 (CH, C-5′), 111.85 (CH, C-2′), 107.65 (C, C-1), 105.95 (CH, C-5), 101.06 (CH, C-3), 55.48 (CH_3, OCH_3), 55.25 (CH_3, OCH_3), 41.96 (CH_2, C-7′) ppm.

2.4-Dihydroxybenzoic Acid *N*-2-(4-Hydroxy-3-methoxyphenyl)ethylamide (13). Starting from 20 mmol of 2,4-dihydroxybenzoic acid and 2-(4-hydroxy-3-methoxyphenyl)ethylamine hydrochloride (22 mmol), the crude product was purified by FC (eluant *n*-hexane/ethyl acetate 3:1 (v/v)) to yield 2.1 g (41%) of colorless crystals. HPLC−MS (RP-18, H_2O/acetonitrile 90:10 to 0:100 in 30 min and then 15 min isocratic, APCI+): t_R 16.34 min, m/z = 303.95 (100%, $[M + H]+$), 606.26 (0.4%, $[2M + H]^+$) >95%. HRMS: calcd $C_{16}H_{17}NO_5$ 303.1107, found 303.1111. ^{1}H NMR (400 MHz, CD_3OD): δ 7.54 (1H, dd, J = 8.7 Hz, J = 0.4 Hz, H-6), 6.81 (1H, dt, J = 1.9 Hz, J = 0.3 Hz, H-2′), 6.72 (1H, dd, J = 8.0 Hz, J = 0.3 Hz, H-5′), 6.67 (1H, ddt, J = 8.0 Hz, J = 1.9 Hz, J = 0.5 Hz, H-6′), 6.30 (1H, dd, J = 8.7 Hz, J = 2.4 Hz, H-5), 6.27 (1H, dd, J = 2.4 Hz, J = 0.4 Hz, H-3), 3.78 (3H, d, J = 0.3 Hz, OCH_3), 3.53 (2H, m, H-8), 2.80 (2H, m, H-7) ppm. ^{13}C NMR (100 MHz; CD_3OD): δ 171.15 (C, C-7), 163.79 (C, C-2), 163.41 (C, C-4), 148.95 (C, C-3′), 146.05 (C, C-4′), 130.25 (CH, C-6), 122.33 (CH, C-6′), 116.24 (CH, C-5′), 113.52 (CH, C-2′), 108.91 (C, C-1), 103.92 (CH, C-3), 56.31 (CH_3, OCH_3), 42.37 (CH_2, C-8), 36.30 (CH_2, C-7) ppm.

2,4-Dihydroxybenzoic Acid *N*-(3-Hydroxy-4-methoxy)benzylamide (14). Starting from 20 mmol of 2,4-dihydroxybenzoic acid and 3-hydroxy-4-methoxybenzylamine hydrochloride (22 mmol), the crude product was purified by FC (eluant *n*-hexane/ethyl acetate 3:1 (v/v)) to yield 1.45 g (30%) of colorless crystals. HPLC−MS (RP-18, H_2O/acetonitrile 95:5 to 0:100 in 30 min and then 15 min isocratic, APCI+): t_R 15.54 min, m/z = 289.89 (100%, $[M + H]^+$). HRMS: calcd for $C_{15}H_{15}NO_5$ 289.0950, found 289.0931. ^{1}H NMR (400 MHz, CD_3OD): δ 7.62 (1H, dd, J = 8.7 Hz, J = 0.3 Hz, H-6), 6.86 (1H, d, J = 8.2 Hz, H-5′), 6.81 (1H, dd, J = 2.2 Hz, J = 0.3 Hz, H-2′), 6.77 (1H, ddt, J = 8.2 Hz, J = 2.2 Hz, J = 0.6 Hz, H-6′), 6.32 (1H, ddd, J = 8.7 Hz, J = 2.4 Hz, J = 0.3 Hz, H-5), 6.28 (1H, dd, J = 2.4 Hz, J = 0.3 Hz, H-3), 4.43 (2H, bs, H-7′), 3.82 (3H, s, OCH_3) ppm. ^{13}C NMR (100 MHz; CD_3OD): δ 171.10 (C, C-7), 163.84 (C, C-2 or C-4), 163.58 (C, C-4 or C-2), 148.29 (C, C-4′), 147.66 (C, C-3′), 133.15 (C, C-1′), 130.25 (CH, C-6), 119.86 (CH, C-6′), 115.68 (CH, C-2′), 112.82 (CH, C-5′), 108.78 (C, C-1), 108.47 (CH, C-5), 103.95 (CH, C-3), 56.49 (CH_3, OCH_3), 43.47 (CH_2, C-7′) ppm.

