Supporting Information

Computational Investigations on the Binding Mode of Ligands for the Cannabinoid-Activated G Protein-Coupled Receptor GPR18

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Table of Contents

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4xnv        1 TGFQF-YYLPAVYILVFIIGFLGNSVAIWMFVFHMKPWSGISVYMFNLALADFLYVLTLP   59
5c1m        1 PSMVTAITIMALYSIVCVVGLFGNFLVMYVIVRYTKMKTATNIYIFNLALADALATSTLP   60
5xsz        1 DNFKYPLYSM-VFSIVFMVGLITNVAAMYIFMCSLKLRNETTTYMMNLVVSDLLFVLTLP   59
GPR18       1 --DEYKIAALVFYSCIFIIGLFVNITALWVFSCTTKKRTTVTIYMMNVALVDLIFIMTLP   58

4xnv       60 ALIFYYFNKTDWIFGDAMCKLQRFIFHVNLYGSILFLTCISAHRYSGVVYP-KSLGRLKK  118
5c1m       61 FQSVNYLMGT-WPFGNILCKIVISIDYYNMFTSIFTLCTMSVDRYIAVCHPVKALDFRTP  119
5xsz       60 LRVFYFVQQN-WPFGSLLCKLSVSLFYTNMYGSILFLTCISVDRFLAIVYPFRSRGLRTK  118
GPR18      59 FRMFYYAKDE-WPFGEYFCQILGALTVFYPSIALWLLAFISADRYMAIVQPKYAKELKNT  117

4xnv      119 KNAICISVLVWLIVVVAISPILFY-SGTGVRKNKTITCYDTTSDEYLRSYFIYSMCTTV-  176
5c1m      120 RNAKIVNVCNWILSSAIGLPVMFM--ATTKYRQGSIDCTLTFSHPTWYWENLLKICVFI-  176
5xsz      119 RNAKIVCAAVWVLVLSGSLPTGFMLNSTNKLENNSISCF------EWK-SHLSKVVIFIE  171
GPR18     118 CKAVLACVGVWIMTLTTTTPLLLLYKDPDK-DSTPATCLKISDIIYLKAVNVLNLTRLT-  175

4xnv      177 -AMFCVPLVLILGCYGLIVRALIYKEPL---------RRKSIYLVIIVLTVFAVSYIPFH  226
5c1m      177 -FAFIMPVLIITVCYGLMILRLKSVRMLSGSKEKDRNLRRITRMVLVVVAVFIVCWTPIH  235
5xsz      172 TVGFLIPLMLNVVCSAMVLQTLRRPNTVL-------NKKKILRMIIVHLFIFCFCFIPYN  224
GPR18     176 -FFFLIPLFIMIGCYLVIIHNLLHGRTSK---LKPKVKEKSIRIIITLLVQVLVCFMPFH  231

4xnv      227 VMKTMNLRARLDFQTPAMCAFNDRVYAT-YQVTRGLASLNSCVNPILYFLAGDTFRRR  283
5c1m      236 I--YVIIKALI---TIP----ETTFQTVSWHFCIALGYTNSCLNPVLYAFLDENFKRC  284
5xsz      225 V--NLVFYSLVRTNTLKGCAAESVVRTI-YPIALCIAVSNCCFDPIVYYFTSETIQNS  279
GPR18     232 I--CFAFLMLGT---------GENSYNPWGAFTTFLMNLSTCLDVILYYIVSKQFQAR  278
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Figure S1. Multiple sequence alignment of the human GPR18 and the templates chosen for homology modeling.

