Antiepileptic Drug Carbamazepine Binds to a Novel Pocket on the Wnt Receptor Frizzled-8

Yuguang Zhao,* Jingshan Ren, James Hillier, Weixian Lu, and E. Yvonne Jones*

ABSTRACT: Misregulation of Wnt signaling is common in human cancer. The development of small molecule inhibitors against the Wnt receptor, frizzled (FZD), may have potential in cancer therapy. During small molecule screens, we observed binding of carbamazepine to the cysteine-rich domain (CRD) of the Wnt receptor FZD8 using surface plasmon resonance (SPR). Cellular functional assays demonstrated that carbamazepine can suppress FZD8-mediated Wnt/β-catenin signaling. We determined the crystal structure of the complex at 1.7 Å resolution, which reveals that carbamazepine binds at a novel pocket on the FZD8 CRD. The unique residue Tyr52 discriminates FZD8 from the closely related FZD5 and other FZDs for carbamazepine binding. The first small molecule-bound FZD structure provides a basis for anti-FZD drug development. Furthermore, the observed carbamazepine-mediated Wnt signaling inhibition may help to explain the phenomenon of bone loss and increased adipogenesis in some patients during long-term carbamazepine treatment.

INTRODUCTION

Ligands belonging to the Wnt family of secreted lipoproteins play central roles in tissue morphogenesis and homeostasis through binding to members of the frizzled (FZD) family of cell surface receptors. Overexpression of FZD proteins has been observed in cancers, and FZD8 has been proposed as a therapeutic target in human lung cancer and renal cell carcinoma. The anti-FZD antibodies vantictumab (OMP-18R5), IgG-2919, and IgG-2921 have been taken into phase 1 clinical trials (ClinicalTrials.gov: NCT01345201) or preclinical trials for targeting speciﬁc FZDs for carbamazepine binding. The first small molecule-bound FZD structure has provided the author and source are cited. This is an open access article published under a Creative Commons Attribution (CC-BY) License, which permits unrestricted use, distribution and reproduction in any medium, provided the author and source are cited.

Results

Screening of Small Molecules for Binding to FZD8CRD. Wnt ligands bind to the FZD receptor CRD to initiate Wnt signaling. Small molecule antagonists that bind to the FZD CRD could therefore have therapeutic potential in cancers with upregulated Wnt signaling. We used SPR to screen for small molecules that bind to FZD8CRD. Small molecules from the Maybridge fragments library (58 compounds) and our own laboratory collection (44 compounds) were screened. All of the compounds screened are listed in Supporting Information, Table S1. Initial screening identified three hits showing SPR responses (Figure 1): carbamazepine, kahweol, and quinacrine (number 79, 87, and 99 in Supporting Information, Table S1). Further analysis of these hits with other irrelevant protein controls eliminated quinacrine as a nonspecific binder. Kahweol and carbamazepine were then carried forward to guided drug design. To date, no structural information about small molecules binding to FZD receptors has been published. We have used surface plasmon resonance (SPR) to screen a set of small molecules and report here the high-resolution crystal structure of the extracellular FZD8 cysteine-rich domain (FZD8CRD) bound with carbamazepine.
co-crystallization with FZD8_{CRD}. While kahweol did not yield a complex structure, the structure of carbamazepine in complex with FZD8_{CRD} was determined (Figure 2). In the absence of further evidence to support kahweol binding, we focused all further work on carbamazepine.

**Structure of FZD8_{CRD} and Its Complex with Carbamazepine.** The amino acid sequence of FZD8_{CRD} is fully conserved between human and mouse. Therefore, although we used a mouse cDNA sequence, the resulting structure is identical to human FZD8_{CRD}. Thus, we refer to it simply as FZD8_{CRD} hereafter. The apo FZD8_{CRD} structure was determined at 2.3 Å resolution in space group P2₁, with four molecules in the asymmetric unit (ASU, Figure 2A). The carbamazepine complex structure (1.7 Å resolution) crystallized in the same condition (see the Experimental Section) with similar lattice contacts to the apo structure, but with higher crystallographic symmetry resulting in space group P2₁₂₁₂₁, with two molecules in each ASU (Figure 2B).