5,7-Dihydroxy-3-(4-hydroxy-3-methoxybenzyl)benzo[*e*][1,3]oxazine-2,4-dione (15). Amide **9** (516 mg, 1.69 mmol) was dissolved in pyridine (10 mL), and after cooling to 0 °C, benzyl chloroformate (1.2 mL, 3.45 mmol) was added under nitrogen atmosphere using a syringe during 10 min. The mixture was stirred at 0 °C for 2 h and between 0 and 20 °C for a further 12 h. After refluxing at 115 °C for 8 h, ice (50 g) was added and the crude product extracted with ethyl acetate. The combined organic phases were washed with brine and saturated $NaHCO_3$ solution, dried with anhydrous Na_2SO_4, filtered, and evaporated to dryness in vacuo. The crude product (1.3 g) was purified using FC (eluant *n*-hexane/ethyl acetate 3:1 to 1:1) to yield 256 mg (46%) of a pale yellow solid. HPLC−MS (RP-18, H_2O/acetonitrile 80:20 to 0:100 in 30 min and then 15 min isocratic, APCI−): t_R 15.30 min, m/z = 330.6 (100%, $[M - H]^-$), 660.9 (0.4%, $[2M - H]^-$) >95%. HRMS: calcd for $C_{16}H_{13}NO_7$ 331.0692, found 331.0702. ^{1}H NMR (400 MHz, CD_3OD): δ 7.06 (1H, d, J = 2 Hz, H-2′), 6.91 (1H, ddt, J = 8.1 Hz, J = 2 Hz, J = 0.5 Hz, H-6′), 6.73 (1H, J = 8.1 Hz, H-5′), 6.18 (1H, d, J = 2.1 Hz, H-3 or H-5), 6.15 (1H, d, J = 2.1 Hz, H-5 or H-3), 4.99 (2H, s, H-7′), 3.83 (3H, d, J = 0.2 Hz, OCH_3) ppm. ^{13}C NMR (100 MHz; CD_3OD): δ 167.50 (C, C-7), 165.91 (C, C-2 or C-4), 163.30 (C, C-4 or C-2), 155.55 (C, C-6), 149.52 (C, carbamate-CO), 148.87 (C, C-3′), 147.59 (C, C-4′), 128.89 (C, C-1′), 123.10 (CH, C-6′), 116.13 (CH, C-5′), 113.97 (CH, C-2′), 100.29 (CH, C-5 or C-3), 95.25 (CH, C-3 or C-5), 94.55 (CH, C-1), 56.13 (CH_3, OCH_3), 45.74 (CH_2, C-7′) ppm.

Sensory Studies. For screening of taste-modulating effects, the test compounds were added directly to an aqueous solution of the appropriate bitter compound; occasionally, the mixture was treated for several minutes in an ultrasonic bath to improve the dissolution process. Panelists (healthy adults, no tasting problems known) were trained on caffeine as bitter standard. Studies were performed in the morning hours 1−2 h after breakfast, during which time they were not allowed to drink black or green tea or coffee due to adaptation to caffeine; only one bitter test per day was performed. A minimum of 10 testers was used in the descriptive test. The bitterness was rated on a scale of 1 (no taste) to 10 (very strong taste). For all experiments, the test solutions were coded and in the case of color or cloudiness the cups were covered using aluminum foil. Panelists were advised to test randomly mixed samples in the given order by the sip and spit method. The raw sensory data were analyzed using the standard functions of Microsoft Excel 97. For calculation of significance, Student's matched pair test was used.

RESULTS AND DISCUSSION

Syntheses. The polyhydroxylated amides were synthesized using a protecting- group-free method described earlier for the preparation of phenolic acid amides (*19*, *17*) with moderate to reasonably good yields as crystalline materials [average 40%, between 18% for aduncamide (**3**) and 68% for salicylic acid vanillylamide (**5**)]. The appropriate acid was esterified with *N*-hydroxysuccinimide using *N*,*N*′-dicyclohexylamide in 1,4-dioxane, and the solution of the activated ester was directly added to the amine or amine hydrochloride dissolved in an aqueous sodium hydrogen carbonate solution (cf. **Scheme 1**). Amide **7** was transferred into the sodium salt **8** by simple reaction with 1 equiv of sodium hydroxide and subsequent evaporation. The sodium content of **8** corresponded to a monosodium salt (calcd 7.40%, found 7.65%). In addition, the proton NMR of **8** is quite similiar to that of **7**; only the signals for H-3 and H-5 were shifted to higher field and that of C-3 and C-5 as well as of C-1 downfield. These shifts indicated the formation of a phenolic anion in position 2 or 4 of the benzoic acid moiety; most probably, a sodium complex was formed between the carbonyl oxygen and the deprotonated hydroxy group at C-2. The cyclic derivative **16** was synthesized starting from 2,4,6-trihydroxybenzoic acid *N*-(4-hydroxy-3-methoxybenzyl)amide (**9**) using benzyl chloroformate in pyridine (cf. **Scheme 1**).