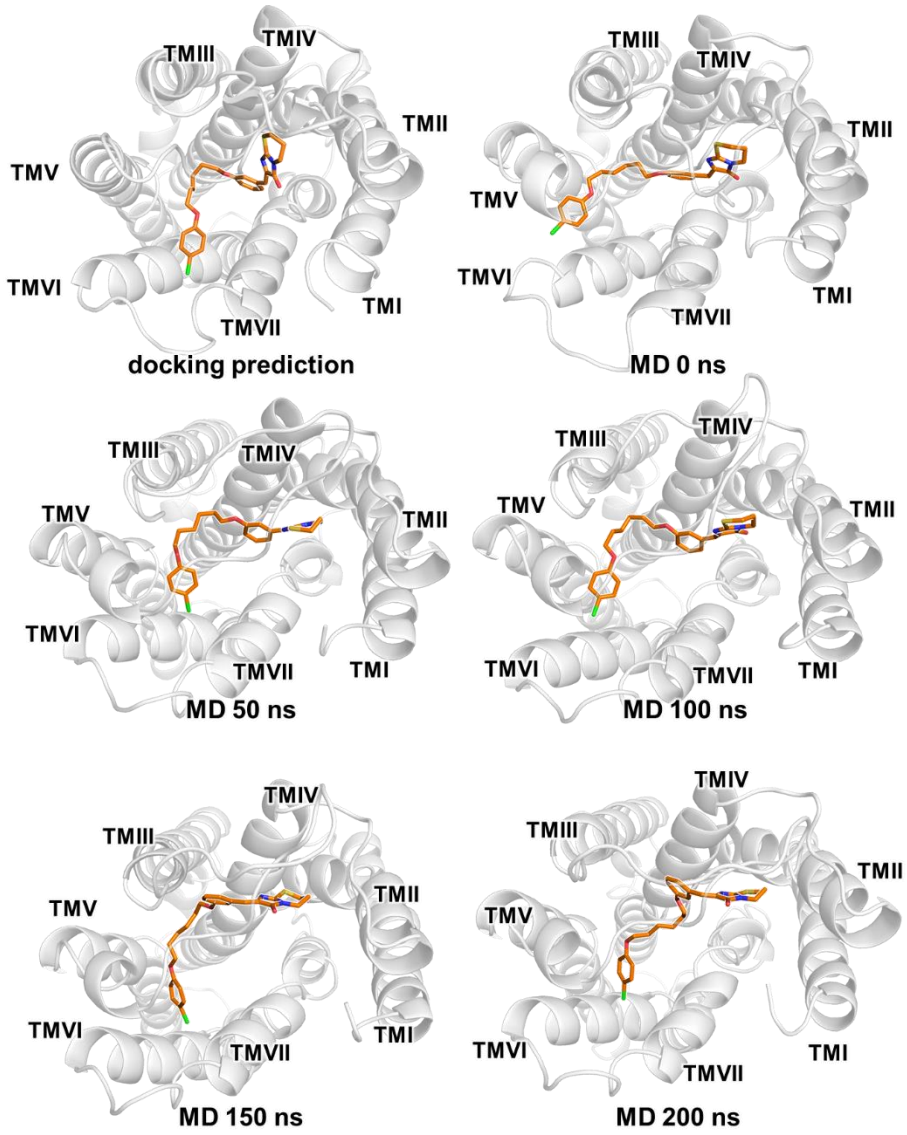

Figure S2. Time scale of the molecular dynamics (denoted 'MD') simulation of GPR18 homology model complex with antagonist **4**. The docking prediction which was used for the simulation run is shown at the top left corner. 0 ns presents the complex after relaxation steps.

