Supporting Materials and Methods

Bioinformatics and Molecular Modeling

Data Mining. The results of the search strategy used in PSSC discussed in the article are briefly outlined in Fig. 6.

Protein Structure Alignment. Structural alignment of the proteins or cores was performed by using an algorithm that involves a combinatorial extension (CE) of an alignment path defined by aligned fragment pairs (AFPs) (1).

Criteria for structure comparison incorporated in the algorithm are, among others, interresidue distances based on the coordinates of C^{α} atoms in the course of finding the longest path of AFPs and optimal structure superposition as rigid bodies and calculation of the root-mean-square deviation (RMSD).

The RMSD is defined as follows:

$$RMSD = \sqrt{\sum_{i=1}^{N} \frac{d_i^2}{N}}$$

in which N = number of C^{α} atoms and d_i = distance between two corresponding atoms i in two structures.

Because RMSD values are size dependent one has to make sure that the structural alignment to which the RMSD value refers is relevant. This means in terms of the concept delineated in the article that the ligand-sensing cores of the aligned proteins are mainly considered. As we observed in our explorations, RMSD values of 4 to 5 Å between two domains or domain cores still may indicate similarity exploitable in terms of the PSSC concept. Remotely related proteins with undetectable sequence relationship

[sequence identity (SI) < 8%] usually are found to show RMSD values of ≈4 Å or smaller (2).

Occasionally, additional information concerning sequence similarity can more easily be deduced from an amino acid sequence alignment derived from a structural alignment. If the active sites are similar and important residues are conserved this might indicate a probable relationship between the respective proteins. In the case of AChE and Cdc25A, for example, the key catalytic residues, Ser-200 and Cys-430, are aligned (see Fig. 7). In the case of Cdc25A and the 11βHSDs, although structurally similarly located, the key catalytic residues Cys-430 (Cdc25A) and Tyr-183 (11βHSD1)/Tyr-232 (11βHSD2) could not be aligned sequentially (see Fig. 8).

Homology Models of 11βHSD1 and 11βHSD2. An unrefined model of the overall shape of 11βHSD1 (assignment to a fold) would be sufficient in terms of the PSSC concept. Still, we tried to get the most accurate and reliable 3D representation of both 11βHSD isozymes. Of major importance for us was to find the best state-of-the-art procedure combining ease of use and reasonable velocity. The 292-aa-long sequence of human 11βHSD1 was taken from the SWISS-PROT protein knowledge base [http://au.expasy.org/sprot (3)]. The sequence was then submitted to the Structure Prediction MetaServer [SPMS, http://bioinfo.pl/meta (4)], from where it was sent to a variety of autonomous prediction servers. We chose a metapredictor because metapredictors are more accurate than any individual server for building the consensus and, above all, the case of 11βHSD1 is not a trivial one, as members of the SDR family are characterized by a low intersequence similarity of <30%. The 3D-Jury system implemented in the SPMS performed as the best metapredictor in the recent CASP5 assessment of methods for protein structure prediction in the field of fold recognition and comparative modeling (5). Even more, it performed just as well as the best human predictors. Also in the LiveBench-6 automated evaluation of protein structure prediction servers the 3D-Jury system was ranked among the best (6), especially the 3D-JuryA-all (3JAa) version. When this approach was used the alignment of the 11βHSD1 sequence to the template structure of levodione reductase (PDB 1IY8) generated by the FFAS03

algorithm (7) was ranked as the highest-scoring solution, with a 3D-Jury score of 175.53. Because the alignment (Fig. 9) dictates the quality of the modeled structure, it was analyzed in detail. It was checked to see if gap positions are reasonable or if secondary structure elements are disrupted. The sequence identity in the aligned part amounts to 17.4%.

This alignment was then used for model construction using MODELLER 6v2 (8). The generated model was analyzed with ProQ (9), which is a neural network to identify correct protein models. The output accuracy measures, LG score and MaxSub, amount to 6.271 and 0.650, respectively, and indicate that the model is very good. Additional WhatCheck (10) and ProCheck (11) analyses were performed for further evaluation.