Scheme 1. Synthesis of Hydroxylated Benzoic Acid Amides **3**–**7** and of Benzo[*e*][1.3]oxazin-2.4-dione **15**

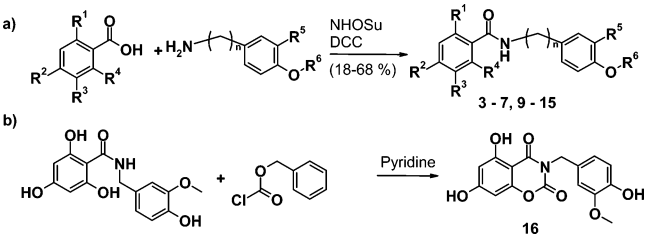

Table 1. Evaluation of Bitter-Masking Effect of Hydroxylated Benzoic Acid Amides Compared to Homoeriodictyol **1** against 500 mg L^{-1} Caffeine

	panelists		bitter rating (1–10)		
compd	all	masking[b]	blank	test	profile (100 mg L^{-1} in 5% sucrose)
1[c]	10	10	7.4 ± 1.6	4.2 ± 1.7*	sweet, vanillic, phenolic
3	16	10	5.0 ± 1.1	5.3 ± 1.2	fresh, fruity, sweet, mouthfeel
4			nd	nd	too bitter
5	15	12	5.3 ± 1.0	4.1 ± 0.9*	vanillic, pepper, pungent, warming
6	12	9	6.1 ± 1.1	4.5 ± 0.5*	fruity, sweet, dry–dusty
7	11	11	6.3 ± 1.2	4.5 ± 0.6*	neutral, slightly vanillic, liquorice
8	16	13	5.9 ± 0.9	4.1 ± 1.0*	cream, sweet, vanillic, caramel, toffee
9	11	8	6.7 ± 0.9	5.1 ± 0.9*	sweet, vanillic, liquorice
10	15	7	5.6 ± 1.0	5.7 ± 1.0	herbal, sweet, rotten
11	15	6	4.6 ± 1.2	4.5 ± 1.2	herbal, cinnamon, dry–dusty, cardboard
12	15	10	5.3 ± 0.9	4.5 ± 1.2	fresh, cooling, sweet
13	12	9	4.6 ± 1.0	3.7 ± 0.8*	vanillic, astringent, phenolic, dry–dusty, long-lasting, weak tingling
14	16	8	4.4 ± 1.1	3.9 ± 0.8	herbal, bitter, phenolic, weak tingling
15	16	7	5.8 ± 1.0	5.1 ± 0.7	astringent, metallic, drying, resin

[a] Test concentration 100 mg L^{-1}; mean values and 95% confidence intervals are given; * means significant ($p < 0.05$); nd means not determined. [b] Number of panelists who rated the bitterness of test solution lower than standard solution. [c] As the sodium salt.

All compounds were purified at least to 95% (HPLC) and thoroughly characterized by ^{1}H/^{13}C NMR, HPLC−MS (APCI mode), and for new compounds also by HRMS. The analytical data obtained for aduncamide (**3**) and salicoyl tyramine (**4**) were in accordance with the data published (*14*, *15*).

Screening for Bitter-Masking Activity. Prior to evaluation as potential bitter masker, the flavor profile of each compound was tested by an expert panel at 100 mg L^{-1} in a 5% sucrose solution. As shown in **Table 1** all compounds show some flavor but the intensities were generally very low and therefore not rated. The influence of the weak intrinsic flavor on masking tests was therefore neglected. The synthesized compounds were