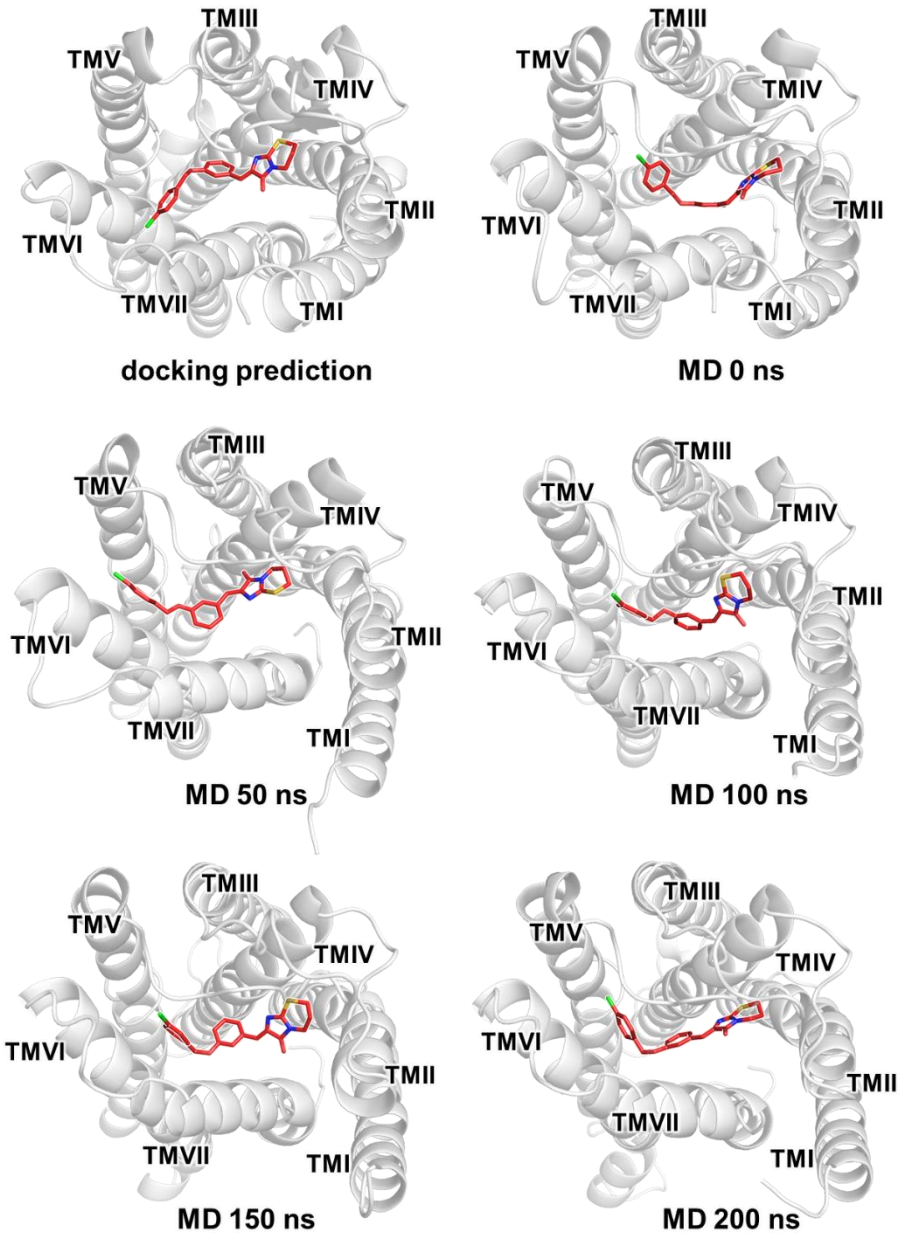

Figure S3. Time scale of the molecular dynamics (denoted 'MD') simulation of GPR18 homology model complex with antagonist **5**. The docking prediction which was used for the simulation run is shown at the top left corner. 0 ns presents the complex after relaxation steps.

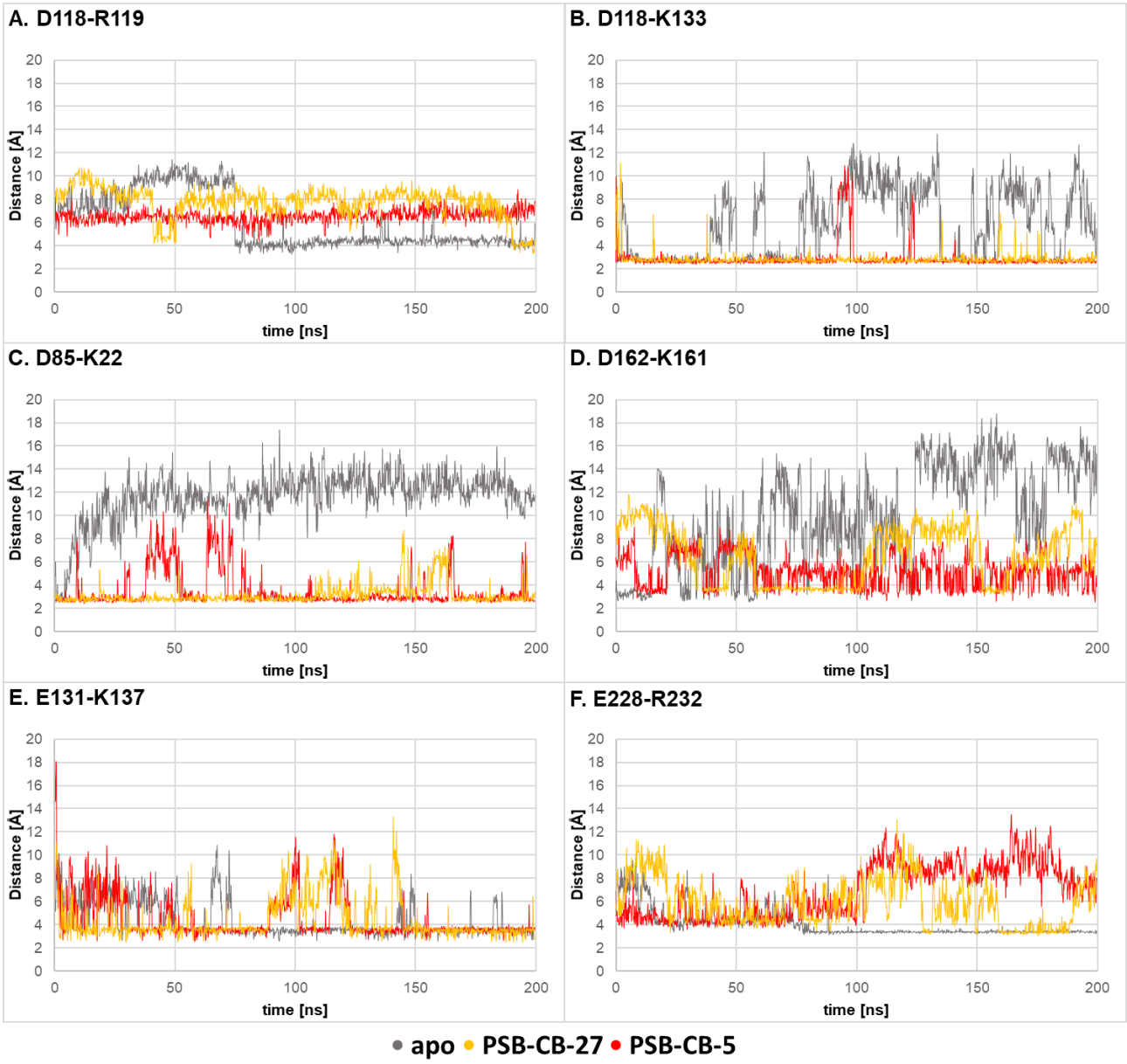

Figure S4. Trajectories of salt bridges during the 200 ns MD simulation runs.