The overall structure of FZD8_{CRD}, either apo or in complex with carbamazepine, is almost identical to those of previously reported apo or Wnt-bound FZD8_{CRD} except at the CRD C-terminus, which forms a β-hairpin with the remaining residues of a Rhinovirus 3C protease cleavage site (used to remove purification tags; Figure 2C and the Experimental Section). In both our structures, the ASUs contain dimers resulting from two-fold noncrystallographic symmetry (Figure 2A,B). This dimeric arrangement, as well as the other lattice packing interactions, differs from the distinctive dimer, mediated by unsaturated fatty acyl–FZD CRD binding, that has been observed for a number of FZD CRD structures, including FZD8.15,17–19

In the apo structure, the four molecules of FZD8_{CRD} in the ASU fall into two conformations (“a” and “b”, Figure 2D) regarding loop_6 (residues R137 to L147). However, both copies of the carbamazepine-bound FZD8_{CRD} are in conformation “b” (Figure 2D). This suggests that carbamazepine may stabilize conformation “b” upon binding. The Xenopus Wnt8-15 or human Wnt3-16 bound FZD8_{CRD} loop_6 corresponds most closely to conformation “a” (Figure 3E). This suggests that Wnt binding may prefer the loop_6 conformation “a.”

**Carbamazepine Binds FZD8_{CRD} at a Novel Pocket.** Carbamazepine (SH-dibenzo[b,f]azepine-5-carboxamide), sold under the trade names Tegretol, Equetro, Carbatrol, Epitol, and Orteril, is a tricyclic compound (Figure 3A). The 1.7 Å resolution complex structure unambiguously showed carbamazepine binding to the FZD8_{CRD}. The simulated annealing omit electron density map showed clear electron density for all atoms of carbamazepine (Figure 3B) and both molecules in the ASU show similar quality density. FZD8_{CRD} possesses two well-documented Wnt binding sites: a hydrophobic groove that binds the palmitoleic acid moiety (PAM) that is appended to the Wnt thumb (site 1) and the Wnt index finger binding site (site 2).15,16 The carbamazepine binding pocket is sandwiched by the two Wnt binding sites (Figures 3C and 4A). This pocket is largely hydrophobic and neutral in surface charge but is surrounded by positively and negatively charged patches (Figure 3D). Aside from Wnt, there are many FZD CRD binding proteins that have been reported, but none of them bind at this pocket. The Wnt mimic Norrin protein
binds at site 2 on FZD4CRD (Figure 4A), while all other reported binders bind at or near site 1. These include the Clostridium difficile toxin B (TcdB22) that binds FZD2CRD (Figure 4B), the Wnt surrogate B12 module that binds FZD8CRD and the peptide FZ7-21 that binds FZD7CRD (Figure 4C), the DARPin module DRPB that binds FZD8CRD and the antibody Fab F2.I that binds FZD5CRD (Figure 4D). Carbamazepine does not overlap with any of the reported FZD CRD ligands when the various structures are superimposed (Figure 4) and instead binds to residues located between the two Wnt binding sites in a novel binding pocket (Figures 3 and 4).

The carbamazepine binding pocket comprises residues from helix α3 (S90, M91), loop_2 (Y52, Q56), loop_4 (P94, P103, L104, P105, P106), and loop_6 (R137). The residues L104 and R137 use their main chain atoms to interact with carbamazepine, while the other interacting residues use their side chains (Figure 5A). A distinctive feature of this pocket is a cluster of four hydrophobic prolines. The interacting residues from the two copies of FZD8CRD in the crystallographic ASU ("a" and "b") show similar interactions with carbamazepine, except for the residue Q56. Q56 of molecule "a" interacts with a carbamazepine nitrogen (N17) atom and azepine ring (C7, Figure 5B), while in the other copy, "b", Q56 interacts with the carbamazepine azepine and benzyl rings (Figure 5C). The difference in the Q56 side-chain conformation may be due to crystal packing, as this area of molecule "a" contacts a neighbor packing molecule. When two representative copies of apo FZD8CRD were superimposed, no overlap was detected with any of the reported FZD CRD ligands.
structures are aligned with carbamazepine-bound structures, the Q56 side chain from the apo structure would sterically hinder carbamazepine binding (Figure 5D). This suggests that Q56 may undergo a conformational change upon carbamazepine binding. The carbamazepine—protein interactions are mainly hydrophobic, especially those involving P105 and P103, which form extensive hydrophobic interactions with the azepine ring and two benzyl rings of carbamazepine (Figure 5E). Y52 forms a hydrogen bond through a water molecule to the nitrogen (N17) of the carboxamide head of carbamazepine (Figure 5A). There are three additional ordered water molecules within the pocket that are conserved between the two molecules in the ASU. The positions of water molecules could guide carbamazepine modification to develop more potent FZD8 inhibitors.

