Modeling of interactions between the human 5-HT7 receptor and ligands

5.årsoppgave i Stadium IV – Profesjonsstudiet i medisin ved Universitetet i Tromsø.

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Nøkkelord : Forskning, Reseptor, Signalstoff, Molekylærbiologi

Innledning

Bakrunnen for valg av denne oppgaven er at jeg ved en tilfeldighet kom over et prosjekt med lignende emne i valgfriperioden på 2. året ved medisinstudiet. Jeg syntes temaet var spennende og er generelt interessert i forskning og nyutvikling. Jeg har også studert ett år IT-grunnfag og har lang erfaring med bruk av datamaskin og programvare. Jeg valgte derfor å spørre min veileder fra 2.års oppgaven om et passende og lignende prosjekt for en 5.års oppgave. Oppgaven er i sin helhet gjennomført og skrevet av meg, med veiledning fra veileder.

Oppgaven bygger på en modellering av den humane 5-HT7 reseptoren og liganders interaksjoner med reseptoren. Ligandene ble valgt ut på bakgrunn av deres høye affinitet til 5-HT7 reseptoren. Ligandene som er brukt er delt inn i tre grupper; agonister, antagonister og partielle agonister. Hovedmålet med denne grafiske fremstillingen er å identifisere hvilke aminosyrer i reseptoren som binder seg med ligandene, og se på hvilke forskjeller det er i interaksjonene med reseptoren i de forskjellige grupper av ligander. Ved å gjøre datasimuleringer på denne måten kan en forsterke tidligere teorier og se mulige nye interaksjoner, uten at man trenger å fysisk bruke reseptorer og ligander i et labratoreforsøk. Sammenligning med tidligere studier av samme type og ikke minst av mutasjonsstudier er viktig for å bedømme resultatet og komme med evt nye teorier.

Dette er en oppgave der den spesifikke interaksjonen mellom ligand og reseptor står i sentrum, et område av forskningen som er spesielt viktig med tanke på utvikling av medikamenter. Jeg har derfor ikke gått særlig dypt inn på reseptorens funksjon og utbredning i menneskekroppen da dette ikke er målet med arbeidet.

Norsk sammendrag

Bakgrunn

Bruk av datasimulerte modeller av reseptorer og ligander er et nyttig verktøy for å forstå den biokjemiske mekanismen bak aktivering eller inaktivering av en resptor, og dermed også nyttig for utvikling av nye medikamenter. Idenifiseringen av hvilke aminosyrer i en reseptor som binder seg til forskjellige ligander kan gi en forståele av hva som må til for å skape stoffer med høyere affinitet og spesifisitet for målreseptoren.

Målet med studiet er å vurdere dockingresultatene med tanke på reseptor-ligand interaksjoner, det vil si hvilke aminosyrer i reseptoren som deltar i bindingen, og hvilke deler av ligandene som er viktig for bindingen. Det skal også gjøres en vurdering av dockingen av de forskjellige typer ligander i modellene basert på de forskjellige krystallstrukturer, for å se på resultatmessige forskjeller ved bruk av de 3 forskjellige krystallstrukturer. Også en sammenligning av resultater fra andre docking-, SAR- (Structure-Activity Relationship) og mutasjonsstudier av 5-HT7 reseptoren gjøres for å få gjort en bedre totalvurdering av dockingresultatene.

Materiale og metode

I dette studiet er det konstruert homologimodeller av human 5-HT7 (5-hydroxytryptamin, serotonin) reseptor basert på 3 forskjellige krystallstrukturer (beta1 adrenerg reseptor bundet med agonist, antagonist og partiell agonist) ved hjelp av homologimodellering og docking av agonister, antagonister og partielle agonister til disse homologimodellene. Krystallstrukturene som er brukt er valgt ut på grunn av deres høye likhet med 5-HT7 reseptoren. Krystallstruktur ble hentet fra PDB databasen [*www.rcsb.org/*], reseptorens aminosyresekvens fra UniProt databasen [*www.uniprot.org/*]. Programmet ICM Pro fra Molsoft ble benyttet til å konstruere homologimodeller. 3D strukturer av ligander med rapportert høy affinitet for 5-HT7 (Ki < 1.0 nM eller tilsvarende) ble importert fra ChEMBL databasen. Sammenlignbare 5-HT7 docking studier ble hentet fra Pubmed.

Resultater og fortolkning

Det var ingen signifikant forskjell på dockingresultatene for docking av ligander til de tre homologimodellene av 5-HT7 reseptoren, med tanke på bedre resultat, for eksempel ved docking av agonister kontra antagonister i agonistmodellen. Partiell

agonist modellen viste generelt dårligere docking resultat.

Reseptor-ligand interaksjoner i transmembrane helixer 3, 5, 6 og 7 sto i samsvar med tidligere studier av 5-HT7 reseptoren som er undersøkt i denne studien. Aminosyrene Val2.60 og Val3.33 er ikke beskrevet som viktig for ligandbinding i noen av de studiene som det er sammenlignet med, men de hadde i dette studiet gjentatte interaksjoner med ligander. Om disse er reelt viktig for ligandinteraksjon og eventuelt affinitet for 5-HT7 reseptoren kan man ikke si noe om på bakrunn av dette studiet. Derfor kan en videre forskning gjennom et mutasjonsstudie på aminosyrer Val2.60 og Val3.33 være nyttig for å avgjøre om disse er viktig for liganders binding og affinitet til 5-HT7 reseptoren.

Abstract

By constructing homology models of the human 5-HT7 (5-hydroxytryptamin, serotonin) receptor based on three different X-ray crystals of the turkey beta1 adrenergic receptor (one with an agonist complex, one with antagonist complex and one with a partial agonist complex) we tried to determine if there are any difference between the docking of agonists, antagonist and partial agonist ligands in the three different models, and look into which amino acids that are important for ligand binding.

The building of the homology models and docking was done using Molsoft ICM pro. X-ray crystal structures where downloaded from the PDB database (www.rcsb.org) and the amino acid sequence from UniProt database (www.uniprot.org). Ligands were selected from was selected from ChEMBL database. Article searches where done in www.pubmed.com.

Results did not indicate a difference in ligand-receptor interactions or energy state of the complexes across the agonist and antagonist models. The model based on the partial agonist complex template yielded less successful dockings and higher energy levels of docking complexes.

Residues included in the binding site, in trans membrane helix's 3 (Asp3.32), 5 (Thr5.43, Ser5.42, Tyr5.38, Phe5.47), 6 (Phe6.51, Phe6.52, Ser6.55), and 7 (Phe7.38), that interact with the ligands in this study, are in accordance with previously published SAR, docking and mutation papers included in this research. Other residues with repeating interaction with ligands include Val2.60, Val3.33 and Tyr5.38. Further investigation on the role of these amino acids in ligand binding could be useful. Agonists and partial agonists tend to bind in the pocket between helix's 4-7, while the antagonists occupy both the pocket between TMH4-6 and the pocket between TMH7-3.