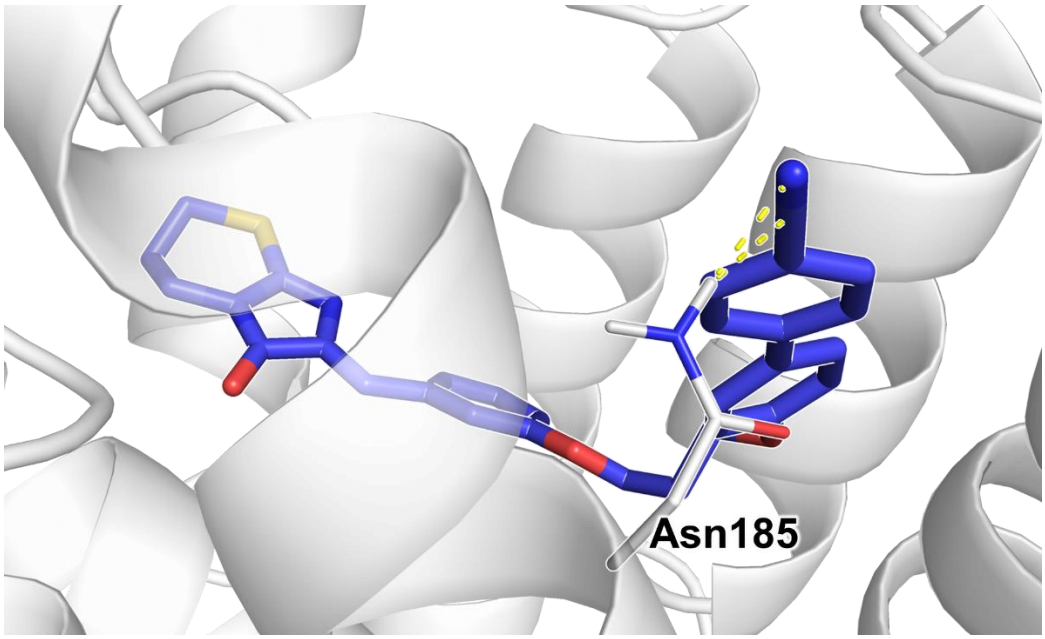

Figure S5. Possible interaction of antagonist **6** with a rotamer of Asn185.

