Amyloid beta 42 peptide (A β 42)-lowering compounds directly bind to A β and interfere with amyloid precursor protein (APP) transmembrane dimerization

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Following ectodomain shedding by β -secretase, successive proteolytic cleavages within the transmembrane sequence (TMS) of the amyloid precursor protein (APP) catalyzed by γ -secretase result in the release of amyloid- β (A β) peptides of variable length. A β peptides with 42 amino acids appear to be the key pathogenic species in Alzheimer's disease, as they are believed to initiate neuronal degeneration. Sulindac sulfide, which is known as a potent γ -secretase modulator (GSM), selectively reduces A β 42 production in favor of shorter A β species, such as A β 38. By studying APP-TMS dimerization we previously showed that an attenuated interaction similarly decreased A β 42 levels and concomitantly increased A β 38 levels. However, the precise molecular mechanism by which GSMs modulate A β production is still unclear. In this study, using a reporter gene-based dimerization assay, we found that APP-TMS dimers are destabilized by sulindac sulfide and related A β 42-lowering compounds in a concentration-dependent manner. By surface plasmon resonance analysis and NMR spectroscopy, we show that sulindac sulfide and novel sulindac-derived compounds directly bind to the A β sequence. Strikingly, the attenuated APP-TMS interaction by GSMs correlated strongly with A β 42-lowering activity and binding strength to the A β sequence. Molecular docking analyses suggest that certain GSMs bind to the GxxxG dimerization motif in the APP-TMS. We conclude that these GSMs decrease A β 42 levels by modulating APP-TMS interactions. This effect specifically emphasizes the importance of the dimeric APP-TMS as a promising drug target in Alzheimer's disease.

Amyloid- β (A β) peptides are produced by a sequential cleavage process involving β - and γ -secretases (1). The most prevalent A β species generated during amyloid precursor protein (APP) processing are the intermediate products A β 40 and A β 42 when A β 48 and A β 49 are converted into smaller A β fragments by a successive release of tri- and tetrapeptides upon stepwise γ -secretase cleavages (2). Indeed, γ -secretase generates a range of A β peptides with variable C termini and the length of A β peptides is critical for the pathogenesis of Alzheimer's disease because the toxic 42-residue isoform causes neurodegeneration that underlies the decline of cognitive functions (3).

Previous attempts to understand γ -secretase cleavage specificity have unraveled that processing of the APP C-terminal fragments (β -CTF) is influenced by the GxxxG dimerization motif of the APP transmembrane sequence (TMS) (4, 5). β -CTF is the only known γ -secretase substrate comprising a GxxxG motif in triplicate, thus making dimerization of the APP-TMS unique (6). Mutational analysis revealed that a disruption of the dimer interface by glycine to alanine mutations at positions 29 and 33 of the A β sequence gradually attenuated the TMS-dimerization strength and diminished the formation of A β 42 in favor of A β 38, whereas A β 40 levels remained largely unaffected (5). In addition, a subset of nonsteroidal antiinflammatory drugs (NSAIDs), including indomethacin and sulindac sulfide, effectively modulate

the production of A β peptides in vitro and in vivo (7). Such compounds, renamed γ -secretase modulators (GSMs), either selectively reduced A β 42 production with a concomitant increase in A β 38 levels or vice versa (8).

The fact that GSMs were found to be active even in cell-free γ -secretase assays raised the possibility that these compounds might alter the γ -site cleavage by directly modifying the conformation of the γ -secretase complex. In particular, presenilin-1 (PS1) was suggested as a candidate molecule to be affected by an allosteric mechanism (9, 10). Other reports assume that GSMs can interact with cellular membranes and alter biophysical properties of the lipid bilayer (11–13). Evidence was also provided that GSMs target the enzyme's substrate when GSM photoprobes labeled APP (14). However, recent NMR results have questioned specific APP binding sites of GSMs (15). Thus, the precise molecular mechanism by which GSMs modulate A β formation is still unclear and the targets will have to be characterized.

