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„Modafinil Analogues: A docking study on the human Dopamine Transporter“

verfasst von / submitted by

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1 Introduction

1.1 The SLC6 transporters – Overview

The members of the SLC6 family are symporters, which use the cotransport of Na^+ and Cl^- down their electrochemical gradient as energy source for the transport of their substrates across biomembranes [1]. Although the function of Cl^- is not fully resolved, it is hypothesized that it might be relevant in regard to strengthen Na^+ affinity [1]. They are furthermore part of the Neurotransmitter Sodium Symporter (NSS) family, which in turn is a division of the amino acid-polyamine-organocation (APC) superfamily [2].

The SLC6 family can be divided into 4 subgroups (Fig. 1) [3]:

- The GABA transporters
- The amino acid/orphan transporters
- The amino acid transporters
- The monoamine transporters: dopamine (DAT), norepinephrine (NET) and serotonin (SERT)

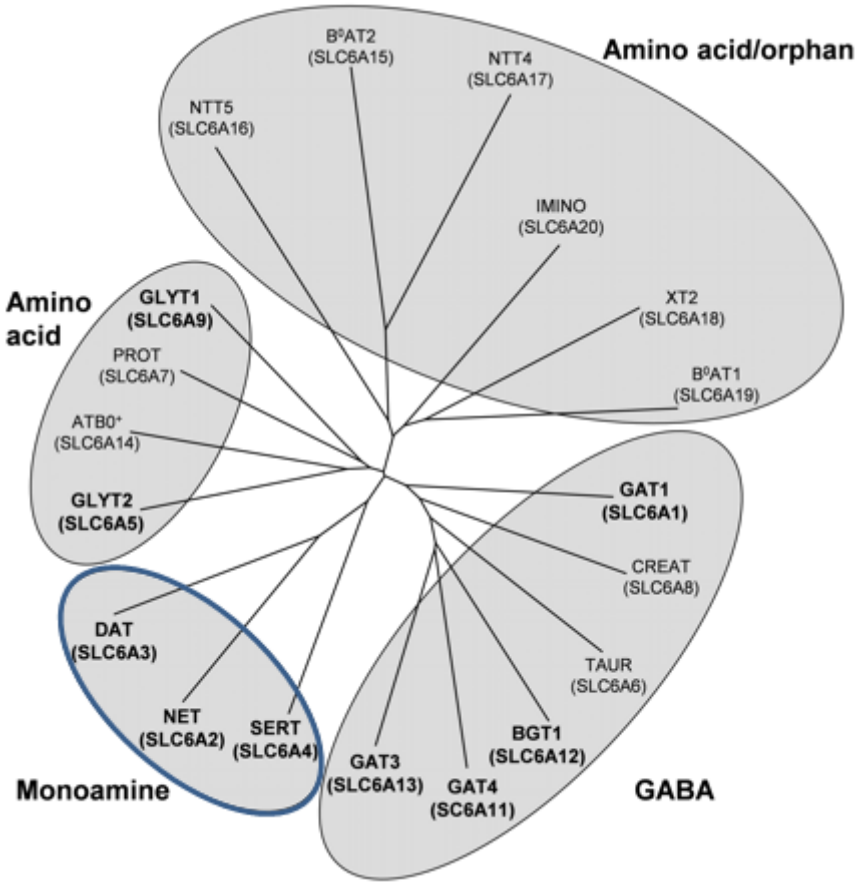


Fig. 1: Phylogenetic tree which shows the SLC6 family members. Adapted from Kristensen et al. [3]

SLC6 transporters can be found in various places in the human body. In the central and the peripheral nervous system, they play a fundamental role in signalling between neurons, and can moreover be found on neurons as well as glia. In addition, they are also located in numerous non-neural tissues such as kidneys, where they are believed to take part in the regulation of osmotic balance, intestine and testis [1].

Because of this significant role in the nervous system, members of the SLC6 transporters are often associated with drug abuse and neurological disorders [1] such as attention deficit hyperactivity disorder (ADHD), mental retardation, Tourette syndrome, schizophrenia, Parkinson disease, addiction, autism and mood disorders like depression, anxiety obsessive compulsive disorder and post-traumatic stress disorder [2]. Hence, SLC6 transporters are considered a prominent target for therapeutic research [1].

1.2 The human dopamine transporter (hDAT)

1.2.1 Structure

As a member of the SLC6 transporter family, the human dopamine transporter consists of 12 membrane spanning domains with intracellularly located N- and C-termini [3]. At the same time this protein can be labelled as a homodimer, which contains 620 amino acids each [1].