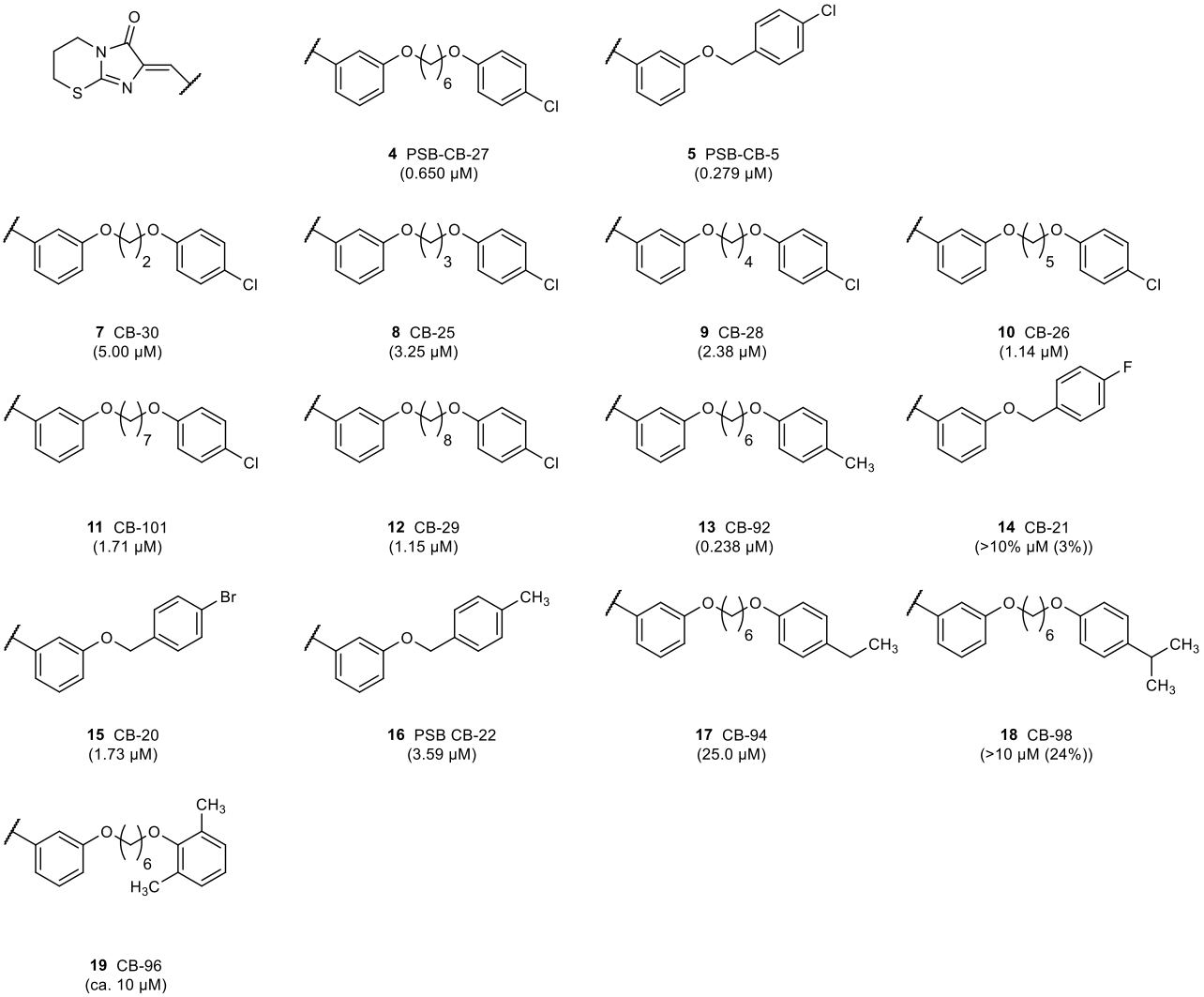

Figure S6. Structures of GPR18 imidazothiazinone antagonists with their respective IC_{50} values in brackets. For IC_{50} values > 10 μM the percent inhibition of agonist-induced luminescence signal at 10 μM is given. Biological results were taken from published studies [1].