In this study, we asked whether a subgroup of GSMs might affect A β 42 generation by binding to the A β sequence and if these GSMs can attenuate APP-TMS dimerization. We show that sulindac sulfide and derived compounds directly bind to A β 42 peptides. Molecular docking experiments suggest that the groove composed of alternating GxxxG glycine residues forms an ideal contact site in an α -helical APP-TMS conformation. In addition, we have found that APP-TMS dimers are destabilized by sulindac sulfide and related A β 42-lowering compounds in a concentration-dependent manner, whereas sulindac and its sulfone derivative that lack A β 42-lowering activity neither bind to the A β sequence nor reduce APP-TMS-dimerization strength. Our data strongly indicate that certain GSMs may act through an inhibition of helix-helix interactions of membrane-spanning A β segments.

Results

Analysis of GSM-A β Interaction by Surface Plasmon Resonance (SPR)

We examined the direct interactions of two A β 42-lowering compounds, indomethacin and sulindac sulfide, and two A β 42-inert compounds, sulindac sulfone and sulindac (Fig. 1), with the A β sequence in a lipid-free environment. Therefore, A β 42 was immobilized to the SPR sensor chip surface and compounds were

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Fig. 1. Chemical structures of sulindac sulfide (A), sulindac sulfone (B), sulindac (C), and indomethacin (D).

passed over the surfaces at indicated concentrations (Fig. 2 and Fig. S1). During sulindac sulfide injection, the response increased rapidly and returned to baseline almost immediately at the end of the injection, indicating fast association and dissociation rates (Fig. 2A). In contrast, sulindac and its sulfone derivative, which only differ from sulindac sulfide in the oxidation state of the sulfur atom, did not show significant binding to immobilized A β 42 (Fig. 2B and C). Kinetic analysis revealed an apparent binding affinity (K_{Dapp}) in the low micromolar range for sulindac sulfide assuming a 1 : 1 stoichiometry, although the stoichiometry might be more complex (Fig. S2). Indomethacin, which is both structurally and pharmacologically related to sulindac sulfide, also reversibly bound to A β 42 though much more weakly (Fig. 2D), thus not permitting a thorough kinetic analysis.

Analysis of Sulindac Sulfide- $\text{A}\beta$ Interactions by NMR Spectroscopy. As sulindac sulfide was the most potent compound to bind to $\text{A}\beta_{42}$, we further analyzed this interaction by NMR spectroscopy. We recorded a solution-state two-dimensional ^1H , ^{15}N correlation spectrum of $\text{A}\beta_{42}$ in the presence and absence of sulindac sulfide (Fig. 3). A change of the chemical shift is indicative for a change in the chemical environment of the peptide backbone, and thus indicates binding. We found resonances of amino acid residues I32, L34, M35, and V39 to be shifted upon ligand binding but not resonances of V40, I41, and A42. In the presence of sulindac sulfide, glycine and serine residues displayed decreased intensities, which is probably due to chemical exchange broadening affecting predominantly C-terminal glycine residues. This result shows that sulindac sulfide preferentially interacts with the $\text{A}\beta$ C terminus in aqueous buffers, further supporting the specificity of this interaction.

Effects of GSMs on APP-TMS Dimerization. For analyzing the ability of selected compounds to affect APP-TMS dimerization, we applied the well-established ToxR system using the APP-TMS residues 29–42 according to A β numbering (Fig. 4A) (5, 16). Briefly, this assay allows monitoring of homophilic interactions of short α -helical TMSs in bacterial membranes, as dimerization of the TMS under study is required for transcription activation of the reporter gene β -galactosidase (β -Gal) (16). Dimerization stability of the APP-TMS was gradually reduced by sulindac sulfide, but not by sulindac sulfone or sulindac (Fig. 4 B–D and

Fig. 2. Interaction of compounds with A β 42 determined by SPR analysis. Overlays of representative SPR sensorgrams obtained from injections of sulindac sulfide (A), sulindac sulfone (B), sulindac (C), or indomethacin (D). Synthetic A β 42 peptide was immobilized yielding 1,700 response units (RUs). Compounds at indicated concentrations were injected for 60 s at a flow rate of 30 μ L/min. All binding curves were double-reference subtracted from DMSO buffer blank and the reference flow cell and adjusted to the molecular weight of the compounds.