**Binding Specificity and Affinity of Carbamazepine.** In addition to the 10 FZDs, the human genome also encodes 5 secreted FZD-related proteins (sFRPs), important Wnt regulatory proteins,26 and all FZDs and sFRPs possess a conserved FZD CRD. Sequence alignment of all FZD and sFRP CRDs shows that only one proline residue (P94) is fully conserved among the residues interacting with carbamazepine (Figure 5F). Another proline, P106, is conserved in all FZDs/sFRPs, except for FZD9/10. The residue S90 is highly conserved but absent in FZD3/6 and sFRP3/4. Other carbamazepine-interacting residues are only partially conserved among FZDs and sFRPs. FZD8 and FZD5 are closely related (>80% sequence identity within the CRD), sharing most of the carbamazepine-interacting residues, including Q56, which is unique to these FZDs. However, the residue Y52 is found only in FZD8 (Figure 5F). The sequence alignment suggests that of all the other FZDs/sFRPs, FZD8 is the most likely candidate to bind to carbamazepine, followed by FZD1, 2, and 7.

We then used biophysical methods to measure the affinity of the interaction between carbamazepine and FZD8CRD, as well as the cross-reactivity of carbamazepine with the CRDs of FZD5 and 7. We have previously used a thermal shift assay (also known as differential scanning fluorimetry) to investigate small molecule—protein interactions.27,28 However, we found that FZD CRDs are highly thermostable (remaining folded at 95 °C), which precluded the measurement of melting curves. We therefore turned to SPR as an alternative method for the detection of small molecule—protein interactions.29,30 All three (FZD8, 5, and 7) CRD constructs yield correctly folded protein samples as evidenced by their gel filtration profiles (Supporting Information, Figure S1). Biotinylated samples of protein samples as evidenced by their gel filtration profiles (Supporting Information, Figure S1) and the HEK293T TOPFlash response to canonical Wnt ligands is dependent mainly on FZD1, 2, and 7.31 To observe the specific response from FZD8, we therefore used an FZD1, 2 and 7 knockout HEK293T cell line31 and introduced the full-length mouse FZD8 expression cassette by lentiviral transduction.32 The cell line was further stably transformed with a T-cell factor/lymphoid enhancer-binding factor 1 luciferase (TCF/LEF, TOPFlash) plasmid to minimize reporter plasmid transfection variations. The cells were then stimulated with a conditioned medium from mouse L-cells expressing Wnt3a, in the presence of a carbamazepine concentration series (Figure 7). Carbamazepine starts to inhibit Wnt3a-induced TOPFlash luciferase activity at a concentration of 8 μM (unpaired t-test, P < 0.0001), with greater inhibition seen at higher concentrations. However, we found that carbamazepine can only partially suppress Wnt3a-induced luciferase activity even at the high concentration of 64 μM, luciferase activity remained around 60% (Figure 7).

**DISCUSSION AND CONCLUSIONS**

FZD proteins, as essential Wnt receptors, are a central point for Wnt signaling intervention in diseases such as cancer. While macromolecules like antibodies, peptides, the FZD5/8-binding B12 protein, and DARPin molecules targeting FZD CRDs have been described,8,18,23,24 small molecules may offer advantages such as being easier to manufacture, more stable, less expensive, and having the potential to be administered orally. Among the methods of screening small molecules for binding to target proteins, SPR provides a measure of direct molecular interactions, allowing an effective triage for candidates with the highest potential for generating complex crystal structures. Structural information forms the basis for rational drug design. We have obtained the first FZD-small molecule structure and discovered that carbamazepine specifically binds to the FZD8CRD and not to the closely related FZD5. To date, no antibodies or synthetic FZD binders have been reported that can distinguish between FZD8 and FZD5. Conceivably, carbamazepine may offer this potential.