1.2.2 Function

The main region where dopamine transporters can be found in the human body is the brain [1], [2] although they can also be observed in the gut [1]. Their most important task is the maintenance of a dopamine homeostasis [3], which is crucial for many functions associated with the nervous system such as learning, mood, attention, movement, appetite, sleep and reward [2].

Through the VMAT (Vesicular Monoamine Transporter) dopamine is stored presynaptically in vesicles. After their release into the synaptic cleft, the neurotransmitter binds to the responding dopamine receptors and causes various effects. In order to regulate this process, the dopamine transporter is responsible for the reuptake of dopamine from the synaptic cleft into the presynaptic region. As shown above, this influx of dopamine is

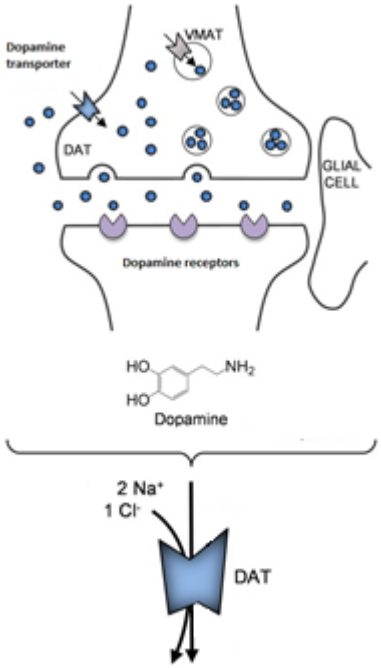


Fig. 2: Illustration showing the regulation of the dopamine transmission adapted from Kristensen et al. [3]

accompanied by an influx of sodium ions [3] (Fig. 2). Furthermore, there are three residues, namely His193, His375 and Glu396, which are associated with Zn^{2+} binding. This ion might be responsible for inhibition of conformational changes necessary for the transport of dopamine. In addition, the residues Asp79 and Tyr335 might also be crucial for the transport of this neurotransmitter, since mutations of these amino acids lead to complications in regard to binding, uptake and equilibrium of translocation [1], [4].

1.2.3 Physiological aspects

The dopamine transporter is responsible for keeping the dopamine level in an equilibrium. If a malfunction occurs in the transport mechanism, drastic effects can be the consequence: Experiments on DAT-KO (knock-out) mice have shown a 5fold increase of dopamine concentration in the EC region whereas, the level of dopamine in the whole brain tissue was decreased by 95% and due to inferior storage of the neurotransmitter in vesicles, the subsequent release was therefore reduced by 75% [3].

Apart from that, DAT-KO-mice showed a distinctive behaviour compared to the wildtype animals. They demonstrated hyperactive behaviour, deficits in cognitive abilities and problems concerning sleep regulation and movement [1]. But as soon as those mice were treated with amphetamine, the hyperactivity decreased, which is a direct opposite to the wild type animals, in which this therapy with amphetamines would actually lead to this condition [3]. Furthermore, it has been shown that DAT-KO mice can survive critical doses of methamphetamine and the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which would normally lead to death. This fact suggests that DAT is essential for the effect of these substances [1].

Some of these expressed symptoms of DAT-KO-mice can be found in patients suffering from ADHD. In accordance to this, the mutation A559V in TM12 of the human dopamine transporter has been associated with two male siblings with ADHD and one patient with bipolar disorder [3].

1.2.4 Pharmaceutical aspects

Because of their involvement in many states of dysregulation in the nervous system, including mental disorders, dopamine transporters are targeted by various substances. The first drugs which were discovered to interact with monoamine transporters are the tricyclic antidepressants [3], which are indicated for depression and neuropathic pain [5]. But since this group of drugs has a broad affinity for a variety of proteins and transporters, other, more specific substances have evolved, including selective serotonin uptake inhibitors (SSRIs),

which only target the SERT and selective norepinephrine reuptake-inhibitors (NRIs), which correspondingly only target the NET [3].

Amphetamine and its analogues are transported as competitive inhibitors, which means they act as substrates. Equally to dopamine, they are transported into the cell by the transporter, where they replace the neurotransmitter in the vesicles. Subsequently, the excess dopamine is released by the transporter back into the synaptic cleft, where it provokes an overstimulation of the cell by binding very frequently to the dopamine receptor.