Fig. S3). Only the highest concentration of sulindac reduced APP-TMS interactions (Fig. 4D), which can presumably be attributed to small amounts of sulindac sulfide generated by the activity of *Escherichia coli* methionine sulfoxide reductases (17). No effect on dimerization was observed for sulindac sulfone (Fig. 4C) because this compound cannot be metabolized to sulindac sulfide. These findings emphasize the specificity of the dimerization-reducing activity of sulindac sulfide. Attenuated dimerization strength was also detected in the presence of indomethacin (Fig. 4E), albeit to a lower extent as compared to sulindac sulfide. The dimerization strength was specifically impaired by 10% at a concentration of 2.5 μM sulindac sulfide or 20 μM indomethacin (Fig. 4 B and E). In addition, we tested the NSAID-type A β 42-lowering GSMs ibuprofen ($\text{IC}_{50} = 250 \mu\text{M}$) and flurbiprofen ($\text{IC}_{50} = 150\text{--}200 \mu\text{M}$) (18) and found a similar propensity to diminish dimerization for both compounds (Fig. S4). Thus, these data imply that the APP-TMS dimerization is significantly impaired in the presence of compounds with A β 42-lowering activity, conceivably through direct binding to the A β sequence.

Analysis of Unique Sulindac Derivatives. To unravel critical substituents for enhanced compound potency, unique sulindac derivatives were synthesized and grouped in pairs with identical substituents in their R¹ position (Fig. 5 *A* and *B*) (19). These substances were analyzed for Aβ₄₂-lowering activity in a sandwich enzyme-linked immunosorbent assay (ELISA) (Fig. 5*C*) as described before (20). From each pair, one compound was

Fig. 3. Interaction between A β 42 and sulindac sulfide monitored by solution-state NMR spectroscopy. two-dimensional ^1H , ^{15}N correlation spectrum obtained for a 100 μM solution of A β 42 in the presence and absence of sulindac sulfide. Chemical shifts are highlighted by dashed circles.

found to be active with similar or increased A β 42-lowering potency, whereas the other compound was inactive, as compared to sulindac sulfide (Fig. 5C). Compound (g) increased A β 42, however, this effect was not significant. Similar to the effects obtained with 60 μM sulindac sulfide, most derivatives did not alter A β 40 secretion (7), except compound (e), which increased A β 40. In addition, treatment with compound (f), which showed the strongest A β 42-lowering activity, also reduced A β 40 levels. When analyzing A β 38 levels (Fig. S5F), high amounts of A β 38 were observed upon treatment with (b), (d), (f), or (h), indicating an inverse relationship between A β 42 and A β 38. Compound (f) led to only moderately elevated A β 38 levels. A possible explanation may be given by GxxxG mutations, which we have previously analyzed and found to attenuate APP-TMS dimerization (5). Comparably to compound (f), the mutation G33I reduced A β 42 as well as A β 40 production, whereas A β 38 levels were only slightly elevated.

Next, we addressed whether these compounds could bind to the A β region (Fig. 5D and Fig. S5A–E) and attenuate APP-TMS interaction (Fig. 5E and Fig. S5G and H). To investigate a direct interaction with A β 42 by SPR, sulindac derivatives were injected at 40 μM . Four substances showed reduced binding activity compared to sulindac sulfide, i.e., (a), (b), (e), and (g); two bound equally well, (c) and (d); and two compounds showed enhanced binding activity, (f) and (h) (Fig. 5D and Fig. S5A–E). The same substances were used in the ToxR assay and compared to the data obtained with sulindac sulfide at a concentration of 10 μM . Most interestingly, APP-TMS dimerization was diminished by three compounds that were shown to lower A β 42, i.e., (d), (f), and (h), and especially (f) was found to be a potent agent (Fig. 5E). In contrast, (a), (c), (e), and (g) had no effect on TMS dimerization and did not lower A β 42 levels. Compound (b) showed a lesser effect although it diminished A β 42 levels. This reducing agent extends to the binding assay, in which (b) exhibited reduced binding compared to sulindac sulfide. Overall, these unique sulindac-related compounds showed gradual effects with