We noticed that the carbamazepine can only partially suppress Wnt3a-induced luciferase activity, even at a high concentration of 64 μM (Figure 7), which is in agreement with the recently reported weak Wnt inhibitory effects in mouse adipose cells.33 The binding pocket that we have identified in

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**Figure 6**. SPR analysis of carbamazepine interaction with FZD5, 7, 8 CRD. Biotinylated mouse FZD5, human FZD7, and mouse FZD8 CRD were immobilized on individual flow cells of a SA chip, respectively. A carbamazepine concentration series was used as an analyte. The SPR sensorgrams are shown in the right panels. ND, not detectable; RU, resonance units.

Carbamazepine only binds to FZD8; however, the commonly used cell line (HEK293T) expresses FZD8 at a very low level, and the HEK293T TOPFlash response to canonical Wnt ligands is dependent mainly on FZD1, 2, and 7.31 To observe the specific response from FZD8, we therefore used an FZD1, 2 and 7 knockout HEK293T cell line31 and introduced the full-length mouse FZD8 expression cassette by lentiviral transduction.32 The cell line was further stably transformed with a T-cell factor/lymphoid enhancer-binding factor 1 luciferase (TCF/LEF, TOPFlash) plasmid to minimize reporter plasmid transfection variations. The cells were then stimulated with a conditioned medium from mouse L-cells expressing Wnt3a, in the presence of a carbamazepine concentration series (Figure 7). Carbamazepine starts to inhibit Wnt3a-induced TOPFlash luciferase activity at a concentration of 8 μM (unpaired t-test, P < 0.0001), with greater inhibition seen at higher concentrations. However, we found that carbamazepine can only partially suppress Wnt3a-induced luciferase activity even at the high concentration of 64 μM, luciferase activity remained around 60% (Figure 7).
there is a significant difference.

FZD8\textsubscript{CRD} does not overlap with the known Wnt binding sites. Thus, the mechanism of action is allosteric. However, the carbamazepine binding pocket may still be important for Wnt signaling. It was previously demonstrated that the disruption of the carbamazepine binding region (insertion of a tripeptide, GSG, before the carbamazepine interacting residues Y52 or R137) abolished Wnt8 binding to FZD8.\textsuperscript{14} The carboxamazine binding affinity $K_d$ value of 17 \textmu M and starting inhibitory concentration of 8 \textmu M are comparable to the reported Wnt/\textbeta-catenin inhibitory concentration of 5–10 \textmu M in adipose cells.\textsuperscript{33} and colon cancer cells.\textsuperscript{34} Carbamazepine is the primary drug used to treat epilepsy. Typical doses are between 400 and 1000 mg per day and the carbamazepine plasma concentration in patients during treatment reaches 20–40 \textmu M,\textsuperscript{35} which is higher than the $K_d$ value and inhibitory concentration we observed.

Epilepsy is a common serious neurological disorder, affecting 1–2\% of the population (over 50 million people) worldwide.\textsuperscript{38} The etiology of epilepsy is multifactorial and the involvement of Wnt signaling misregulation has recently received attention.\textsuperscript{37} Wnt/\textbeta-catenin signaling may play a role in the development of temporal lobe epilepsy.\textsuperscript{38} Conditional knock-out of the major Wnt/\textbeta-catenin negative regulator adenomatosus polyposis coli (APC) gene in mice, which results in elevated Wnt/\textbeta-catenin signaling, causes chronic seizures\textsuperscript{39} with similar features to those seen in humans with infantile spasms, a common syndrome in childhood epilepsy.\textsuperscript{40} In a mouse model, both deletion and overexpression of \textbeta-catenin have a significant impact on seizure susceptibility.\textsuperscript{41} The kainic acid-induced epilepsy animal model shows upregulated Wnt/\textbeta-catenin signaling.\textsuperscript{42} Increased Wnt/\textbeta-catenin signaling is associated with the increased number of neuronal stimuli,\textsuperscript{43} while decreased Wnt/\textbeta-catenin signaling by the Wnt antagonist Dickkopf-related protein 1 (DKK1) has been shown to be able to protect against the development of hippocampal sclerosis, which is a hallmark of temporal lobe epilepsy.\textsuperscript{44} The antiepileptic drug carbamazepine (an inhibitor of voltage-gated sodium channels\textsuperscript{45}) has been shown to decrease Wnt/\textbeta-catenin signaling in the human colon adenocarcinoma SW480 cell line\textsuperscript{51} and mouse adipocyte 3T3-L1 cells.\textsuperscript{39} The results presented here contribute further evidence that Wnt signaling modulation may be involved in carbamazepine treatment of epilepsy. This warrants further studies of the role of Wnt-FZD8 signaling modulation in the therapeutic mechanism of carbamazepine against epilepsy.