Similarly, the homology model of 11βHSD2 was constructed. The alignment of the 11βHSD2 sequence (Arg-171 to Arg-405) to the template structure of glucose dehydrogenase (PDB 1GCO) generated by the mGenTHREADER algorithm (12) was ranked as the highest-scoring solution, with a 3D-Jury score of 167.42 (Fig. 10). Sequence identities amount to 17.9%. After careful analysis, model building with MODELLER 6v2 was performed. Model evaluation using ProQ gave an LG score of 5.088 and a MaxSub score of 0.427. WhatCheck and ProCheck analyses confirmed the good quality of the model.

Chemistry

General Methods and Procedures. *Analytical Methods.* ^{1}H and ^{13}C spectra were recorded on a Varian Mercury 400 spectrometer. Fast atom bombardment (FAB)-MS and electron impact (EI)-MS were measured on a Finnigan MAT MS 70 spectrometer. GC-MS spectra were measured on an HP 6890 Series GC connected to an HP 5973 mass selective detector.

Materials. TLC was performed on Merck silica gel 60 F_{254} aluminum sheets. For flash chromatography Baker silica gel (40-70 μm) was used. Solvents were distilled by using

standard procedures. All reactions were carried out under an argon atmosphere. All photochemical oxidations were carried out under a dry oxygen atmosphere. Commercial reagents were used without further purification.

Synthesis of 3-Substituted Furans (Procedure A). A 2.5 M solution of *n*-BuLi in hexane (2.8 eq) was added dropwise to a stirred solution of 3-bromofuran (3 eq) in dry Et_2O (10 ml) at –78°C. After 30 min, a solution of the aldehyde (1-4 mmol, 1 eq) in Et_2O (5 ml) cooled to –78°C was added and the mixture was stirred for 90 min at this temperature. The reaction mixture was allowed to warm to room temperature. The reaction was quenched with saturated aqueous NH_4Cl (10 ml) and extracted with Et_2O (five times with 20 ml). The combined organic layers were washed with saturated aqueous NH_4Cl (three times with 10 ml), water (twice with 10 ml), and brine (twice with 10 ml) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using cyclohexane/EtOAc (10:1, vol/vol) as eluent to afford the 3-substituted furan (yields: 65–87%).

Synthesis of 3-Substituted Bisfurans (Procedure B). A 2.5 M solution of *n*-BuLi in hexane (5.8 eq) was added dropwise to a stirred solution of 3-bromofuran (6 eq) in dry Et_2O (10 ml) at –78°C. After 30 min, a solution of carboxylic ester (0.1-0.2 mmol, 1 eq) in THF (5 ml) cooled to –78°C was added and the mixture was stirred for 90 min at this temperature. The reaction mixture was allowed to warm to room temperature and was then quenched with saturated aqueous NH_4Cl (10 ml) and extracted with Et_2O (five times with 20 ml). The combined organic layers were washed with saturated aqueous NH_4Cl (three times with 10 ml), water (twice with 10 ml), and brine (twice with 10 ml) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using cyclohexane/EtOAc (10:1, vol/vol) as eluent to afford the 3-substituted bisfuran (yields: 19–82%).

Synthesis of 3-Substituted Furyl Ketones (Procedure C). A 2.5 M solution of *n*-BuLi in hexane (0.95 eq) was added dropwise to a stirred solution of 3-bromofuran (1 eq) in dry Et_2O (40 ml) at –78°C. After 30 min, this mixture was added to a solution of

carboxylic acid chloride (≈13 mmol, 1 eq) in Et_2O (50 ml) cooled to –10°C. The reaction mixture was stirred for 1 h at this temperature and then allowed to warm to room temperature. The reaction was quenched with saturated aqueous NH_4Cl (10 ml) and extracted with Et_2O (five times with 20 ml). The combined organic layers were washed with saturated aqueous NH_4Cl (three times with 10 ml), water (twice with 10 ml), and brine (twice with 10 ml) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using cyclohexane/EtOAc (10:1, vol/vol) as eluent to afford the 3-substituted furyl ketones (yields: 70–73%).