Contents

Introduction

Serotonin mediate many different physiological functions by interacting with 14 serotoninergic receptor subtypes, further classified into seven families (5-HT1-7)(1). The 5-HT7 receptor is part of the G-protein coupled receptor (GPCR) superfamily. It consists of 7 trans membrane helixes, 3 extracellular loops and 3 intracellular loops (2) (fig.1A fig.2A). It is the latest of the 5-HT receptors family to be discovered (3). Studies have revealed a high concentration of the 5-HT7 receptor in hippocampus, thalamus, hypothalamus, cerebral cortex, and also in gastrointestinal and cardiovascular smooth muscle tissue (4, 17). Functions of the receptor has been proposed to be involvement in regulation of body temperature, circadian rhythms, learning and memory, neuronal excitability, inflammatory processes in the brain and smooth muscle relaxation of cerebral arteries among others(4). Because of its many various important functions it has become an attractive target for drug discovery (treatment of depression, inflammatory bowel disease, migraine, sleep disorders)

In this study three dimensional models of the 5-HT7 receptor will be constructed. Docking studies with known 5-HT7 receptor agonists, partial agonists and antagonists will be performed for the 3 different homology models of the 5-HT7 receptor. Results will be compared to other pharmacophore and docking studies that have been done for the 5-HT7 receptor. Also a comparison will be done to see if there is a significant difference in successful docking when docking the different groups of ligands to the 3 different homology models of the 5-HT7 receptor based on the the agonist, antagonist and partial agonist crystal structure. I.e. will the antagonists dock with better results in the receptor based on antagonist bound crystal then on the other two receptors.

Method

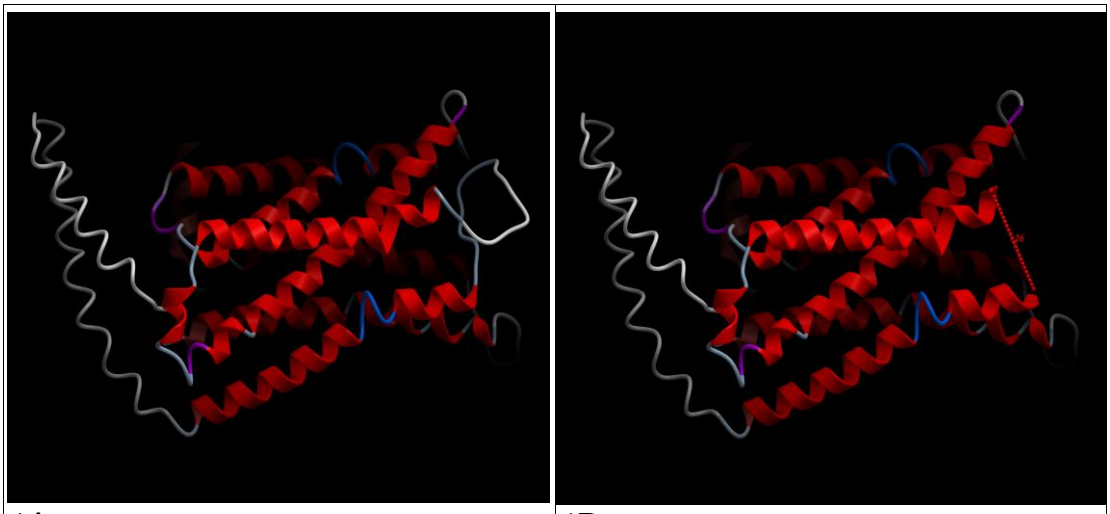
21 ligands (table 1) that is known to have high affinity for the human 5-HT7 receptor was selected from ChEMBL database [https://www.ebi.ac.uk/chembldb/]. These where imported to Molsoft ICM (5) and converted into an sdf file (table 1).

The receptors used are based on X-ray crystal structures of three turkey beta1 adrenergic receptor (6, 7). The crystal structures differ by what type of ligand that was bound to the receptor when the crystallization was done; an agonist, an antagonist and a partial agonist. Using Molsoft ICM (5) the models was built after

they were aligned with the 5-HT7 amino sequence (imported from the protein knowledge base, accession code: P34969). The homology models were refined by optimizing side chain conformations of non-conserved residues (residues that are different in the target 5-HT7 from the template beta1 adrenergic receptor) by Monte Carlo simulation and annealing the backbone by energy minimization.

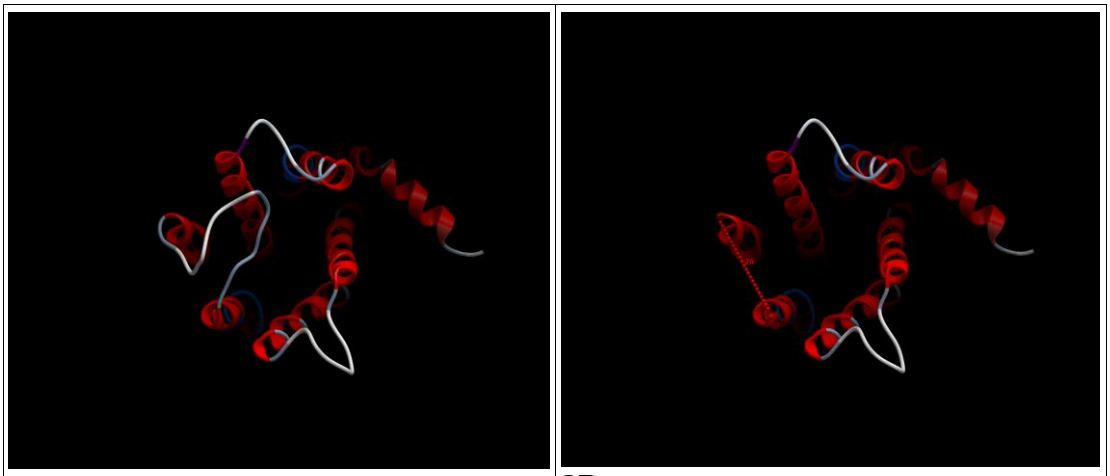
Docking was done with a rigid receptor using Molsoft ICM (5). The binding site was identified using IcmPocketFinder. The docking with the 21 ligands was done in a batch docking. Top ten results, based on energy score was saved for each ligand.

Very few ligands connected to Asp3.32 by an H-bond and low energy score, so it was decided to attempt to make the binding pocket slightly bigger. This was done by mutating Y333 (Tyr7.43) and L329 (Leu7.39) to alanine, then running a flexible receptor docking with previously successfully docked ligand number 4, and then mutating the Y333 and L329 back, hoping that this would create a slightly bigger binding pocket. New docking batches with the 21 ligands where done but no significant improvement in results was seen. Still trying to improve the docking, the second extracellular loop (ECL2) in its entirety was removed from the receptor models, in an attempt to make more room for the larger ligands in the binding site (fig1 and fig 2). This did improve the overall score and interactions of all ligands to all receptor models. Two docking batches were done for all three receptor models with the removed ECL2.



1A | 1B

Fig 1. 5-HT7 receptor model 2 based on x-ray structure of beta 1 adrenergic receptor – antagonist complex. 1A: before removing the ECL2. 1B: After removing the ECL2.



2A 2B

Fig 2. 5-HT7 receptor model 2 based on x-ray structure of beta 1 adrenergic receptor – antagonist complex. Seen from extracellular side. 2A: before removing the ECL2. 2B: After removing the ECL2.

Amino acid numbering in figures are numbered according to the amino acid sequence of the human 5-HT7 receptor (UniProt database:P34969). In the text Ballesteros-Weinstein nomenclature (8) is used (Conversion table is shown in table 2). This is based on the very few and highly conserved residues in the transmembrane helix's of almost all of the family A GPCRs. The most conserved residue in each helix is given the number 50 (Asn in helix 1, Asp in helix 2, Arg in helix 3, Trp in helix 4, Pro in helix's 5-7). For each amino acid in a transmembrane helix the position is numbered relative to the highly conserved residue. For example in TMH3 where the reference residue is Arg3.50, the amino acid in the position before is Asp3.49, and the one after is Tyr3.51.