It is noteworthy that long-term antiepileptic drug (AED) treatment can have side effects, including disorders of bone metabolism leading to bone loss.\textsuperscript{46–50} The exact mechanism of this pathological change is not completely understood. It is commonly accepted that intact Wnt signaling is essential for proper bone formation and remodeling.\textsuperscript{51} The strength and integrity of the human skeleton depend on a delicate equilibrium between bone resorption by osteoclasts and bone formation by osteoblasts. Wnt/\textbeta-catenin signaling can inhibit osteoclastogenesis\textsuperscript{52} and increase bone mass. On the other hand, inhibition of Wnt signaling can lead to bone loss. Among the 10 FZD receptors, FZD8 and FZD9 are particularly relevant to bone metabolism. While FZD9 regulates osteoblast function through noncanonical Wnt signaling,\textsuperscript{53,54} FZD8 mediates canonical Wnt/\textbeta-catenin signaling and inhibits osteoclastogenesis.\textsuperscript{52} The discovery that carbamazepine can inhibit FZD8-mediated Wnt signaling may help to explain the loss of bone density associated with long-term treatment with carbamazepine. In addition, weight gain affecting patients treated with carbamazepine has also been linked to Wnt signaling inhibition.\textsuperscript{53} Carbamazepine use has also been shown to reduce the risk of prostate cancer,\textsuperscript{55,56} and synergistically inhibits breast cancer cell proliferation when combined with other anticancer treatment,\textsuperscript{57} although the exact mechanisms remain to be investigated.

In summary, we have identified carbamazepine as a specific ligand for the Wnt receptor FZD8 using an SPR screen of small molecules. The high-resolution crystal structure of the complex reveals a novel binding site in FZD8\textsubscript{CRD} that allows small molecule interactions to discriminate between closely related FZDs. As well as potentially explaining the loss of bone density observed in patients following long-term treatment with carbamazepine, our carbamazepine–FZD8\textsubscript{CRD} structure also provides a new avenue to explore the design and development of FZD specific inhibitors.

**EXPERIMENTAL SECTION**

**Protein Production and Crystallization.** Mouse FZD8 (UniProtKB Q61091) residues A28-R153 (identical amino acid sequence to human FZD8\textsubscript{CRD}) with glycosylation site mutations (N49E and N152E) were PCR amplified from the template cDNA (Source Bioscience, Nottingham U.K.; clone ID 40130820) and cloned into a stable cell line vector pNeo-sec\textsuperscript{58} with a Rhinovirus 3C cleavage site, a monovenus fluorescent protein, and 6xHis tags. The resulting 3C protease treated protein contains two additional amino acids (GT) from KpnI cloning site and six amino acids (LEVLFAQ) from the 3C cleavage site at the C-terminus. HEK293S GnT1– cells
were co-transfected with this plasmid and a PhiC31 integrase expression vector (pCB92/pgk-ϕC31). The polyclonal population of cells following G418 (1 mg/mL) selection was cultured in a CompacT automated cell culture system. The secreted proteins were captured by Talon beads, to remove the monoVenus and His tags, and concentrated to 10 mg/mL using the Superdex 200 16/60 column (GE Healthcare). The resulting cell line was further transformed using a Super TOPFlash plasmid (pRL-IRES-GFP sequence after the expression cassette. The resulting cell line was further transformed using a Super TOPFlash firefly luciferase expression cassette in a stable cell line, followed by blastocidin selection. The polyclonal population from the blastocidin selection (20 µg/mL−1) was seeded in a 96-well plate (104 cells/well) and transfected with a constitutive Renilla luciferase plasmid (pRL-IRES-K; Promega) at a concentration of 10 ng/mL−1 with lipofectamine 2000 (Invitrogen). Twenty-four hours after transfection, the media were replaced with carbamazepine dilution series diluted in Wnt3a conditional media from the Wnt3a producing mouse L Wnt-3A cell line (ATCC CRL-2647, ref 67). The culture medium from L-cells served as the Wnt3a control medium. The firefly and Renilla luciferase activities were measured 24 h later using the Dual-Glo luciferase reporter assay system (Promega) with an Ascent luminoskan luminometer (Labsystems). The firefly luciferase activity was normalized with the constitutive Renilla luciferase activity.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.9b02020.