Synthesis of 2-Substituted Furans (Procedure D). A 2.5 M solution of *n*-BuLi in hexane (2.8 eq) was added dropwise to a stirred solution of diispropylamine (3 eq) in dry Et_2O (10 ml) at –78°C. After 30 min, a solution of furan (3 eq) in dry Et_2O (5 ml) cooled to –78°C was added and stirred for additional 30 min at this temperature. A solution of the aldehyde (≈3 mmol, 1 eq) in Et_2O (5 ml) cooled to –78°C was added and the mixture was stirred for 1 h at this temperature. Finally, the reaction mixture was allowed to warm to room temperature. The reaction was quenched with saturated aqueous NH_4Cl (10 ml) and extracted with Et_2O (five times with 20 ml). The combined organic layers were washed with saturated aqueous NH_4Cl (three times with 10 ml), water (twice with 10 ml), and brine (twice with 10 ml) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using cyclohexane/EtOAc (10:1, vol/vol) as eluent to afford the 2-substituted furan (yields: 79–91%).

Oxidation of Furans to γ-Hydroxybutenolides (Procedure E). A solution of a 2- or 3-substituted furan, ketofuran, or bisfuran (0.04-0.4 mmol, 1 eq), diisopropylethylamine (10 eq), and Bengal rose (≈2 mg) in CH_2Cl_2 (20 ml) was saturated with anhydrous oxygen, cooled to –78°C, and irradiated with a tungsten filament lamp (200 W) at this temperature under an oxygen atmosphere for 2 h. After warming up to room temperature, saturated aqueous oxalic acid (2 ml) was added and the mixture was vigorously stirred for 30 min. The mixture was diluted with water (10 ml) and extracted with $CHCl_3$/EtOAc

(1:2, vol/vol, three times with 20 ml). The combined organic layers were dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using cyclohexane/EtOAc (10:1, vol/vol) to CH_2Cl_2/MeOH (95:5, vol/vol) as eluent to afford the γ-hydroxybutenolide (yields: 25–94%).

Typical Procedure for the Preparation of Butenolides Starting from 2-(Trimethylsilyloxy)furan (TMSOF) (Procedure F1). A solution of TMSOF (1 eq) in CH_2Cl_2 was added dropwise to a stirred solution of an aldehyde (0.3-0.4 mmol, 1 eq) and BF_3•OEt_2 (1 eq) in dry CH_2Cl_2 (10 ml) cooled to –78°C. After 1 h of stirring at this temperature, the reaction mixture was allowed to warm to room temperature. The reaction was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (three times with 20 ml). The combined organic layers were washed with brine (three times with 10 ml) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using cyclohexane/EtOAc (10:1 to 1:2, vol/vol) as eluent to afford the corresponding butenolide (yields: 51–75%).

Typical Procedure for the Preparation of Bisbutenolides Starting from TMSOF (Procedure F2). The bisbutenolides were synthesized by following procedure **F1** using 3 eq of TMSOF and 3 eq of BF_3•OEt_2 (yields: 69–74%).

Oxidation with 1-Hydroxy-1,2-Benziodoxol-3(1*H*)-one 1-Oxide (IBX) (Procedure G). A solution of a furyl alcohol (0.04-0.09 mmol, 1 eq) in THF (5 ml) was added dropwise to a stirred solution of IBX (10 eq) in DMSO (5 ml) at room temperature. After 6 h, the reaction was quenched with water (10 ml) and the mixture was stirred for 30 min. The precipitate was filtered off. The aqueous phase was then extracted with cold Et_2O (three times with 20 ml). The combined organic layers were washed with brine (three times with 20 ml) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using cyclohexane/EtOAc (30:1 to 1:1, vol/vol) as eluent to afford the corresponding (yields: 87–94%).

Addition of Grignard Reagents (Procedure H). A Grignard reagent (4 eq) was added to a stirred solution of a ketone (0.4-0.5 mmol, 1 eq) in Et_2O (10 ml) at –78°C. After 1 h, the reaction mixture was allowed to warm to room temperature. The reaction was quenched with aqueous NH_4Cl solution (10 ml) and extracted with Et_2O (five times with 20 ml). The combined organic layers were washed with brine (10 ml) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using cyclohexane/EtOAc (30:1 to 5:1, vol/vol) as eluent to afford the corresponding alcohol (yields: 88–90%).