Amino acid residue in human 5-HT7 receptor	Ballesteros-Weinstein
V98	Val2.60
D121	Asp3.32
V122	Val3.33
F117	Phe3.28
I118	Ile3.29
Y207	Tyr5.38
T208	Thr5.39
S211	Ser5.42
T212	Thr5.43
F216	Phe5.47
F299	Phe6.44
T300	Thr6.45
W303	Trp6.48
F306	Phe6.51
F307	Phe6.52
S310	Ser6.55
L322	Leu7.32
R326	Arg7.36
F328	Phe7.38
L329	Leu7.39
W330	Trp7.40
L332	Leu7.42
Y333	Tyr7.43

Table2. Conversion table for Ballesteros-Weinstein nomenclature.

Results

After completing 2 batches of docking on each receptor model, all with the same 21 ligands, we can see that the overall results where not to different between the three receptor models (Table 3), although a higher average energy score can be seen in the docking results for the partial agonist model. The majority of ligands docked successfully, with a hydrogen/ionic bond to Asp3.32, though ligands 3, 4 and 21 did not make any successful docking (binding with Asp3.32) on any of the models.

Table 3. Ligand nr shows the ligand number and the stack number from the docking batch. In a stack, number 1 has the lowest energy score, while 10 has the highest energy score. At bottom the average for all ligands, agonists, antagonists and partial agonists are shown. Only stacks with the lowest energy for each ligand (represented in table 3) is included in the average.

Dock1 Agonist model	
Ligand nr/stack nr	Energy (kcal/mol)
1 - 1	-17,35
2	
3	
4	
5 - 2	-18,97
6	
7 - 6	-13,86
8	
9 - 1	-15,46
10 - 7	-4,93
11 - 3	-17,00
12 - 1	-17,38
13	
14	
15 - 9	-10,17
16 -4	-11,82
17 - 1	-18,07
18 - 1	-18,62
19 - 1	-18,28
20 - 1	-23,28
21	
All ligands	-15,78
Agonists	-17,35
Antagonist	-16,75
Partial ago.	-10,20

Dock2 Agonist model	
Ligand nr/stack nr	Energy (kcal/mol)
1 - 4	-9,39
2 - 1	-22,25
3	
4	
5	
6	
7	
8 - 2	-14,03
9 - 2	-13,17
10 - 1	-13,75
11 - 1	-17,87
12 - 4	-15,01
13 - 1	-17,15
14	
15 - 7	-10,89
16 - 4	-15,38
17 - 1	-14,55
18	
19	
20 - 1	-20,42
21	
All ligands	-15,32
Agonists	-9,39
Antagonist	-16,39
Partial ago.	-13,46

Dock1 Antagonist model	
Ligand nr/stack nr	Energy (kcal/mol)
1 - 4	-12,90
2	
3	
4	
5	
6	
7 - 3	-13,52
8 - 1	-20,77
9 - 6	-14,48
10 - 1	-18,61
11 - 1	-17,33
12	
13 - 5	-18,35
14 - 3	-16,53
15 - 2	-17,21
16	
17	
18 - 3	-16,39
19	
20 - 1	-25,24
21	
All ligands	-17,39
Agonists	-12,90
Antagonist	-18,17
Partial ago.	-16,55

Dock2 Antagonist model	
Ligand nr/stack nr	Energy (kcal/mol)
1 - 1	-18,03
2	
3	
4	
5 - 8	-9,10
6	
7 - 1	-19,03
8 - 2	-14,69
9 - 5	-12,10
10 - 1	-11,20
11 - 1	-15,01
12 - 2	-18,30
13 - 1	-17,76
14 - 1	-19,30
15 - 2	-14,95
16 - 3	-16,39
17	
18 - 1	-17,39
19 - 1	-17,87
20 - 1	-18,62
21	
All ligands	-15,98
Agonists	-18,03
Antagonist	-16,53
Partial ago.	-11,65

Average for all models	
All ligands	-14,97
Agonists	-14,50
Antagnoist	-15,42
Partial ago.	-13,13

Dock1 Partial agonist model	
Ligand nr/stack nr	Energy (kcal/mol)
1 - 3	-13,97
2 - 2	-15,82
3	
4	
5 - 3	-10,23
6 - 2	-13,75
7 - 4	-9,77
8 - 1	-21,53
9 - 4	-11,05
10 - 2	-12,99
11 - 2	-14,32
12 - 3	-11,44
13 - 6	-11,97
14 - 4	-11,60
15 - 3	-13,48
16	
17 - 3	-10,63
18 - 2	-15,20
19	
20 - 1	-10,06
21	
All ligands	-12,99
Agonists	-13,97
Antagonist	-13,06
Partial ago.	-12,02

Dock2 Partial agonist model	
Ligand nr/stack nr	Energy (kcal/mol)
1 - 2	-15,37
2 - 9	-5,66
3	
4	
5 - 3	-10,30
6	
7 - 4	-10,47
8 - 1	-21,34
9 - 1	-14,20
10 - 1	-15,66
10 - 7	-9,23
12 - 5	-12,45
13 - 3	-13,51
14	
15	
16	
17 - 2	-10,03
18 - 1	-12,28
19 - 4	-9,21
20 - 4	-13,02
21	
All ligands	-12,34
Agonists	-15,37
Antagonist	-11,59
Partial ago.	-14,93

5-HT7 receptor model 1 based on x-ray structure of beta 1 adrenergic receptor – full agonist complex

The lowest energy score of the agonists in the agonist model came from ligand 1 (the full agonist 5-carboxamido-tryptamine), with a score of -17.35 kcal/mol (fig. 3). Almost all of the agonists docked with its hydrophobic part in the pocket between TMH4-6 and the nitrogen of the carboxamido moiety in close proximity to Ser5.42, and Thr5.43. Van der Waals interactions could be seen between the hydrophobic parts of the ligands and Val.3.33, Phe6.51, Phe6.52 and Leu7.39. Some results showed a hydrogen bond between the ligand and Thr5.43, and some to Ser5.42. The ligand with the lowest energy score had an H-bond interaction with Thr5.43.

Ligand 20 scored the lowest on energy out of the antagonists, -23.28 kcal/mol (fig. 4). As expected it placed itself with a hydrogen bond to Asp3.32 while occupying the two pockets on either side. Also the Cl-terminal end is placed deep in the hydrophob

pocket between TMH4-6, showing van der Waals interactions with Phe6.51, Phe6.52 and Val3.33. The other hydrophob end is placed in the pocket between TMH7-3 with the same type of interactions with Leu7.39, Leu7.32, Phe3.28 and Val2.60. None of the results showed any hydrogen-bond interaction between ligand and residues in the pocket between THM7-3.