List of screen compounds (Table S1) and size-exclusion chromatography of FZD_CRD profiles (Figure S1) (PDF)

Analytical data (CSV)

Accession Codes

PDB codes for structures of FZD8CRD and its complex with carbamazepine are 6TFM and 6TFB, respectively. The authors would like to thank Professor Michel Boutrous (DFKZ, Germany) for the human full-length FZD8 plasmid (pHL-Avi3 vector). To produce biotinylated proteins, these plasmids were co-transfected with the pDisplay_BirA-ER plasmid into HEK293T cells with the media supplemented with 20 µM biotin. This procedure allows in vivo biotinylation to occur. The dialyzed conditioned media were directly used for the immobilization of ligands. The affinity was measured at 25 °C in 10 mM HEPES, pH 7.4, 150 mM NaCl, 0.005% Tween20, and 2% DMSO using a Biacore S200 machine (GE Healthcare). The biotinylated ligands (1000 RU each) were coupled to a SA sensor chip (GE Healthcare), and the analyte carbamazepine was tested using a two-fold serial dilution. The resulting cell line was further transformed using a Super TOPFlash firefly luciferase expression cassette in a stable cell line, followed by blastocidin selection. The polyclonal population from the blastocidin selection (20 µg/mL−1) was seeded in a 96-well plate (104 cells/well) and transfected with a constitutive Renilla luciferase plasmid (pRL-IRES-K; Promega) at a concentration of 10 ng/mL−1 with lipofectamine 2000 (Invitrogen). Twenty-four hours after transfection, the media were replaced with carbamazepine dilution series diluted in Wnt3a conditional media from the Wnt3a producing mouse L Wnt-3A cell line (ATCC CRL-2647, ref 67). The culture medium from L-cells served as the Wnt3a control medium. The firefly and Renilla luciferase activities were measured 24 h later using the Dual-Glo luciferase reporter assay system (Promega) with an Ascent luminoskan luminometer (Labsystems). The firefly luciferase activity was normalized with the constitutive Renilla luciferase activity.

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**Table 1. Data Collection and Refinement Statistics**

| Description                          | FZD8CRD | FZD8CRD-carbamazepine |
|--------------------------------------|---------|------------------------|
| data set                             | FZD8CRD| FZD8CRD-carbamazepine |
| PDB code                             | 6TFM    | 6TFB                   |
| wavelength (Å)                       | 0.916   | 0.916                  |
| space group                          | P2₁     | P2₁                    |
| unit cell dimensions (Å)             | a = 52.3, b = 66.0, c = 72.7; α = β = γ = 90° | a = 52.0, b = 68.1, c = 73.8; α = β = γ = 90° |
| resolution (Å)                       | 72.7–2.34 | 2.38–2.34             |
| unique reflections                   | 20741 (1024) | 25589 (1212)          |
| Rmerge (%)                           | 0.19 (0.83) | 0.13 (---)             |
| < I > / < σ(I)                        | 7.3 (2.3) | 9.4 (1.0)              |
| CC half                              | 0.99 (0.89) | 0.99 (0.84)           |
| completeness (%)                     | 98.9 (98.5) | 99.2 (95.3)           |
| redundancy                            | 6.5 (5.9) | 11.5 (8.5)            |

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**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.9b02020.

- List of screen compounds (Table S1) and size-exclusion chromatography of FZD_CRD profiles (Figure S1) (PDF)
- Analytical data (CSV)

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**Accession Codes**

PDB codes for structures of FZD8CRD and its complex with carbamazepine are 6TFM and 6TFB, respectively. The authors will release the atomic coordinates and experimental data upon article publication.

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Y.Z. and E.Y.J. designed the project and wrote the manuscript together with J.H. and J.R., Y.Z. performed experiments and analyzed data with J.R. and J.H. W.L. helped with tissue culture.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

FZD<sub>CRD</sub>, frizzled cysteine-rich domain; ASU, asymmetric unit; SPR, surface plasmon resonance; TCF/LEF, T-cell factor/lymphoid enhancer-binding factor 1

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