Fig. 4. Dimerization of the APP-TMS in the presence of compounds. (A) A β residues 23–54. The TMS is highlighted by the light gray box. Glycine residues of the GxxxG motifs are shown in dark gray. The indicated sequence A β 29–42 comprises the amino acids which were inserted into the ToxR fusion protein. (B–E) ToxR assays. Dimerization in the presence of sulindac sulfide (B), sulindac sulfone (C), sulindac (D), and indomethacin (E) measured as β -Gal activity. DMSO control was set as 100% (mean $\pm$ SEM, $n = 8$ –15). Asterisks indicate significant difference from DMSO control (* $P < 0.0001$, Student's t test).

regard to diminishing A β 42 generation, binding to the A β 42 sequence, and attenuating APP-TMS dimerization. Compound (d) was similar, and compounds (f) and (h) were superior to sulindac sulfide considering these three aspects. Accordingly, we can conclude that the R^2 substituent has a predominant impact on the compounds' activity. Although this position can accommodate large substituents, they should be ideally of electron-withdrawing nature. The R^1 position appeared to be very sensitive to the lipophilicity of the substituent, as depicted by the loss of activity with sulindac sulfone. Grossly, these data indicate a consistent tendency between A β 42 binding, APP-TMS dimerization, and A β production, especially in consideration that diverse experimental approaches were applied.

Molecular Modeling of GSMs Binding to A β 42. We used flexible docking to determine the binding sites of the four compounds sulindac sulfide, sulindac sulfone, sulindac, and indomethacin in the APP-TMS and to map the most likely conformation of these complexes (Fig. 6 and Figs. S6 and S7). Because the TMS is most likely

Fig. 5. Sulindac derivatives and their effects on A β production, binding to A β 42, and APP-TMS dimerization. (A) Unique scaffold from which sulindac-derived agents (A–H) have been evolved. R¹, R²: substituents. (B) Overview of unique sulindac derivatives. Structural features are listed. Compounds with A β 42-lowering activity are classified as active, otherwise as inactive. (C) ELISA: A β 40 and A β 42 levels detected upon treatment of CHO cells with 60 μ M compound. DMSO-treated cells served as control (set as 100%, mean $\pm$ SEM, $n = 3$, * $P < 0.05$, Student's t test). Sixty micromolar sulindac sulfide reduced A β 42 by about 60% (gray line) without affecting A β 40 levels (dashed black line) (7). (D) SPR analysis: Compounds at 40 μ M were injected, the association phase was monitored for 30 s. Double-reference subtracted sensorgrams were adjusted to the respective molecular weight of the compounds. Response unit (RU) values at the end of the injection were displayed as percentage of RUs obtained upon injections of 40 μ M sulindac sulfide in the same run (set as 100%, indicated by the horizontal gray line, mean $\pm$ SEM, $n = 3$, * $P < 0.01$, Student's t test). For sensorgrams, see Fig. S5. (E) ToxR assay: Compounds were tested at 10 μ M. At this concentration, sulindac sulfide reduced dimerization strength by 33% as marked by the horizontal gray line. DMSO control was set as 100% (mean $\pm$ SEM, $n = 3$, * $P < 0.01$, Student's t test).