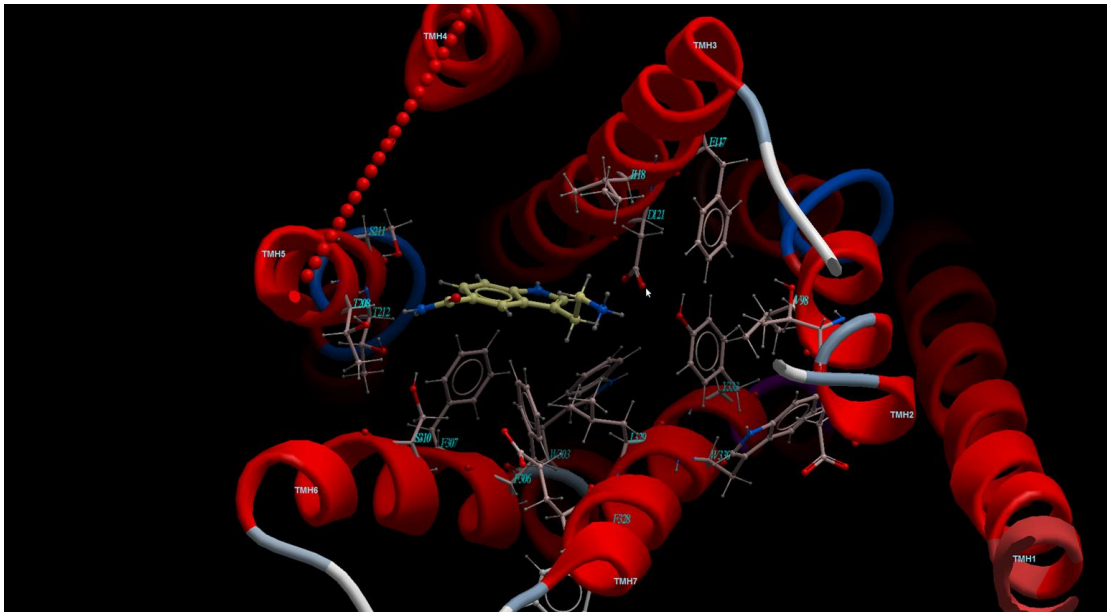


Fig 3. Ligand 1, CHEMBL 18840, 5-HT7 agonist, docked with agonist model. Seen from the extracellular side.

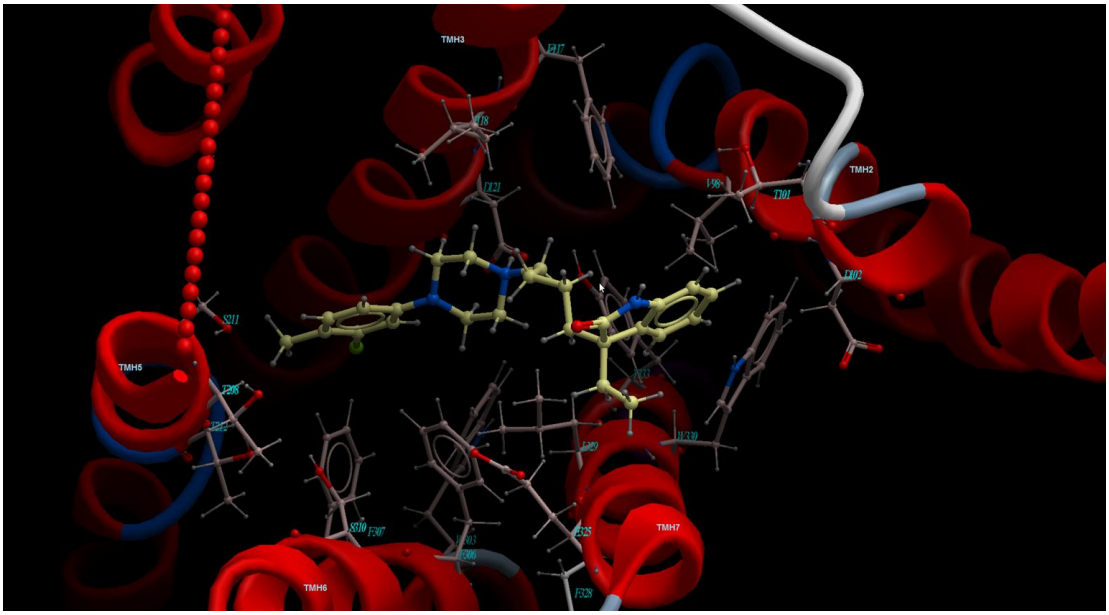


Fig 4. Ligand 20, CHEMBL 259086, 5-HT7 antagonist, docked with agonist model. Seen from the extracellular side.

The partial agonists had the highest average energy score of all the ligands groups. In the agonist model ligand 9 had the lowest score, -15.46 kcal/mol. The majority of the agonists were placed in the pocket between TMH4-6, in addition to the hydrogen/ionic bond to Asp3.32. Also the partial agonists favored the pocket, between TMH4-6 (fig.6). Though ligand 9 placed itself in the pocket between TMH7-3 with the ionic/ hydrogen bond to Asp3.32 and van der Waals bonds to Leu7.39 and Leu 7.32 (fig 5).

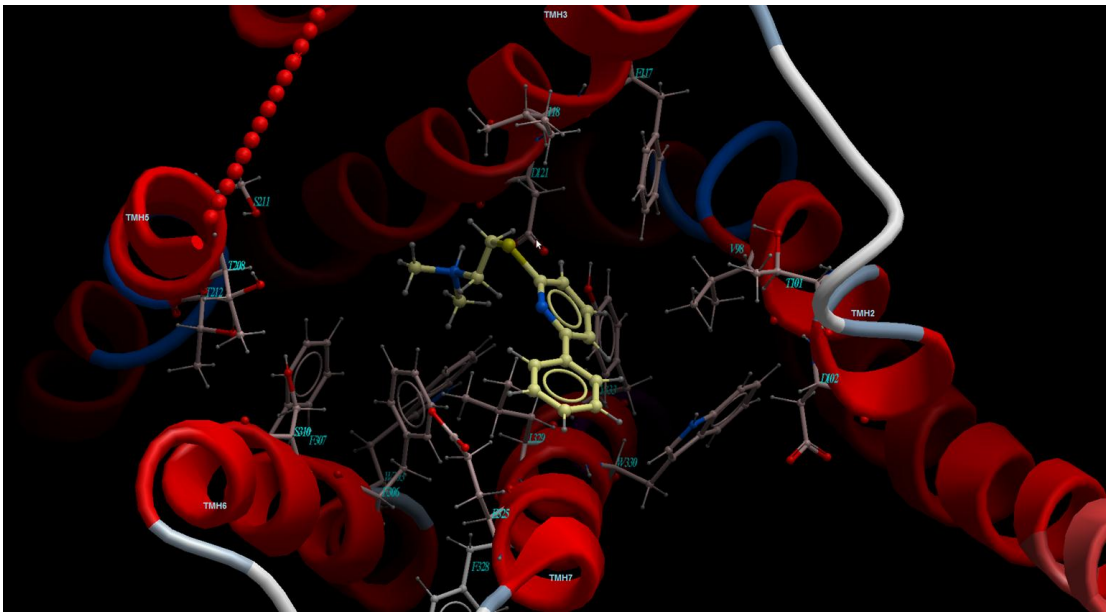


Fig 5. Ligand 9, CHEMBL 161765, 5-HT7 partial agonist, docked with agonist model. Seen from the extracellular side.