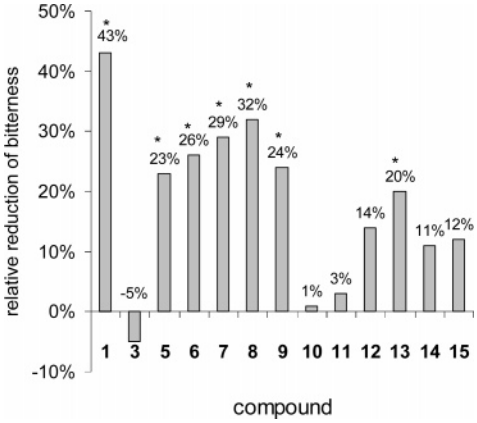

Figure 3. Masking effect of hydroxylated benzoic acid amides compared to homoeriodictyol **1** against 500 mg L^{-1} caffeine (test concentration 100 mg L^{-1}). Relative masking effect was calculated starting from averaged bitter ratings of blank and test solution (cf. **Table 1**); * significant ($p < 0.05$).

screened for their bitter masking activity by comparison with a 500 mg L^{-1} caffeine solution and a test solution containing the caffeine and the test compound (cf. **Table 1**). To avoid adaptation, the samples were anonymously coded and given to the panelist in randomized order. Data from at least 10 panelists, and in most cases of 15 or 16 panelists, were used. All ratings were used for calculations, even in cases of possible adaptation effects due to prior coffee or tea consumption. The bitterness rating of a 500 mg L^{-1} caffeine solution is constantly between 4.4 and 6.7. The raw data were averaged for blank and for the test solution, respectively, and the relative bitter inhibition was calculated starting from the mean values (cf. **Figure 3**).

The naturally occurring aduncamide (**3**) showed no masking effect, and salicoyl tyramine (**4**) showed an intrinsic bitterness. In contrast to these discouraging results, the benzyl amides **5**, **6**, **7**, and **9**, which are much closer structural relatives to homoeriodictyol (**1**) (they contain the vanillyl moiety and at least parts of the substitution pattern of flavanone ring A) were able to reduce significantly the bitterness of caffeine by 20−30%. As a result, it seemed to be possible to reduce the complexity of the parent bitter masker without loss of activity. When parent compound **7** was methylated at the benzoic part to yield vanillylamide **12**, which resembled the moderate bitter masker sterubin (eriodictyol-7-methyl ether, 20% activity, *10*), the activity to inhibit the bitterness of caffeine was slightly reduced.

In our previous study (*10*) we had found that the monosodium salt of homoeriodictyol was more active (43%) than the parent flavanone (28%). Thus, we prepared the corresponding sodium salt **8** of the most active vanillylamide **7**. In contrast to homoeriodictyol, the activity is only slightly increased.

Table 2. Bitter-Masking Effect of 100 mg L^{-1} 2,4-Dihydroxybenzoic Acid Vanillylamide (**7**) against Various Bitter Compounds[a]

	panelists		bitter rating (1–10)		% reduction of bitter
bitter compound	all	masking	blank	test	rating
caffeine, 500 mg L^{-1}	11	11	6.3 ± 1.2	4.5 ± 0.6	29*
quinine, 5 mg L^{-1}	16	12	5.5 ± 1.3	4.1 ± 0.8	25*
salicin, 250 mg L^{-1}	16	12	6.5 ± 1.1	5.1 ± 0.9	21*
H-Leu-Trp-OH, 2000 mg L^{-1}	16	8	6.8 ± 1.0	6.5 ± 1.2	4

[a] Test concentration 100 mg L^{-1}; mean values and 95% confidence intervals are given; * means significant ($p < 0.05$); nd means not determined.

To get more information about structural elements that may be necessary or decrease the activity, we synthesized the amide **10** as the closest relative to eriodictyol (**2**) which was the most active bitter-masking flavanone in our previous study (47% activity, *10*). Surprisingly, the benzamide **10**, which contains the catechol moiety, showed no activity at all. On the other hand, amide **11**, which is the structural analogue to the more or less inactive naringenin, was also not able to reduce the bitterness of caffeine. Interestingly, the reducing power of the phenethylamide **13**, which is a homologue to **7**, is only slightly decreased. Amide **14**, containing an isovanillyl unit, was synthesized as an analogue of hesperetin. As in the case of the flavanone (12% activity, *10*), the masking activity was only weak and not significant. Last but not least, we synthesized a more constrained analogue of amide **9**; the activity is largely reduced but not fully eliminated.

Thus, the combination of free hydroxy groups in the benzoic acid part which corresponds to ring A of flavanones and a vanillyl unit in the amine moiety which corresponds to ring B of flavanones seemed to be necessary for a masking activity toward caffeine. In most cases, changes of the substitution pattern at the amine part caused decrease or loss of activity, as did methylations at the benzoic acid part.