Although methylphenidate is an amphetamine analogue, its effect on the dopamine transporter is significantly different, since it is a non-transported inhibitor. As ADHD is considered to be caused by a dysregulation of the dopamine homeostasis, it is nowadays treated with amphetamines, as it is the case with narcolepsy [3]. Another treatment for the mentioned diseases is Modafinil [6], which has a similar mechanism of action as methylphenidate and will be discussed in depth later on in chapter 1.3.

Equally important is the effect of cocaine and its analogues, since it has led to a widespread drug abuse in today's society. Being a non-transported inhibitor, cocaine directly blocks the dopamine transporter, which leads to higher levels of the neurotransmitter in the synaptic cleft because of the ongoing exocytosis. This also leads to an excessive binding to the receptors. Despite cocaine binding to all of the monoamine transporters, it has been suggested that the rewarding effects following its abuse may mostly be associated with the dopamine transporter [3].

Even though cocaine abuse is a serious contemporary problem, a suitable substitution therapy has not yet been discovered. There might be a new approach in using benztropines as therapeutics in this area, as they interact with the transporter too, but are not as highly potent as cocaine analogues [2].

A lot of research is also going on regarding Parkinson's disease, as this condition is linked to the degeneration of dopaminergic cells. Substances with the purpose to terminate dopamine uptake into degenerated cells are used on the dopamine transporter, as well as compounds for diagnostic positron emission tomography (PET) [1].

1.2.5 dDAT and the primary binding pocket

Penmatsa et al. managed to create an x-ray crystal structure of the dopamine transporter derived from the Drosophila melanogaster (dDAT) in complex with nortriptyline, a tricyclic antidepressant. Since this template shows a sequence identity of over 50% to its mammalian pendant [5], it can be useful in order to find out more about the mode of action and structural features of molecules that unfold their effects on hDAT. The complex with nortriptyline (Fig. 3)

shows, that the primary binding site of dDAT can be divided into three subsites, which harbour specific moieties of the inhibitors according to their own properties.

- *Subsite A* includes residues Asp46, Phe43, Gly322, and Ser421 and mostly interacts with the polar, amine part of the structure.
- *Subsite B* includes the residues Phe325, Val120, Ala117, Asp121, Gly425, Ser422, Tyr124
- *Subsite C* is defined by Phe319, Asp475 and Ala479 and together with the residues of subsite B they interact with the tricyclic, hydrophobic moiety of the molecule.

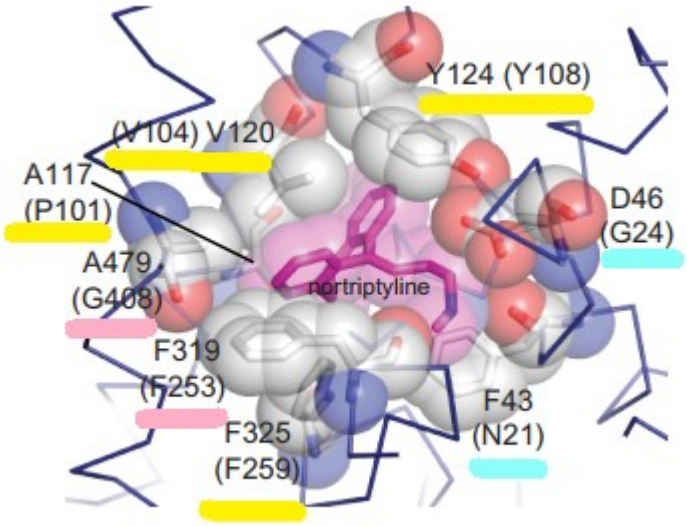


Fig. 3: dDAT in complex with nortriptyline adapted from Penmatsa et al. [5]. Subsite A is shown in turquoise, subsite B in yellow and subsite C in pink

1.3 Modafinil, (*RS*)-2-[(Diphenylmethyl)sulfinyl]acetamid

1.3.1 Structure and medical use

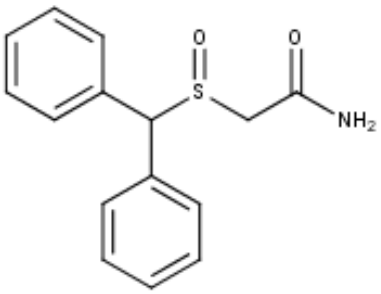


Fig. 4: Modafinil

Modafinil (Fig. 4 - Marvin was used for drawing, displaying and characterizing chemical structures, substructures and reactions, Marvin 15.2.23, 2015, ChemAxon http://www.chemaxon.com) has been widely used as treatment for various sleep disorders such as narcolepsy [6] due to the fact that it has a positive effect on wakefulness and prolongs the attention span [7]. For this reason the drug is also used in military surroundings as it has been discovered further that

although it might be a wake-promoting agent, it does not prevent individuals from sleep when an opportunity arises [8]. Given this fact, it is the drug of choice in military campaigns, where individuals often have to cope with sleep deprivation [6].