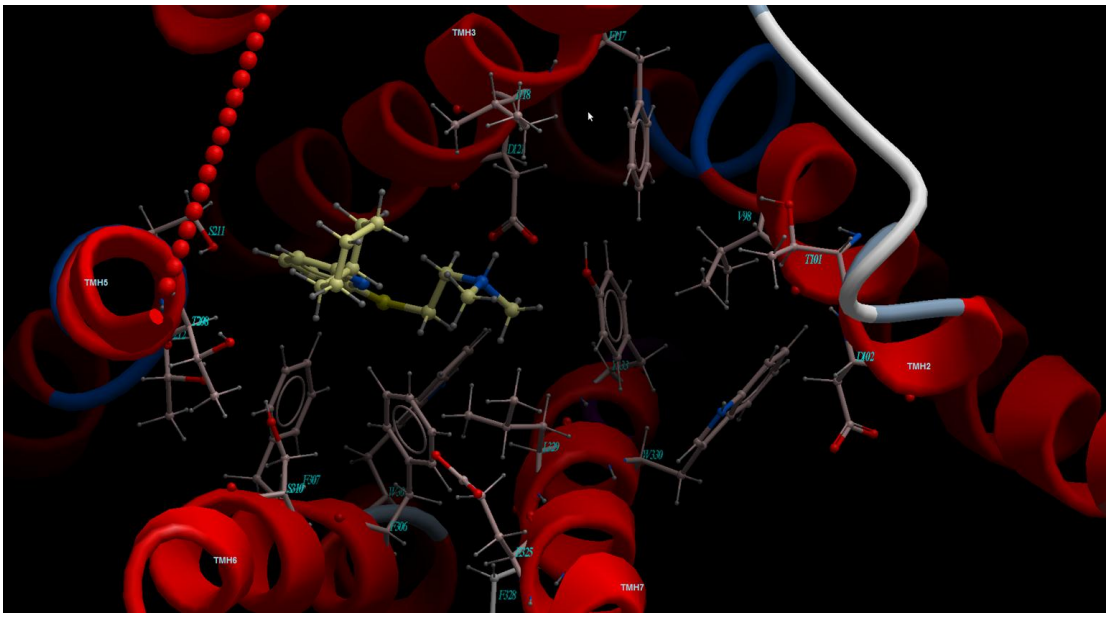


Fig 6. Ligand 10, CHEMBL 158402, 5-HT7 partial agonist, docked with agonist model. Seen from the extracellular side.

5-HT7 receptor model 2 based on x-ray structure of beta 1 adrenergic receptor – antagonist complex

Agonist ligand 1, with an energy score of -18.03 kcal/mol, docked with a hydrogen/ionic bond to Asp3.32 and the carboxamido moiety in the pocket between TMH4-6 (fig 7). The ligand also made a H-bond to Thr5.43, van der Waals interactions with Val3.33, Phe6.51 and Phe6.52. The majority of the dockings by this ligand made a hydrogen-bond to Ser5.42. A few results indicated a ligand – Ser6.55 interaction via a hydrogen bond.

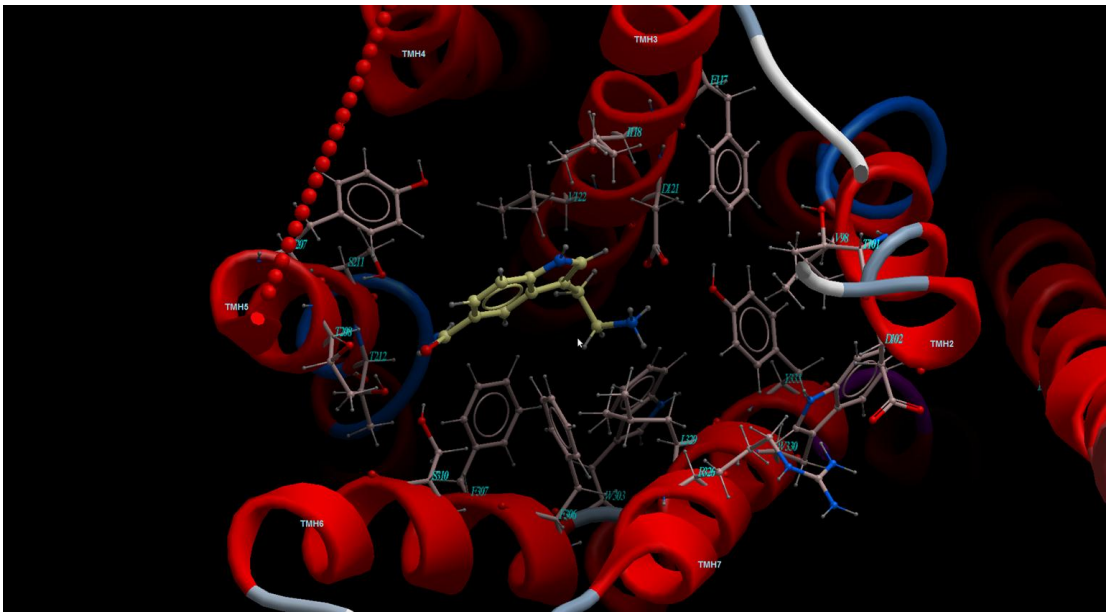


Fig 7. Ligand 1, CHEMBL 18840, 5-HT7 agonist, docked with antagonist model. Seen from the extracellular side.

Antagonist ligand 20 showed an overall low energy score on both agonist and antagonist model. With -25.24 kcal/mol it had the lowest score of all docking results. In addition to the Asp3.32 bond the ligand had a hydrogen bond to Tyr5.38 in the TMH4-6 pocket and hydrophob interactions with Val3.33, Phe6.51 and Phe6.52, with the Cl-terminal of the ligand placed in the pocket between THM7-3, where it bonded to Phe3.28, Leu7.39 and Val2.60 via van der Waals (fig 8). Other results indicated ligand-residue interaction by H-bonds to Ser5.42 and Trp7.40. In the case where it connected to Trp7.40 the Cl-terminal end was placed in the pocket formed by THM4-6, but this specific result had a higher energy score (-15.97 kcal/mol).

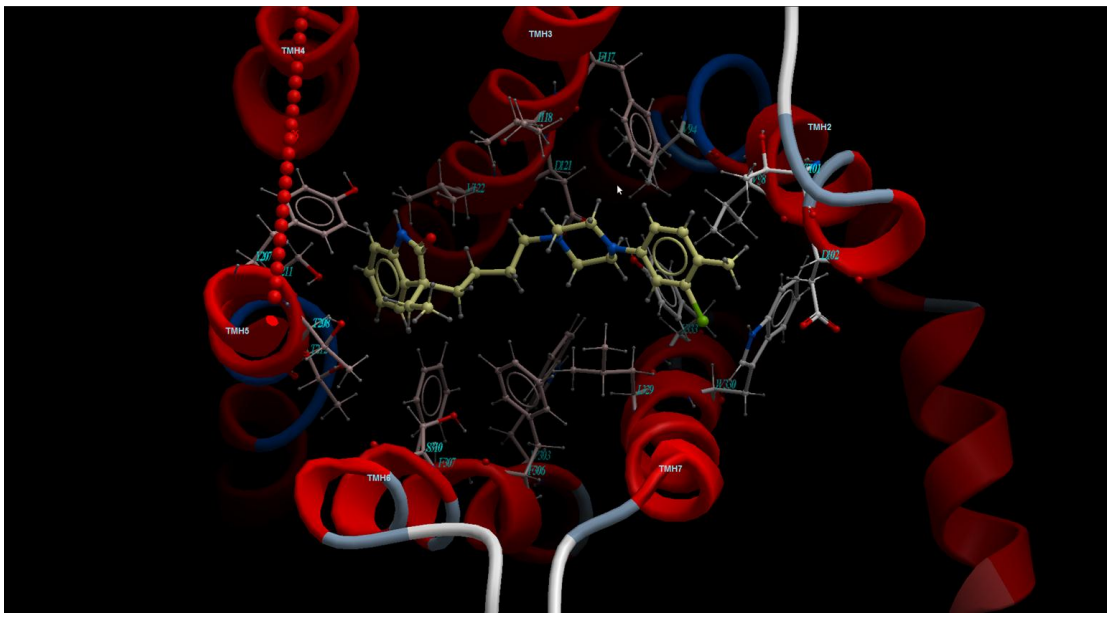


Fig 8. Ligand 20, CHEMBL 259086, 5-HT7 antagonist, docked with antagonist model. Seen from the extracellular side.