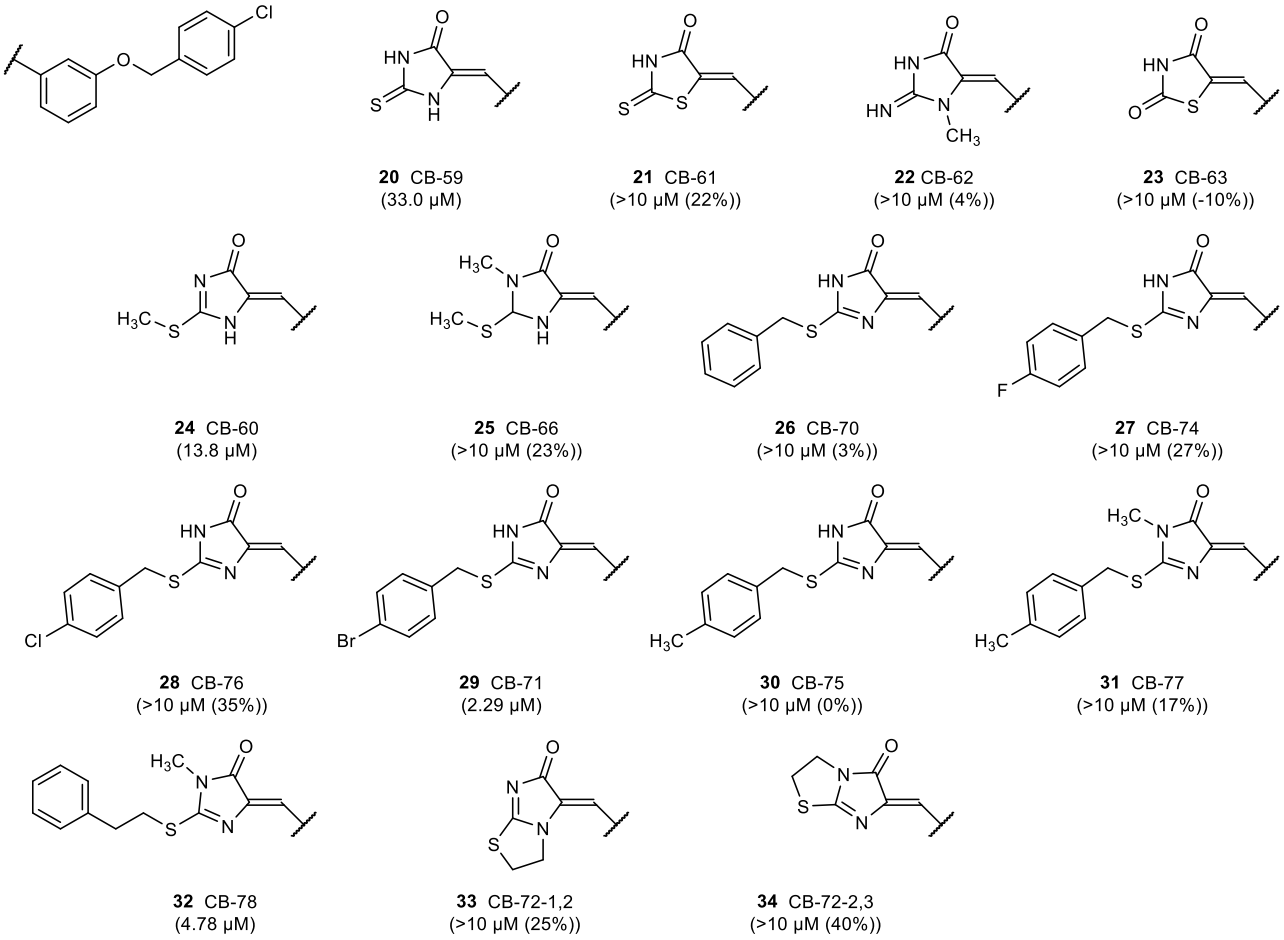

Figure S7. Structures of GPR18 antagonists with modification of the core structure with their respective IC_{50} values in brackets. For IC_{50} values > 10 μM the percent inhibition of agonist-induced luminescence signal at 10 μM is given. Biological results were taken from published studies [1].

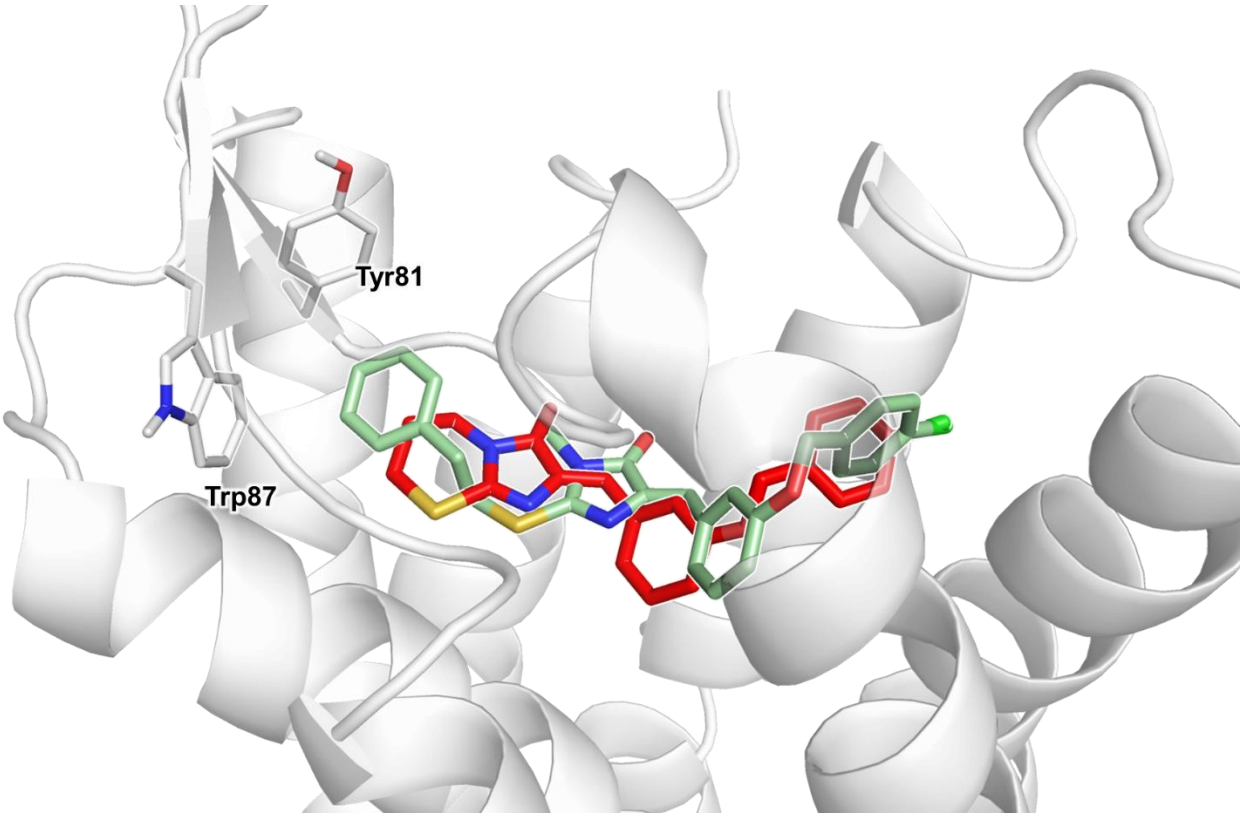

Figure S8. Comparison of the putative binding mode of antagonist **32** (green) and predicted binding mode of antagonist **5**.