The drug is also used as treatment for ADHD, though the mostly used substances for this disorder are methylphenidate and amphetamines [6]. However, Modafinil might gain more popularity since it does not display the same high liability to addiction as the previous mentioned medications, although it does improve symptoms linked to said sickness [9].

Furthermore, this drug may have positive effects on patients dealing with cognitive impairments caused by mental disorders such as schizophrenia and substance abuse. Most drugs associated with addiction work through the dopaminergic system in the brain area, where the continued exposure to drugs like cocaine and amphetamine leads to modulation of the dopamine release. This condition leads to diverse side effects of drug abuse concerning the state of mind such as problems with memory and attention. Modafinil might become a suitable substitution as it does help with those effects and does not display the high abuse liability of amphetamines and methylphenidate [6].

On the other hand, Modafinil is nowadays widely abused by healthy individuals as cognitive enhancer. Mainly high school and university students use Modafinil (Provigil) or Methylphenidate (Ritalin) as “smart drugs” in order to increase performance in attention, memory and learning skills [10], [6].

1.3.2 Modafinil’s effect on DAT

After binding to the dopamine transporter, Modafinil induces an inhibition of the dopamine re-uptake, which leads to an increased amount of dopamine in the synaptic cleft. This situation leads to an ongoing stimulation of dopaminergic receptors resulting in Modafinil’s described effect [11]. Though studies have shown that this drug has a lower affinity to DAT than methylphenidate, the occupation takes place at a similar level [6]. Furthermore it has been shown that Modafinil causes a higher dopamine concentration in the frontal cortex, which leads to the assumption that this brain area might be crucial for cognitive enhancement [6], [12]. Moreover, studies have shown that DAT knock-out mice do not display an increased wakefulness after Modafinil administration, strengthening this assumption [6], [13].

1.3.3 Published Modafinil pose

The R-enantiomer of Modafinil has already been described as the more stable and more active enantiomer and therefore is of higher interest than the S-enantiomer [7], [14]. After creating a homology model of hDAT using the MODELLER algorithm with the crystal structure of LeuT, the bacterial counterpart of the dopamine transporter as a template, Schmitt et al. [7] performed a docking study using MOE [15] in order to discover Modafinil's most probable pose in the binding site of the human dopamine transporter.

Studies performed on LeuT have confirmed the existence of an S1 primary binding site in the center of the protein and a second binding site called S2 above the S1 binding site, located in the extracellular pathway of the transporter. Those binding sites were also identified by using the "Site Finder" in MOE [15], and thus also used as binding sites for the docking study. Using the standard scoring method (London dG) the following docking pose was acquired:

In the S1 site of hDAT, Modafinil was located in a way that the diphenyl ring system faced Val152, Gly153, Tyr156 (TM3), which are part of the subsite B, while the sulfinylacetamide moiety pointed towards Phe76, Ala77, Asp79 (TM1), which belong to subsite A, and Phe320, Ser321, Leu322 (TM6), which are located in subsite C (Fig. 5) [7].