In the antagonist model the partial agonists docked in the pocket between TMH4-6. Most results from this group of ligands did not make a successful dock, and none of the ligands made any other H-bonds while retaining the bond to Asp3.32. Ligand 10, with the lowest energy score -18.61 kcal/mol, docked with an H-bond to Asp3.32 and van der Waals bonds to Phe6.52, Phe6.52 and Val3.33, filling the TMH4-6 pocket (fig 9).

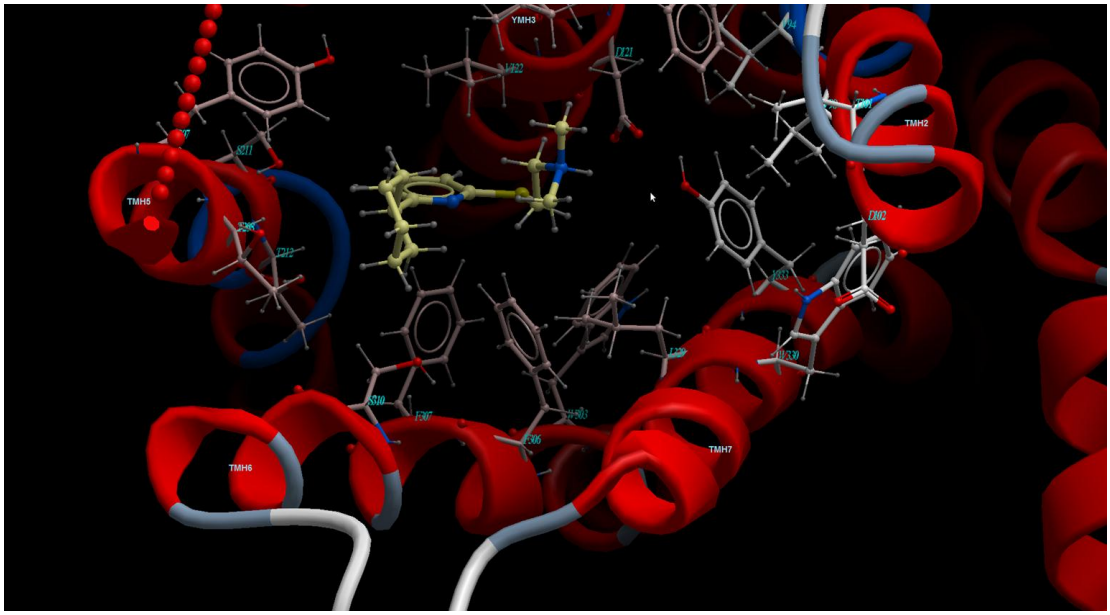


Fig 9. Ligand 10, CHEMBL 158402, 5-HT7 partial agonist, docked with antagonist model. Seen from the extracellular side.

5-HT7 receptor model 3 based on x-ray structure of beta 1 adrenergic receptor – partial agonist complex

The overall results on the partial agonist model were not as good as on the other two models. The best results came from ligand 8 in the first docking with an energy score of -21.53 kcal/mol (fig 10). While keeping the Asp3.32 ionic/hydrogen bond it also had an H-bond to Tyr5.38. Van der Waals interaction between the ligand and residues in the receptor included Val3.33, Phe6.51 and Phe6.52 in the pocket between TMH4-6, Phe3.28, Leu7.39 and Ile3.29 in the pocket between TMH7-3. It also scored low in the second docking, -21.34 kcal/mol. Although it did not make many successful dockings while maintaining the Asp3.32 bond and occupying the two pockets on either side, the ligand did make a hydrogen-bond with Ser5.42 in two of the docking simulations.

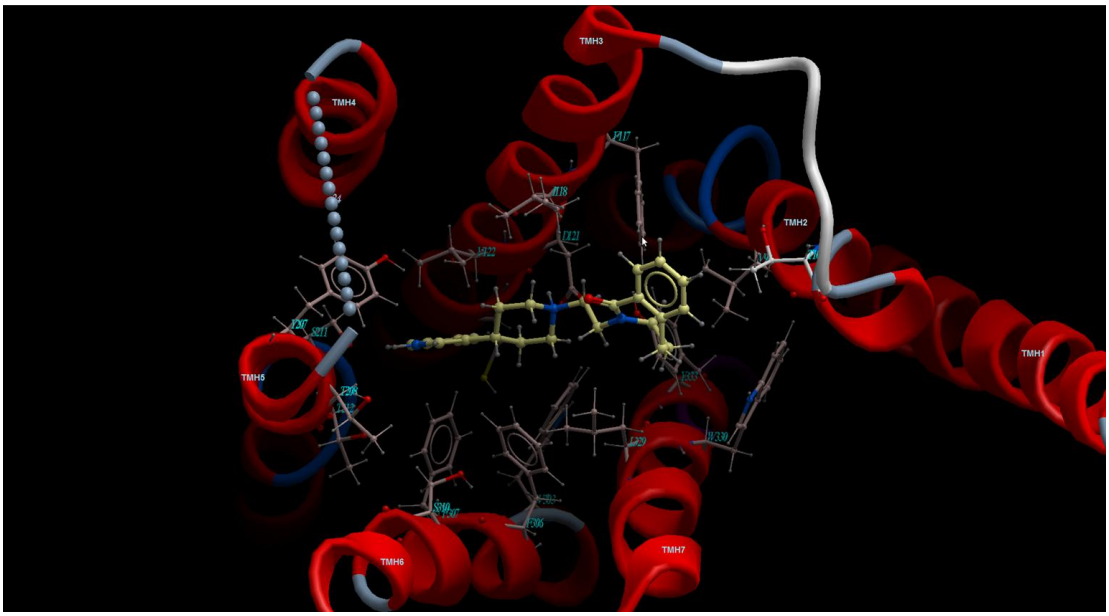


Fig 10. Ligand 8, CHEMBL 267062, 5-HT7 antagonist, docked with partial agonist model. Seen from the extracellular side.

Discussion

It should be mentioned that residues in the extracellular loop 2 (ECL2) is known to be involved with ligands binding to aminergic GPCRs (as well as residues in trans membrane helix's 3, 5, 6 and 7) (11, 15). The second extracellular loop has also been suggested to be important for selectivity over the 5HT-1a receptor, by reducing the size of certain cavities where ligands bind in the 5-HT7 receptor (11). The ECL2 in this study was removed to create a larger pocket for the ligands to be placed in during the docking simulations. Therefor we cannot say anything about the role of amino

acids in the ECL2 regarding binding of ligands to the receptors in this study.

Many ligands did not make many successful dockings with the homology receptor models, and a few did not make any at all. Some of the reasons for this might be that we did a rigid receptor docking. Doing a flexible receptor docking might have shown more successful results. Also there are small discrepancies in the homology model. The similarity of the amino acid sequence of the template used (beta1 adrenergic receptor with ligand complex) and the resolution of the template contribute to the quality of the homology model. The template used in this research has one of the highest resemblances to the amino acid sequence of the human 5-HT7 receptor.