α -helical in the native membrane environment in the ToxR assay, the helical section from E23 to V45 of one APP-TMS was scanned with the docking application GOLD. Even though the docking site was not restricted to one specific face of the helix, all compounds cluster along the flat surface built up by the four glycine residues G25, G29, G33, and G37 of the GxxxG motif, exactly at the proposed dimer interface (Fig. 6). In general, these compounds are very flat and rigid, due to their aromatic character and the conjugated π -electron system along the backbone atoms and are therefore quite prone to binding to flat protein surfaces. Furthermore, the compounds are approximately 10–12.5 Å in length thereby fitting exactly between the amide groups of two adjacent glycine residues. Moreover, π -interactions between the aromatic molecules and the electrostatic surface of the APP-TMS may enhance binding affinity.

To evaluate the binding affinity of the four compounds, the geometrical fit, π -interactions, and hydrogen bonds between the compounds and the APP-TMS must be taken into account. The carboxylic acid terminus of sulindac sulfide (Fig. 6A) and sulindac (Fig. 6C) are found to be hydrogen bonded to the backbone amide group of G29 or to the amino group of K28. However, repulsive forces between sulindac's sulfoxide group and the acyl group of G33 may account for the weak binding affinity to the APP-TMS. Sulindac sulfone (Fig. 6B) seems not to be involved in any hydrogen bonding network also attributable to a possible repulsion by the sulfone group. The predicted complex with indomethacin (Fig. 6D) follows this scheme, though indomethacin is bound in a different orientation as compared to the other compounds. The carboxylic terminus of indomethacin is found to be hydrogen bonded to the backbone amide group of G33 and the hydrophobic group at the opposed end avoids

repulsive forces. Taken together, the polarity of the aromatic substituents and their hydrogen bonding capacity appear to strongly influence the binding affinity. Thus, the flexible docking supports our experimental data.

Fig. 6. Sulindac sulfide (A), sulindac sulfone (B), sulindac (C), and indomethacin (D) flexibly docked to the APP-TMS. All compounds cluster at the smooth surface provided by glycines arranged in GxxxG motifs. Oxygen is depicted in red, nitrogen in blue, sulfur in dark yellow, fluorine in deep purple, and chlorine in lime green. Potential hydrogen bonds are indicated as black dashed lines.

Discussion

There is accumulating evidence that NSAIDs of a new subclass, called GSMs, including indomethacin and sulindac sulfide, are able to specifically modulate the A β peptide length during APP processing and to selectively lower the level of neurotoxic A β 42 (2, 7). However, the underlying mechanisms of GSM activities are still unclear.

We found a distinct interaction of sulindac sulfide and indomethacin with the peptide backbone of A β 42 peptides, whereas neither sulindac nor sulindac sulfone showed specific binding. The binding affinity for sulindac sulfide was found to be in the micromolar range, which is in line with results obtained from cell culture assays where an effective A β 42-lowering activity was observed with concentrations of 25–50 μ M (18). Our thorough SPR analysis indicated an increasing stoichiometry between sulindac sulfide and A β 42, ranging from 0.2:1 to 2:1 (Fig. S2), pointing to an extended binding site, as illustrated by the overlay of different clusters of the molecular docking (Figs. S6 and S7). The occurrence of A β 42 oligomers on the chip surface is unlikely because, under acidic conditions used in the immobilization procedure, A β 42 is mainly monomeric (Fig. S1).

Recently, we and others have shown that APP forms dimers via at least three dimerization sites: two different sites in the ectodomain (21, 22) and a third dimerization site formed by three consecutive GxxxG motifs as part of the APP-TMS (5). Here, we found that sulindac sulfide and derived compounds are able to reduce helix–helix interaction of the third dimerization site in a native bacterial membrane. Thus, these dimer-affecting compounds need to have the capacity to enter the lipid bilayer, which was indeed shown for sulindac sulfide and indomethacin but not for sulindac or sulindac sulfone (11, 12). The structures of sulindac sulfide and its two metabolites only differ in the oxidation state of the sulfur atom, assuming that the strong interaction of sulindac sulfide with membranes may be attributed to its smaller dipole moment (dipole moments of sulindac sulfide, sulindac sulfone, and sulindac are 1.50, 4.49, and 3.96, respectively) (11, 12).