Addition of Aryllithium Reagents (Procedure I). A 2.5 M solution of *n*-BuLi in hexane (4.3 eq) was added to a stirred solution of an aryl bromide compound (4.5 eq) in Et_2O (10 ml) at –78°C. After 30 min, a solution of a ketone (0.3-0.4 mmol, 1 eq) in Et_2O (5 ml) was added and the mixture was allowed to warm to 0°C. After 2 h of stirring at this temperature, the reaction mixture was allowed to warm to room temperature. The reaction was quenched with aqueous NH_4Cl solution (10 ml) and extracted with Et_2O (five times with 20 ml). The combined organic layers were washed with brine (10 ml) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using cyclohexane/EtOAc (30:1 to 5:1, vol/vol) as eluent to afford the corresponding alcohol (yields: 75–85%).

Synthesis and Analytical Data of Selected Compounds

Bis-5-hydroxy-5*H*-furan-2-on-4-yl-(6-undecylnaphthalene-2-yl)methanol (1). According to general procedure B, to 3-bromofuran (0.14 ml, 1.59 mmol, 9 eq) were added 2.5 M *n*-BuLi in hexane (0.62 ml, 1.55 mmol, 8.8 eq) and methyl 6-undecylnaphthalene-2-carboxylate (60 mg, 0.176 mmol, 1 eq). The white solid was purified by flash chromatography (yield: 38 mg, 49%). This product (20 mg, 0.045 mmol, 1 eq), $(i\text{Pr})_2\text{NEt}$ (0.079 ml, 0.45 mmol, 10 eq), and Bengal rose (5 mg) were subsequently reacted according to **E** under oxygen atmosphere. The resulting solid was purified by flash chromatography (yield: 9 mg, 37%). R_f = 0.36 [cyclohexane/ethyl acetate 1:1 (vol/vol)]. ^{1}H-NMR (400-MHz, $CDCl_3$): δ = 7.28-8.00 (m, 6H), 5.90-6.40 (m, 4H), 2.69-

2.75 (m, 2H), 1.60-1.67 (m, 2H), 1.18-1.35 (m, 14H), 0.84-0.87 (t, J = 6.84 Hz, 3H). ^{13}C NMR (100.6-MHz, $CDCl_3$): δ = 170.83, 158.67, 137.11, 136.28, 131.33, 131.14, 128.44, 128.20, 127.65, 127.13, 125.99, 125.24, 114.84, 93.66, 82.48, 36.20, 32.01, 31.42, 29.80, 29.74, 29.73, 29.66, 29.52, 29.46, 22.81, 14.26. High-resolution (HR)-MS [FAB in 3-nitrobenzyl alcohol (3-NBA)]: 508.2506 (6.8) $[M]^+$, 490.2504 (50.8) $[M - H_2O]^+$ ($C_{30}H_{36}O_7$: 508.2461 g/mol).