Binding site description and pharmacophore models

Several pharmacophore models for the 5ht7 have been published (9,10,11,4,12). Most suggest at least four pharmacophore features; a positive ion (PI), a hydrogen-bond acceptor (HBA) and two hydrophobic regions (HYD). The PI, a basic nitrogen, interacts with Asp3.32, and works as the main anchoring point for the ligand. The two hydrophobic regions are located on each "side" of the PI. Located in the receptor binding site it would be one between helix's 4-6 (HYD1) (Phe5.47, Phe6.52, Phe6.51, Trp6.48 (10,11,13)) and the other between helix's 7-3 (HYD2) (Phe3.28, Ile3.29, Phe7.38, Trp7.40, Leu7.39 (10,13,14)). Theories about a more central placed hydrophob pocked have also been published(4, 13). It has also been suggested that the two pockets formed by TMH4-6 and TMH7-3 contains residues that bonds with a ligands HBA moiety (Ser5.42, Ser6.55, Thr5.43 between TMH4-6 (11,12,13) and Tyr7.43 (14) between TMH7-3).

Comparing the three models

As most of the ligands used in the docking are antagonists, one could expect an average lower energy score from the dockings to the 5-HT7 receptor model based on x-ray structure of beta 1 adrenergic receptor with an antagonist complex. But as we can see from table 3 the results are not significantly better from the antagonist model compared to the agonist model. The 5-HT7 receptor model based on x-ray structure of beta 1 adrenergic receptor with a partial agonist complex yielded a higher energy score on most of the ligands, suggesting that in this study the crystal structure with the partial agonist complex might be less suited for docking with 5-HT7 antagonists.

Agonists

The agonist in this study made many successful dockings (connecting with an hydrogen/ionic-bond to Asp3.32) while scoring a low energy. The agonist ligand occupied the pocket between TMH4-6 in almost every case of a successful docking. Residues included in the hydrophob pocket are located in TMH5 (Phe5.47 (11)) and TMH6 (Phe6.44, Phe6.51, Phe6.52, Trp6.48 (10)) as suggested in previously published papers. In this study we also noticed that many of the agonist results showed van der Waals interaction with Val3.33. In most cases it did make an H-bond, mainly with Thr5.43 (which has been suggested as one of the residues connecting with an HBA part of ligands (12)), and in several occasions H-bonds between ligand and Ser5.42 and Ser6.55 where seen. These two residues have also been suggested to be HBA connecting amino acids in the binding of both agonists and antagonists (10, 11). It has been suggested that the pocket formed between TMH4-6 is where the agonist's hydrophob/aromatic part bind, by interaction with hydrophob residues and hydrogen-bond (12).

There was no significant difference in the way the agonist docked in the three different models, and there were minimal differences between the agonist and antagonist model. Even so, the dockings in the receptor model with full agonist complex are probably more correct models, as it represents the receptor in its active state.

Antagonists

Most of the ligands used in the docking studies are 5-HT7 antagonists (table 3). Despite the large number of antagonists docked, none of them made a successful docking while connecting with an hydrogen-bond in the TMH7-3 pocket (mainly Tyr7.43 (14)). Many hydrophobic interactions where seen, mainly with Leu7.39, Leu7.32 in TMH7, Phe3.28 and Val2.60 in TMH3 and 2 respectively. The importance of residue Val2.60 might be worth investigating further. The less conserved area between TMH7-3 has been proposed to be important in antagonist selectivity over other monoamine GPCRs, especially for ligands with the presence of hydrophob aromatic- and H-bond accepting moieties that interact with this part of the receptor(14). Some of the residues suggested to be important in this selectivity are Tyr7.43, Phe3.28, Arg7.36 (11, 14).

In many of the results we could see an interaction by an HBA in the pocket between TMH4-6, in addition to van der Waal bonds to Phe6.52, Phe6.52 and Val3.33. Residue S6.55 in this pocket is believed to be important for antagonist selectivity over the 5HT-1a receptor (11). In this study antagonists mostly interacted with Ser5.42, but also Thr5.43 and Tyr5.38. While it has been proposed that S6.55 connects to a ligands HBA in the 5-HT7 receptor, the 5HT-1a receptor has an alanine at this position, and it is believed that this is one of the reasons for the selectivity. Ligands with selectivity over 5HT1a must have the correct structure and spacing allowing it to make the connection to S6.55 rather than S5.42 in the 5-HT7 receptor. None of our antagonists did make a successful docking while connecting to S6.55 with a hydrogen-bond, but a few of the agonist results did show an H-bond to this residue. Ligands 7 and 8, both antagonists, has been proved to be selective antagonists over other the human 5-HT2A receptor (16(ref2)).

Partial agonists

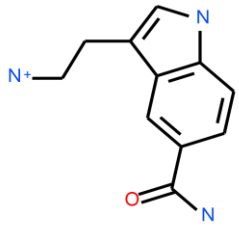
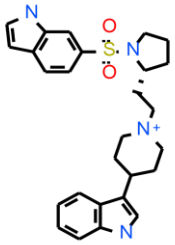
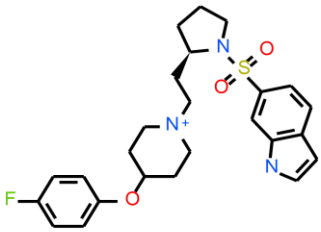
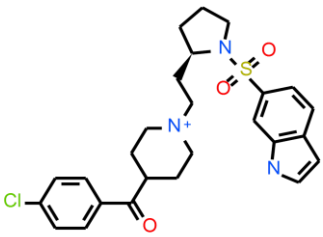
The few partial agonists used in the docking had an overall high energy score (table 3), and they did not show any pattern of better docking results in the docking with the model based on the crystal structure with a partial agonist complex.
Since ligand 9 and 10 doesn't have a suitable HBA moiety, neither made any other H-bonds to other parts of the binding site then the mandatory Asp3.32 connection. Ligand 21 didn't make any successful dockings.

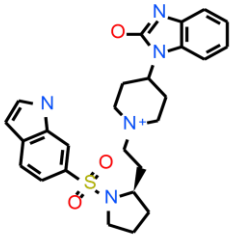
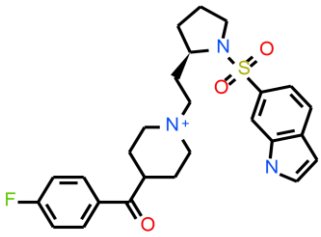
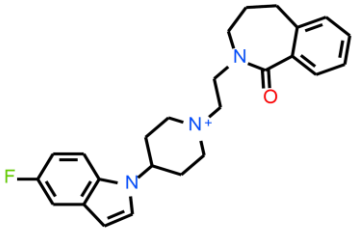
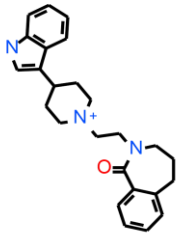
Most of the dockings resulted in a placement of the partial agonists in the pocket between TMH4-6. But the ligand with the highest score in the agonist model was placed more in the center, connecting via van der Waals bonds to Leu7.32 and Leu7.39 between TMH7-3, with its aromatic hydrophobic terminal end in close proximity of Phe7.38, though no interaction could be seen with that residue. This might be because of the placement of Phe7.38 in this specific homology model, as its aromatic ring is pointing outwards, and not in towards the expected binding site. Phe7.38 has been suggested to be an important residue for selectivity for some partial agonists (13). Along with residues Phe6.51 and Phe6.52, it is thought to create a hydrophobic pocket in the central part of the binding site (13). The placement of ligand 9, in the first docking in the model based on the crystal structure with an agonist complex (fig3), may support this theory. A few other results like this where also seen with ligand 10, though with a much higher energy score.