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sp|Q14330|GPR18_HUMAN      ------------------------------------------------------------      0
sp|P21554|CNR1_HUMAN       MKSILDGLADTTFRTITTDLLYVGSNDIQYEDIKGDMASKLGYFPQKFPLTSFRGSPFQE      60
sp|P34972|CNR2_HUMAN       ------------------------------------------------------------      0

                                                                                   TMI
sp|Q14330|GPR18_HUMAN      MITLNNQDQPVPFNS-----------------------------------SHPDEYKIA      24
sp|P21554|CNR1_HUMAN       KMTAGDNPQLVPADQVNITEFYNKSLSSFKENEENIQCGENFMDIECFMVLNPSQQLAIA      120
sp|P34972|CNR2_HUMAN       -----------------MEECWVT------EIANGSKDGLDSNPMKDYMILSGPQKTAVA37
                                                                          .  ::  :*
                                                                   TMII
                           ------------------------------------------------------------
sp|Q14330|GPR18_HUMAN      ALVFYSCIFIIGLFVNIT---ALWVFSCTTK-KRTTVTIYMMNVALVDLI----FIMT-L      75
sp|P21554|CNR1_HUMAN       VL-----SLTLGTFTVLENLLVLCVILHSRSLRCRPSYHFIGSLAVADLLGSVIFVYSFI      175
sp|P34972|CNR2_HUMAN       VL-----CTLLGLLSALENVAVLYLILSSHQLRRKPSYLFIGSLAGADFLASVVFACSFV      92
                           .*        :* :  :    .* ::  : . :      :: .:* .*::    *  : :
                                        TMIII
                           ------------------------------------------------------------
sp|Q14330|GPR18_HUMAN      PFRMFYYAKDEWPFGEYFCQILGALT-VFYPSIALWLLAFISADRYMAIVQPKYAKELKN      134
sp|P21554|CNR1_HUMAN       DFHVFHR-KDS---RNVFLFKLGGVTASFTASVGS--LFLTAIDRYISIHRPLAYKRIVT      229
sp|P34972|CNR2_HUMAN       NFHVFHG-VDS---KAVFLLKIGSVTMTFTASVGS--LLLTAIDRYLCLRYPPSYKALLT      146
                            *::*:   *.      *   ::*.:*  *  *:.   * : : ***:.:  *   * : .
                                TMIV                                          TMV
                           ------------------------------------------------------------
sp|Q14330|GPR18_HUMAN      TCKAVLACVGVWIMTLTTTTPLLLLYKDPDKDSTPATCLKISDIIYLKAVNVLNLTRLTF      194
sp|P21554|CNR1_HUMAN       RPKAVVAFCLMWTIAIVIAVLPLLGWNCEK---LQSVCSDIFPHI--------DETYLMF      278
sp|P34972|CNR2_HUMAN       RGRALVTLGIMWVLSALVSYLPLMGWTCCP-----RPCSELFPLI--------PNDYLLS      193
                             :*:::   :* ::   :   *: :.          * .:   *           *

                           ------------------------------------------------------------
sp|Q14330|GPR18_HUMAN      FFLIPLFIMIG---CYLV---------------------IIHNLLHGRTSKLKPKVKEKS      230
sp|P21554|CNR1_HUMAN       WIGVTSVLLLFIVYAYMYILWKAHSHAVRMIQRGTQKSIIIHTSEDGKVQVTRPDQARMD      338
sp|P34972|CNR2_HUMAN       WLLFIAFLFSGIIYTYGHVLWKAHQHVASLSGH-------------QDRQVPGMARMRLD      240
                           :: .  .::      *                                .       . .
                           TMVI                                   TMVII
                           ------------------------------------------------------------
sp|Q14330|GPR18_HUMAN      IR---IIITLLVQVLVCFMPFHICFAFLMLGTGENSYNPWGAFTTFLMNLSTCLDVILYY      287
sp|P21554|CNR1_HUMAN       IRLAKTLVLILVVLIICWGPLLAIMVYDVFGKMNKLIKTVFAFCSMLCLLNSTVNPIIYA      398
sp|P34972|CNR2_HUMAN       VRLAKTLGLVLAVLLICWFPVLALMAHSLATTLSDQVKKAFAFCSMLCLINSMVNPVIYA      300
                           :*    :  :*. :::*: *.   :.. :  . ..  :  ** ::*  :.: :: ::*

                           ------------------------------------------------------------
sp|Q14330|GPR18_HUMAN      IVSKQFQARVISVMLYRNYLRSMRRKSFRSGSLRSLSNINSEML----------------      331
sp|P21554|CNR1_HUMAN       LRSKDLRHAFRSMFP--SC----------EGTAQPLDNSMGD----SDCLHKHANN----      438
sp|P34972|CNR2_HUMAN       LRSGEIRSSAHHCLA--HW----------KKCVRGLGSEAKEEAPRSSVTETEADGKITP      348
                           : * :::      :               .   : *..   :

sp|Q14330|GPR18_HUMAN      -----------------------------------  331
sp|P21554|CNR1_HUMAN       -AASVHRAAESCIKSTVKIAKVTMSVSTDTSAEAL  472
sp|P34972|CNR2_HUMAN       WPDSRDLDLSDC-----------------------  360
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Figure S9. Multiple sequence alignment of human GPR18 and the cannabinoid receptors CB_1 and CB_2. Residue positions involved in the binding of cannabinoid agonists in the X-ray crystal structure of CB_1 receptor are highlighted.