Evaluation of 2,4-Dihydroxybenzoic Acid Vanillylamide (7). The most promising candidate, 2,4-dihydroxybenzoic acid *N*-(4-hydroxy-3-methoxybenzyl)amide (**7**), was evaluated more in depth. First, the activity against other bitter components was tested (cf. **Table 2**). As a prototypical bitter component quinine was chosen; salicin was tested because the bitter receptor for this class of compounds is now known (*5*). In addition, *N*-L-leucyl-L-tryptophan (H-Leu-Trp-OH) was evaluated as a typical bitter peptide; such hydrophobic peptides can cause a lot of bitter taste problems in processed food applications (*20*). Similiar to the results for homoeriodictyol (*10*), amide **7** was able to reduce the perceived bitterness of quinine and salicin by about 20% but was not active toward H-Leu-Trp-OH. Additionally, the dose-

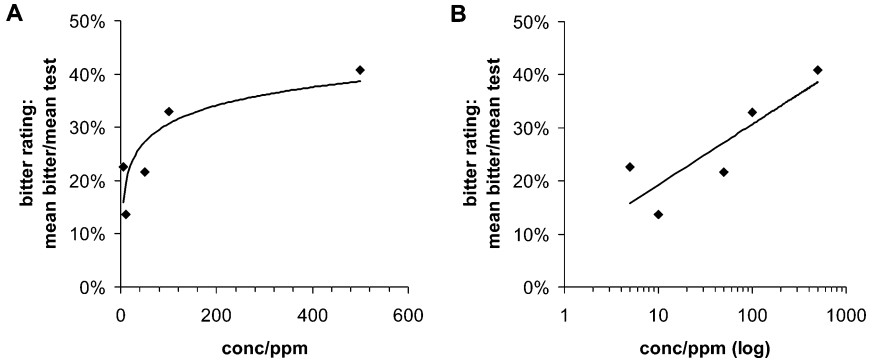

Figure 4. Dose–response plot of masking effect of 2,4-dihydroxybenzoic acid vanillylamide (**7**) in 500 mg L^{-1} caffeine solution. (**A**) linear plot and (**B**) semilogarithmic plot.

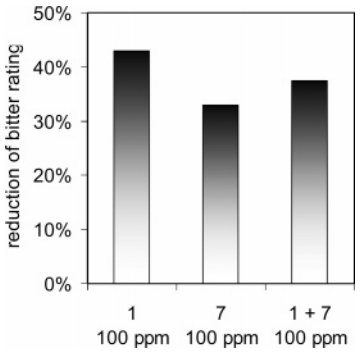

Figure 5. Masking effect of a combination of homoeriodictyol (**1**) and vanillylamide (**7**) toward 500 mg L^{-1} caffeine compared to the single compounds.

dependent activity of **7** toward the bitterness of a 500 mg L^{-1} caffeine solution was determined. The results (cf. **Figure 4A**) indicated clearly a dose-dependent activity that reached a plateau at 500 mg L^{-1} and a more or less linear relationship between 5 and 500 mg L^{-1} when plotted semilogarithmically (cf. **Figure 4B**). As a further experiment, the activity of a 1:1 mixture of homoeridictyol (**1**) and amide **7** was compared with the values obtained for the single compounds. As shown in **Figure 5**, there was only a more or less additive effect, and no significant synergism or antagonism could be found. These results suggest that both compounds act as competitors on the molecular level.

In conclusion, we have found structural analogues to bitter-masking flavanones that also were able to reduce the bitterness of caffeine solutions. The most interesting compounds were the hydroxylated benzoic acid vanillylamides **5**−**9**. The compounds, which have only a weak flavor profile, show activities comparable to homoeriodictyol (**1**). All further variations in structure caused a decrease or loss in bitter-masking ability. Summarizing the data for the most active amide **7**, we conclude that the hitherto unknown mechanism of inhibition of flavanones and the new amides is probably the same, because the behavior of both structural classes was nearly the same. In the future, the new amides may be alternatives for the expensive flavanones to create flavor solutions to mask bitterness of pharmaceuticals or foodstuffs. Besides these possible applications, the new structures may be used as a tool to find the molecular mechanism responsible for the bitter masking effect.

ABBREVIATIONS USED

AMP, adenosine monophosphate; APCI, atmospheric pressure chemical ionization; FC, flash chromatography; H-Leu-Trp-OH, *N*-L-leucyl-L-tryptophan; t_R, retention time.

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