4-[1-(4-Chlorophenyl)-1-hydroxyhexadecyl]-5-hydroxyfuran-2(5*H*)-one (2).
According to general procedure C, 3-bromofuran (1.16 ml, 13 mmol, 1 eq) was reacted with 2.5 M *n*-BuLi in hexane (5.23 ml, 0.013 mol, 1 eq) and palmitoyl chloride (3.58 g, 0.013 mol, 1 eq) to give 1-(furan-3-yl)hexadecan-1-one as a solid in 71% yield (2.84 g) after purification by flash chromatography. This product (0.13 g, 0.42 mmol, 1 eq) was then treated with 4-chlorophenylmagnesium bromide (1.7 ml, 1.7 mmol, 4 eq) according to general procedure H and the resulting white solid was purified by flash chromatography (0.156 g, 89% yield). Finally, 1-(4-chlorophenyl)-1-(furan-3-yl)hexadecan-1-ol (20 mg, 0.048 mmol, 1 eq), (iPr)$_2$NEt (0.83 ml, 0.48 mmol, 10 eq), and Bengal rose (5 mg) were reacted under oxygen atmosphere according to general procedure E to give 4-[1-(4-chlorophenyl)-1-hydroxyhexadecyl]-5-hydroxyfuran-2(5*H*)-one. The white solid was purified by flash chromatography over silica gel (10 mg, 49% yield). R_f = 0.35 [cyclohexane/ethyl acetate 1:1 (vol/vol)]. ^{1}H NMR (400-MHz, $CDCl_3$): δ = 7.04-7.38 (m, 4H), 6.48-6.56 (m, 1H), 6.33 (s, 1H), 1.99-2.30 (m, 2H), 1.19-1.40 (m, 28H), 0.84-0.87 (t, J = 7.03 Hz, 3H). ^{13}C NMR (100.6-MHz, $CDCl_3$): δ = 171.04, 163.89, 142.49, 132.55, 130.30, 130.30, 128.83, 128.66, 116.55, 97.56, 75.32, 40.67, 31.98, 30.13, 29.77, 29.68, 29.65, 29.56, 29.42, 29.39, 29.01, 22.79, 14.25. HR-MS (FAB in 3-NBA): 433.1868 (100) $[M - H_2O]^+$ ($C_{26}H_{39}O_4Cl$: 451.2537 g/mol).

5-Hydroxy-4-[1-hydroxy-1-(3-methoxyphenyl)dodecyl]furan-2(5*H*)-one (3).
According to general procedure C, 3-bromofuran (1.16 ml, 13 mmol, 1 eq) was reacted with 2.5 M *n*-BuLi in hexane (5.23 ml, 0.013 mol, 1 eq) and dodecanoyl chloride (2.85 g, 0.013 mol, 1 eq) to give 1-(furan-3-yl)dodecan-1-one as a solid in 70% yield (2.27 g) after purification by flash chromatography. Subsequently, according to general procedure

H, 1-(furan-3-yl)dodecan-1-one (0.13 g, 0.52 mmol, 1 eq) was reacted with 3-methoxyphenylmagnesium bromide (2.08 ml, 2.08 mmol, 4 eq), and the resulting white solid was purified by flash chromatography (0.167 g, 90% yield). Finally, 1-(4-chlorophenyl)-1-(furan-3-yl)dodecan-1-ol (20 mg, 0.056 mmol, 1 eq), (iPr)$_2$NEt (0.1 ml, 0.56 mmol, 10 eq), and Bengal rose (5 mg) were reacted under oxygen atmosphere according to general procedure E. The resulting white solid was purified by flash chromatography (10 mg, 49% yield). R_f = 0.35 [cyclohexane/ethyl acetate 1:1 (vol/vol)]. ^{1}H NMR (400-MHz, $CDCl_3$): δ = 6.78-7.29 (m, 4H), 6.09 (s, 1H), 5.83 (s, 1H), 3.79 (s, 3H), 1.97-2.14 (m, 2H), 1.19-1.27 (m, 18H), 0.83-0.87 (t, J = 7.03 Hz, 3H). ^{13}C NMR (100.6-MHz, $CDCl_3$): δ = 171.05, 162.39, 159.61, 143.90, 129.61, 118.44, 117.45 112.53, 111.42, 97.5, 75.5, 55.3, 40.67, 31.98, 29.84, 29.81, 29.69, 29.69, 29.68, 29.65, 29.55, 29.42, 22.79, 14.25. HR-MS (FAB in 3-NBA): 413.2302 $[M + Na]^+$, 390.2429 (20.2) $[M]^+$, 373.2416 (92.6) $[M – H_2O]^+$ ($C_{23}H_{34}O_5$: 390.2406 g/mol).