Importantly, a slightly attenuated dimerization had a tremendous effect on A β 42 generation that we could show earlier for the GxxxG mutants G29A and G33A, which caused a 7% and 23% dimer reduction, respectively, and lowered A β 42 levels by approximately 60%. In this respect, the effects of 20 μ M indomethacin and 2.5 μ M sulindac sulfide can be directly compared to GxxxG mutants G29A and G33A (5, 7). From previous NMR measurements with purified and monodisperse C99 in lyso-myristoylphosphatidylglycerol micelles, the authors have concluded that interactions with sulindac sulfide are of nonspecific nature (15), which is seemingly in contradiction with our results obtained from the ToxR assay. Here, in a natural lipid bilayer, A β 42-lowering compounds affected APP-TMS dimerization, although we cannot specify whether existing dimers are disrupted or if binding to monomers inhibits the reassociation and the dimer formation. Like any other noncovalent TMS association, the assembly of APP-TMS helices is believed to occur in a dynamic equilibrium of monomers and dimers within the bacterial membrane. Accordingly, our data suggest that the APP-TMS helix–helix interaction is competed for by a helix–compound interaction. GSM binding may shift the monomer-dimer equilibrium toward the monomeric state and thus affects APP processing as shown previously (5, 7). It is also tempting to speculate that there is a unifying mechanism explaining the striking correlation between the attenuated APP-TMS interaction, A β 42-lowering activity, and binding strength to the A β sequence.

Why does sulindac sulfide bind to the APP-TMS but not sulindac sulfone or sulindac? Our model is taking into consideration all low-energy conformations of the complex (Figs. S6 and S7) and is based on the assumption that GSMs bind to monomeric A β in aqueous solutions (BIACORE and NMR data, see Figs. 2 and 3) or affect APP-TMS dimerization in an α -helical

conformation (ToxR data, see Fig. 5). According to this model, an ideal compound to modulate A β generation would have to be hydrophobic enough to enter the membrane and possess the substituents and hydrogen bonding capacity to build up stable complexes with the A β sequence within the APP-TMS. These requirements are fulfilled by sulindac sulfide having a rigid flat geometry and an optimal size to sterically accommodate to the GxxxG motifs, with substituents permitting hydrogen bonds and avoiding repulsions. Collectively, our data imply that sulindac sulfide and derived compounds lower the A β 42 production through interaction with the APP-TMS and modulation of TMS-dimerization.

Similar to APP, numerous other γ -secretase substrates dimerize via their TMS, e.g., E-cadherin (23), ErbB4 (24), or Notch (25), and it is reasonable to speculate that certain GSMs may similarly modulate processing of those substrates. Indeed, sulindac sulfide and indomethacin decreased the relative level of released Notch-1 A β -like peptide species, i.e., N β 25, without changing the total N β level (26). Thus, we assume that binding to the substrate is determined by conformational characteristics like an α -helical flat protein surface, rather than by a specific primary sequence. In any case, if processing of other γ -secretase substrates is affected by GSMs, signaling via the intracellular domains might not be impaired as the ϵ -cleavage still occurred in the presence of those compounds (7, 9, 27).

From 12 sulindac-derived compounds analyzed in this study, only one compound, i.e., (b), diminished A β 42 levels but was a relatively weak binder and showed almost no effect on dimerization. At least, this finding shows that, besides substrate targeting of GSMs, other mechanisms may exist. However, the APP-TMS might be especially attractive for GSMs because APP is the only γ -secretase substrate with three consecutive GxxxG motifs providing an extended groove suitable for binding. In conclusion, our results suggest a unique strategy including a high-throughput screen for the identification and for the development of new compounds with optimized pharmacological characteristics superior to well-known GSMs.