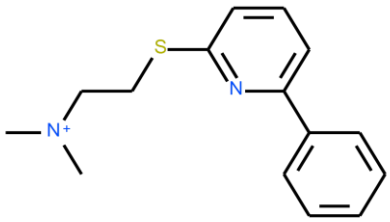
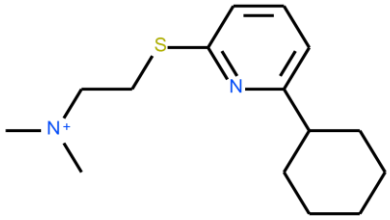
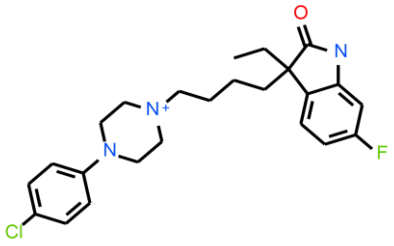
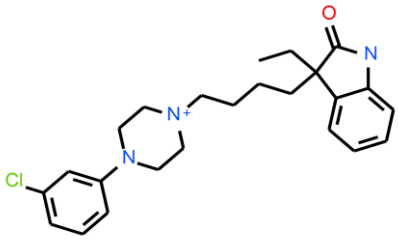
Conclusion

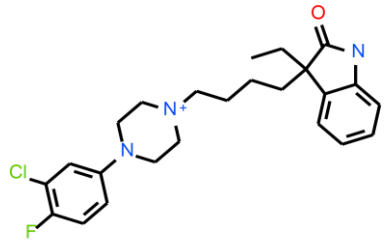
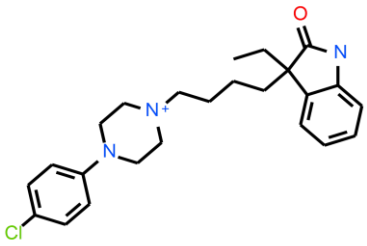
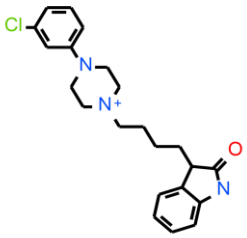
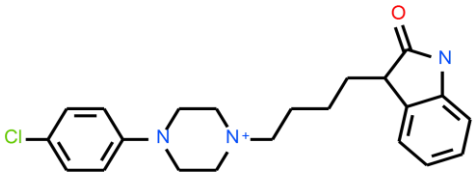
The ligands used for docking in this study fitted in to the binding site of the 3 different homology models of the human 5-HT7 receptor as we had expected, and mostly in accordance with previously published SAR, docking and mutation studies investigated. This includes the Asp3.32 ionic bond, hydrogen bonds to residues Ser5.42, Thr5.43 and Ser6.55 in the pocket between TMH4-6 and van der Waals interactions. Only one H-bond to residues in the area between TMH7-3 was seen, and that was to Trp7.40. This only happened on one single result, and the energy of the complex was high. Agonists and partial agonists docked mostly in the pocket between TMH4-6, with a few exceptions of the partial agonist where it was more centrally placed. The larger antagonists filled out both the pocket between TMH4-6 and TMH7-3. Residues that repeatedly had hydrophobic interactions to ligands (via van der Waals) include Val2.60, Phe3.28, Val3.33, Ile3.29, Phe6.51, Phe6.52, Leu7.32, Leu7.39. The repeating antagonist interaction with Val2.60, and both agonist and antagonist interaction with Val3.33, might be worth further investigation to clarify their role in ligand binding. Also a few results indicated hydrogen bond between Tyr5.38 and antagonists.

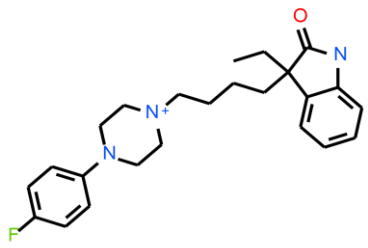
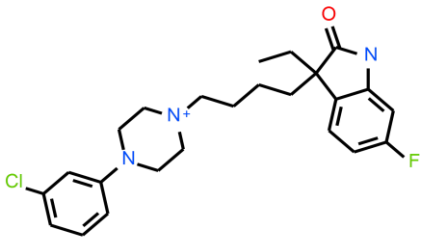
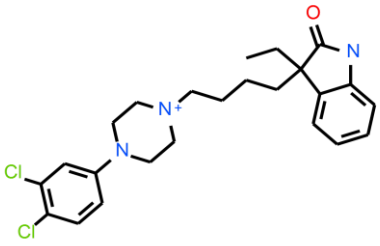
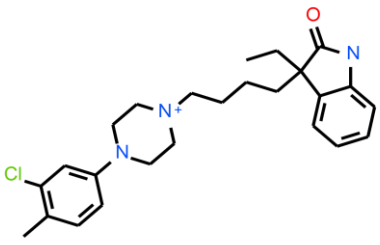
The only significant difference we could register between the results from the 3 different homology models, was that the one based on beta1 adrenergic receptor with a partial agonist complex yielded less successful dockings and in general a higher energy score. This might suggest that this crystal structure is less suited to be used as template for homology models intended for docking with antagonists.

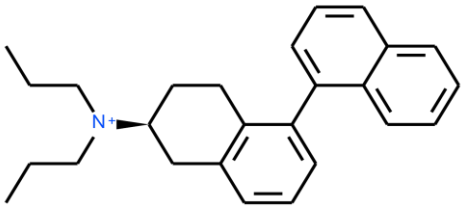
Ligands used in the dockingbatch			
Ligand Nr	**Formula**	**CHEMBL ID**	**Ligand type**
 1	C11H14N3O	CHEMBL18840	Agonist
 2	C27H33N4O2S	CHEMBL430706	Antagonist
 3	C25H31FN3O3S	CHEMBL413707	Antagonist
 4	C26H31ClN3O3S	CHEMBL116292	Antagonist

5 	C26H32N5O3S	CHEMBL115262	Antagonist
6 	C26H31FN3O3S	CHEMBL114345	Antagonist
7 	C25H29FN3O	CHEMBL9951	Antagonist
8 	C25H30N3O	CHEMBL267062	Antagonist

9 	C15H19N2S	CHEMBL161765	Partial agonist
10 	C15H25N2S	CHEMBL158402	Partial agonist
11 	C24H30ClFN3O	CHEMBL409662	Antagonist
12 	C24H31ClN3O	CHEMBL408976	Antagonist

13 	C24H30ClFN3O	CHEMBL406414	Antagonist
14 	C24H31ClN3O	CHEMBL261719	Antagonist
15 	C22H27ClN3O	CHEMBL261209	Antagonist
16 	C22H27ClN3O	CHEMBL260994	Antagonist

 17	C24H31FN3O	CHEMBL260872	Antagonist
 18	C24H30ClFN3O	CHEMBL259549	Antagonist
 19	C24H30Cl2N3O	CHEMBL259087	Antagonist
 20	C25H33ClN3O	CHEMBL259086	Antagonist

 21	C26H32N	CHEMBL596959	Partial agonist

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