4-[1-(4-Fluorophenyl)-1-hydroxydodecyl]-5-hydroxyfuran-2(5*H*)-one (4). According to general procedure C.16 ml, 13 mmol, 1 eq) was reacted with 2.5 M n-BuLi in hexane (5.23 ml, 0.013 mol, 1 eq) and dodecanoyl chloride (2.85 g, 0.013 mol, 1 eq) to give 1-(furan-3-yl)dodecan-1-one as a solid in 70% yield (2.27 g) after purification by flash chromatography. Then, according to general procedure H, 1-(furan-3-yl)dodecan-1-one (0.13 g, 0.52 mmol, 1 eq) was treated with 4-fluorophenylmagnesium bromide (1.04 ml, 2.1 mmol, 4 eq). The resulting white solid was purified by flash chromatography to give 1-(4-fluorophenyl)-1-(furan-3-yl)dodecan-1-ol in 90% yield (0.16 g). In the last step, this furyl alcohol (20 mg, 0.058 mmol, 1 eq) was reacted with (iPr)$_2$NEt (0.1 ml, 0.58 mmol, 10 eq) and Bengal rose (5 mg) under oxygen atmosphere according to general procedure E. The resulting white solid was purified by flash chromatography (50% yield, 10 mg). R_f = 0.35 [cyclohexane/ethyl acetate 1:1 (vol/vol)]. ^{1}H NMR (400-MHz, $CDCl_3$): δ = 7.00-7.42 (m, 4H), 6.08 (s, 1H), 5.83 (s, 1H,), 1.94-2.15 (m, 2H), 1.20-1.39 (m, 18H), 0.83-0.86 (t, J = 7.03 Hz, 3H). ^{13}C NMR (100.6-MHz, $CDCl_3$): δ = 170.24, 169.33, 162.19, 137.99, 127.00, 126.92, 117.80, 115.31, 115.25, 97.69, 75.31, 40.80, 31.97, 29.78, 29.70, 29.68, 29.67, 29.64, 29.63, 29.55, 29.41, 22.78, 14.24. HR-MS (FAB in 3-NBA): 401.2095 (100) $[M + Na]^+$, 361.2168 (92.2) $[M – H_2O]^+$ ($C_{22}H_{31}O_4F$: 378.2206 g/mol).

4-[(2-Fluoro-4-hexadecylphenyl)(hydroxy)methyl]-5-hydroxyfuran-2(5*H*)-one (5). According to general procedure A, to 3-bromofuran (0.172 ml, 1.94 mmol, 4.5 eq) and 2.5 M *n*-BuLi in hexane (0.75 ml, 1.82 mmol, 4.2 eq) was added 2-fluoro-4-hexadecylbenzaldehyde (0.15 g, 0.43 mmol, 1 eq) to give (2-fluoro-4-hexadecylphenyl)(furan-3-yl)methanol as a white solid (0.12 g, 67%). After purification by flash chromatography, this product (20 mg, 0.048 mmol, 1 eq) was reacted according to general procedure E with (iPr)$_2$NEt (0.084 ml, 0.48 mmol, 10 eq) and Bengal rose (5 mg) under oxygen atmosphere to give **3** in 49% yield (10 mg). R_f = 0.40 [cyclohexane/ethyl acetate 1:1 (vol/vol)]. ^{1}H NMR (400-MHz, $CDCl_3$): δ = 6.85-7.34 (m, 3H), 6.17 (s, 1H), 5.86 (s, 1H), 5.79 (s, 1H), 2.53-2.58 (m, 2H), 1.52-1.58 (m, 2H), 1.20-1.30 (m, 16H), 0.84-0.87 (t, J = 7.04 Hz, 3H). ^{13}C NMR (100.6-MHz, $CDCl_3$): δ = 169.36, 160.84, 156.22, 146.60, 128.07, 124.86, 122.72, 117.66, 115.48, 97.69, 64.77, 35.59, 31.99, 31.12, 29.80, 29.77, 29.75, 29.73, 29.71, 29.69, 29.66, 29.58, 29.54, 29.43, 29.35, 22.79, 14.24. HR-MS (FAB in 3-NBA): 448.2945 (16.7) $[M]^+$ ($C_{27}H_{41}O_4F$: 448.2989 g/mol).