Materials and Methods

Compounds and Peptides. Standard compounds were purchased from Sigma-Aldrich. For the synthesis of sulindac analogues, the structure of sulindac as guiding scaffold has been chemically modified by solid phase synthesis (19, 28). For peptide synthesis, see refs. 21 and 29. A β 42 was monomerized as described before (30, 31). Briefly, A β 42 was dissolved in 98% formic acid. After immediate evaporation of the solvent the peptide was stored at -20°C .

Surface Plasmon Resonance Analysis. SPR experiments were performed using a BIACORE 3000 instrument and CM5 sensor chips (GE Healthcare) as described previously (31). In brief, PBS was used as running buffer at 25°C . Monomerized A β 42 peptide was dissolved in 0.12% ammonia to 1 mg/mL, diluted 1:10 with 10 mM sodium acetate (pH 3.4), and subsequently immobilized to the sensor chip surface by amine coupling (30, 31). Compounds were diluted from DMSO stock solutions in running buffer and injected for at least 1 min at a flow rate of 30 μ L/min using the KINJECT command. After the dissociation phase the chip was rinsed with 20 mM HCl. Corresponding DMSO dilutions were used as buffer blank. The W0-2 antibody (Genetics Company) diluted in PBS (3.4 μ g/mL) was injected before and after sample runs to control the integrity of the A β 42 surface. For more information see [SI Text](#).

NMR Spectroscopy. NMR measurements were carried out on a Bruker 600-MHz AVANCE NMR spectrometer equipped with a triple channel cryoprobe at 5°C . Monomerized A β 42 peptide was dissolved in 20 mM NaOH, diluted with 50 mM phosphate buffer (pH 7.0) to approximately 100 μ M, and measured immediately in the absence or presence of sulindac sulfide (3-fold molar excess).

ToxR Assay. The TMS under study was fused between the ToxR transcription activator and the maltose binding protein as described before (5, 16). One milliliter cultures were inoculated in quadruplicate with precultures of transformed *E. coli* FHK12 cells and grown in 24-well dishes in the presence of compounds or DMSO for 6 h at 37°C . Ten micrograms per milliliter Polymyxin

B nonapeptide (Sigma-Aldrich) was added to achieve permeabilization of the bacterial outer membrane (32). Culture aliquots were lysed, the β -Gal substrate orthonitrophenyl- β -D-galactopyranoside (Sigma-Aldrich) was added and products were measured at 405 nm using a microplate reader (Anthos HT2, Anthos). Values were normalized to the cell density measured at 620 nm (OD₆₂₀).

Cell Lines and A β Detection by ELISA. CHO cells stably overexpressing wild-type human APP751 and wild-type human PS1 were cultured as described previously (20). Cells were treated with compounds at 60 μ M or DMSO for 24 h. Aliquots of medium (10 μ L for detection of A β 40, 100 μ L for A β 42) were analyzed for secreted A β peptides by ELISA using the monoclonal A β antibody IC16 as capture antibody and HRP-labeled antibodies against A β 40 (BAP-24) or A β 42 (BAP-15) (33) for detection. Statistics were performed using GraphPad Prism software (GraphPad Software).

Flexible Docking of GSMs to the APP-TMS Tertiary Structure Model. The APP-TMS model, that is based on the tertiary structure of glycophorin A was used from our previous analyses (5). Tertiary structures of compounds were taken from the PubChem structural database and were cleaned with DS viewer (Accelrys). All side chains from E23 to V45 of the APP-TMS model and

compounds were rendered flexible during the docking process. We used *GoldScore* (search efficiency: 100%, no additional constraints) as the scoring function. The function (and final ranking of the ligand poses) is based on (i) protein-ligand hydrogen bond energy, (ii) protein-ligand van der Waals energy, (iii) ligand internal van der Waals energy, and (iv) ligand torsional strain energy. The final complex structures were selected from the top 10 lists ranking the energetically most plausible docking modes of each run. All figures were produced with PyMOL Molecular Graphics System.

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