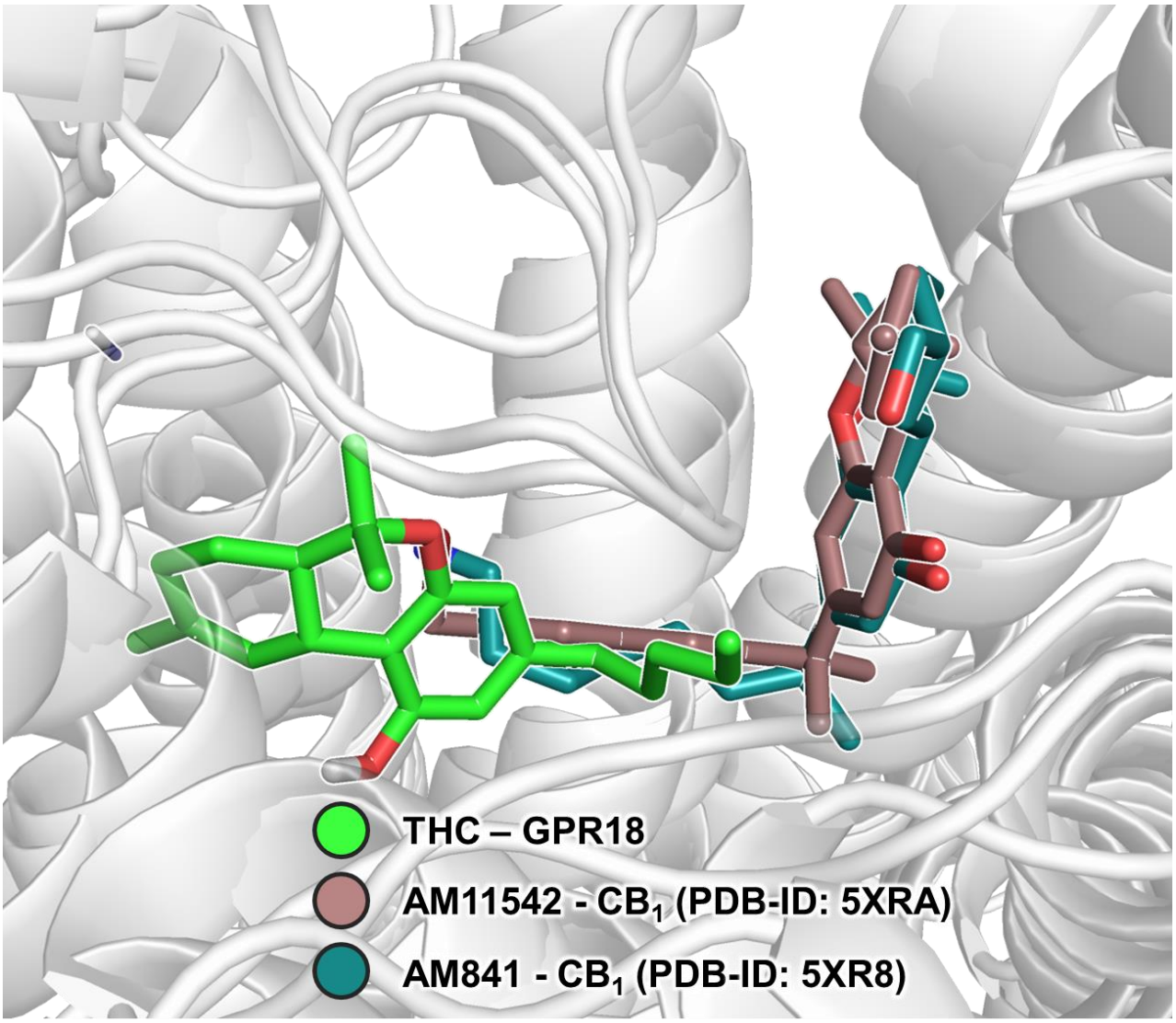

Figure S10. Comparison of the proposed binding mode of THC to GPR18 with the binding of THC derivatives to the CB_1 receptor as observed in the crystal structure [2].

References

1. Schoeder, C. T.; Kaleta, M.; Mahardhika, A. B.; Olejarz-Maciej, A.; Łażewska, D.; Kieć-Kononowicz, K.; Müller, C. E. Structure-activity relationships of imidazothiazinones and analogs as antagonists of the cannabinoid-activated orphan G protein-coupled receptor GPR18. *Eur. J. Med. Chem.* **2018**, *155*, 381–397.
2. Hua, T.; Vemuri, K.; Nikas, S. P.; Laprairie, R. B.; Wu, Y.; Qu, L.; Pu, M.; Korde, A.; Jiang, S.; Ho, J.-H.; *et al.* Crystal structures of agonist-bound human cannabinoid receptor CB1. *Nature* **2017**, *547*, 468–471.