1,3-Bis(5-oxo-2,5-dihydrofuran-2-yl)tetradecanol (6). According to general procedure F2, tetradec-2-enal (25 mg, 0.12 mmol, 1 eq) was treated with BF_3•OEt_2 (52 mg, 0.36 mmol, 3 eq), and TMSOF (57 mg, 0.36 mmol, 3 eq). The resulting yellow oil was purified by flash chromatography to give **6** as a white solid in 72% yield (77 mg). R_f = 0.23 [cyclohexane/ethyl acetate 5:1 (vol/vol)]. ^{1}H NMR (400-MHz, $CDCl_3$): δ = 7.53-7.56 (dt, J = 1.56, 5.86 Hz, 2H); 6.07-6.09 (dt, J = 2.15, 5.87 Hz, 2H), 4.84-4.85 (m, 2H), 4.10 (m, 1H), 1.49-1.98 (m, 2H), 1.16-1.38 (m, 22H); 0.77-0.81 (t, J = 7.04 Hz, 3H). ^{13}C NMR (100.6-MHz, $CDCl_3$): δ = 173.45, 152.84, 121.10, 84.54, 72.09, 35.44, 31.80, 29.75, 29.69, 29.62, 29.54, 29.52, 29.49, 29.46, 29.31, 29.24, 22.60, 14.08. HR-MS (FAB in 3-NBA): 389.2690 (4.0) $[M - H_2O]^+$ ($C_{24}H_{38}O_5$: 406.2719 g/mol).

Furan-3-yl-(5-hexadecyl-2-methoxyphenyl)methanol (7). According to general procedure A, 3-bromofuran (0.17 ml, 1.9 mmol, 4.5 eq) was treated with 2.5 M *n*-BuLi in hexane (0.7 ml, 1.75 mmol, 4.2 eq) and reacted with 5-hexadecyl-2-

methoxybenzaldehyde (150 mg, 0.42 mmol, 1 eq) to afford furan-3-yl-(5-hexadecyl-2-methoxyphenyl)methanol as a white solid after flash chromatography in 71% yield. R_f = 0.51 [cyclohexane/ethyl acetate 5:1 (vol/vol)]. ^{1}H NMR (400-MHz, $CDCl_3$): δ = 6.79-7.35 (m, 5H), 6.36 (d, *J* = 0.98 Hz, 1H), 5.93 (s, 1H), 3.79 (s, 3H), 2.49-2.55 (m, 2H), 1.51-1.59 (m, 2H) 1.19-1.29 (m, 26H), 0.85-0.89 (t, *J* = 7.04 Hz, 3H). ^{13}C NMR (100.6-MHz, $CDCl_3$): δ = 154.45, 142.66, 139.35, 135.05, 130.62, 128.15, 127.30, 124.28 110.48, 109.41, 60.34, 55.43, 35.15, 31.97, 31.74, 29.75, 29.73, 29.71, 29.69, 29.67, 29.62, 29.56, 29.52, 29.41, 29.33, 29.22, 22.75, 14.19. GC-MS (*m/z*): 428 $[M]^+$. HR-MS (FAB in 3-NBA): 411.0031 (38.4) $[M - H_2O]^+$ ($C_{28}H_{44}O_3$: 428.3290 g/mol).

Biological Testing

Inhibition of Cdc25A. The clone pET9d/His-Cdc25A was expressed in the *Escherichia coli* strain BL21-DE3 and purified in the presence of 8 M urea (13). A 30-μg sample of the purified enzyme was preincubated with the inhibitors in 50 μl of a buffer containing 50 mM Tris, 50 mM NaCl, and 2 mM dithioerythritol, pH 8.0, for 15 min at room temperature (14). Then, 50 μl of a buffer containing 50 mM Tris, 50 mM NaCl, 1 mM EDTA, and 100 mM *p*-nitrophenyl phosphate (*p*-NPP, Calbiochem), pH 8.0, was added to give a final assay volume of 100 μl and an end concentration of 50 mM *p*-NPP. Similarly, control experiments in the presence of 0.001% Triton X-100 were performed (15). The read-out (405 nm) was recorded on a microplate reader at 37.0 ± 0.1°C continuously for 80 min. The reaction rate was determined from the absorption difference between 30 min and 60 min reaction time. After screening the whole library, IC_{50} values of the most promising candidates (best percent inhibition) were determined. Different concentrations of each test compound were assayed, and the percent inhibition due to the presence of test compound was calculated. IC_{50} values were determined graphically from log concentration vs. inhibition curves. Results are shown in Table 2.

Inhibition of 11βHSD1 and 11βHSD2. *Materials.* Cell culture reagents were purchased from Invitrogen, [1,2,6,7-^{3}H]Cortisone was from American Radiolabeled Chemicals (St. Louis), and [1,2,6,7-^{3}H]cortisol was from Amersham Pharmacia Biosciences. Thin-layer

chromatography (TLC) plates (SIL G-25 UV254) were purchased from Macherey-Nagel (Oensingen, Switzerland).

Assay. 11βHSD1-dependent oxoreduction of cortisone and 11βHSD2-dependent oxidation of cortisol were measured in lysates of stably transfected HEK-293 cells as described previously (16, 17). Briefly, cells were incubated for 16 h in steroid-free medium, washed once with PBS, detached, and centrifuged for 3 min at 150 × *g*. The supernatant was removed and the cell pellet was quick-frozen in a dry-ice/ethanol bath. At the day of experiment, cell pellets were resuspended in buffer TG1 (20 mM Tris•HCl, pH 7.4/1 mM EGTA/1 mM EDTA/1 mM $MgCl_2$/100 mM NaCl/20% glycerol) and sonicated, and activities were determined immediately. The rate of conversion of cortisol to cortisone or the reverse reaction was determined in 96-well optical PCR plates (Applied Biosystems) in a final volume of 22 μl. Reactions were started by mixing 10 μl of cell lysate with 12 μl of reaction mixture (TG1 buffer supplemented to give a final concentration of 400 μM $NADP^+$ or NADPH, 30 nCi of ^{3}H-labeled substrate, 200 nM unlabeled cortisone or 25 nM unlabeled cortisol, and inhibitor between 0 and 200 μM), followed by incubation for 10 min at 37°C. Similarly, control experiments in the presence of 0.01% Triton X-100 were performed (15). Because of inactivation of 11βHSD2 due to solubilization of the enzyme out of the membrane by detergents (18), the control experiment could not be carried out with 11βHSD2. The reactions were stopped by adding 10 μl of methanol containing 2 mM of unlabeled steroids, followed by separation of steroids by TLC as described (16). Enzyme kinetics were analyzed by nonlinear regression using the Data Analysis Toolbox (Elsevier MDL, San Leandro, CA) assuming first-order rate kinetics. Results are shown in Table 2.

Inhibition of Acetylcholinesterase (AChE). *Materials.* AChE (type III, electric eel), acetylthiocholine iodide (ATC), and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich.

Assay. Inhibitory activity was measured by using the spectrophotometric method of Ellman *et al.* (19, 20). ATC was used as the substrate of the enzymatic reaction and

DTNB for the measurement of AChE activity. The assay was performed in 96-well microtiter plates. In this procedure, the enzyme (0.25 unit/ml) was preincubated with the inhibitors in 21 μl of 100 mM sodium phosphate buffer, pH 7.2, for 15 min at room temperature. Then, 79 μl of a solution of ATC and DTNB in 100 mM phosphate buffer, pH 8.0, was added to the assay solution to give a final volume of 100 μl and end concentrations of 200 μM ATC (21) and 300 μM DTNB (22). Similarly, control experiments in the presence of 0.01% Triton X-100 were performed (15). The change in absorbance at 405 nm was recorded during 5 min at 30-s intervals at 25.0 ± 0.1°C. The reaction rate was calculated from the absorption difference between 30 s and 90 s reaction time and was determined as to be 0.059 ± 0.003 ΔA/min in the absence of inhibitor (22). After screening the whole library, IC_{50} values of the most promising candidates (best percent inhibition) were determined. Different concentrations of each test compound were assayed, and the percent inhibition due to the presence of test compound was calculated. IC_{50} values were determined from log concentration vs. inhibition curves by using the IC_{50} fit function that is implemented in the program GRAFIT version 5.0.4 (Erithacus, Surrey, U.K.). Results are shown in Table 2.

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