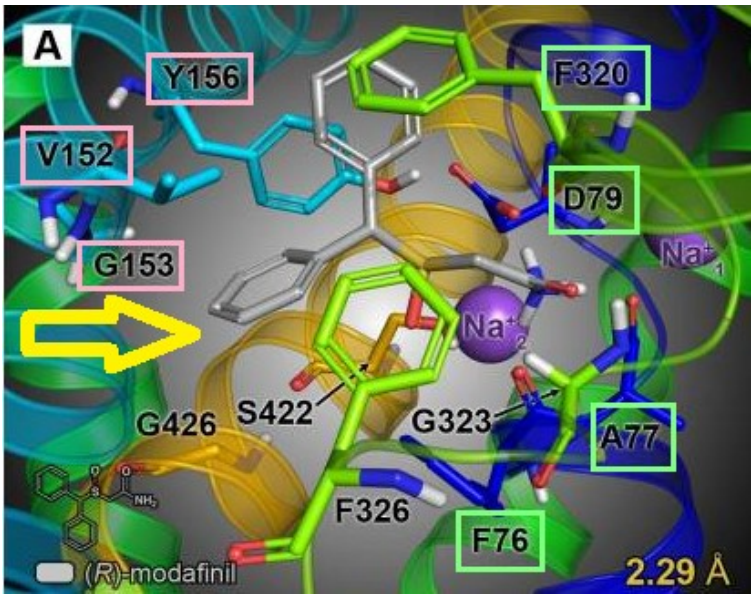


Fig. 5: Modafinil (grey) inside the hDAT S1 binding site. The diphenyl ring system faces the amino acids highlighted in pink (subsite B) and the sulfinylacetamide moiety is facing the amino acids highlighted in green (subsites A & C) Adapted from Schmitt et al. [7]

1.3.4 Published Modafinil analogues

Previous studies already provided the effect of Modafinil analogues on the dopamine transporter: Cao et al. [14] synthesized a set of analogues and evaluated them in regard to their binding affinity to the dopamine transporters of rat brains. Since the sequence identity of the rat dopamine transporters and the human dopamine transporter make up ~93.1% according to an alignment performed with UniProt [16], it can be assumed that the transporters correspond in their activity. Their work shows further, that the R-enantiomer of Modafinil is the more stable one and has a prolonged activity compared to the S-enantiomer. Since a previous study [17] had shown that para-halo-substituted analogues of benztropine structures, which have a biphenyl moiety comparable to Modafinil, have an increased affinity at DAT, Cao et al. [14] have chosen a similar approach.

The synthesized Modafinil analogues include halogens in the para positions of both biphenyl rings, which indeed did mostly lead to a higher binding affinity on DAT. As could be seen, mere H atoms had a lower affinity than an F, which in return had a lower affinity than Cl, which was only surpassed by Br atoms. Remarkably, this assay showed a different outcome than the one with the benztropine analogues, as this approach produced the following order of binding affinity on DAT: Br<H<Cl<F [15].

Moreover, it has been shown that the sulfoxide function may be ideal for binding to the dopamine transporter, if the adjacent primary amide function is not substituted. But removing the S=O function does not decrease the binding affinity significantly [14].

Finally, another group of analogues was synthesized in which the original primary amide function was replaced by tertiary amides and amines. While the tertiary amides decreased the binding affinity, the amines which also showed a higher water solubility by forming salts, induced a higher activity and also contained the compound with the highest potency concerning DAT [14].

2 Aim of the thesis

As described above, Modafinil has a prominent role concerning its effect on the dopamine transporter which in turn has an important impact on the human nervous system because of its significant influence on the physiological and psychological health of human beings. For this reason, there is a lot of research going on in that area and new substances are being synthesized and tested to explore their activity.

Up today, Modafinil's mechanism of action is not fully understood. It is therefore highly important to investigate the drug-transporter interactions on a molecular level. Computational

approaches can be very helpful to understand the context between the structural characteristics of compounds and their measured activity values. The 3D structure of the dopamine transporter (Drosophila melanogaster) was published in 2013 [5], therefore it is also possible to conduct structure-based studies allowing to gain deeper insights into the interactions of this prominent compound and its target.

A set of Modafinil analogues was synthesized and tested in vivo to determine their IC50 values by Saroja et al. [18] and subsequently used for this study to analyse their structure-activity relationships. Afterwards several in silico procedures such as a docking study and common scaffold clustering were performed on handpicked compounds to compare the resulting scores with the in vitro experiments.

The evaluation of the structure-activity relationship shows how much the biological activity of the compounds is dependent on the alteration of the original Modafinil structure. Simple modifications have a visible effect on the IC50 value since certain interactions in the Modafinil binding site of the human dopamine transporter can be lost or gained by adding or removing specific moieties. According to this, a docking study was performed with various compounds to elucidate the circumstances in the binding site. It shows explicitly which pose of the used analogues has the highest score and therefore the best binding affinity due to interactions with the amino acids shaping the human dopamine transporter. Thereupon a Common Scaffold Clustering was conducted in order to unite similar poses into individual clusters, which were then compared to the Modafinil pose already published by Schmitt et al. [7].

Thus, the aim of this diploma thesis is to analyse this set of Modafinil analogues in multiple ways and to find a correlation between their bioactivity and the discoveries from the computational experiments on hDAT.

3 Methods

3.1 The Modafinil Analogues

The research group of Prof. Gert Lubec (Medical University of Vienna) provided us a dataset consisting of 55 compounds containing the Modafinil scaffold. Out of these, 15 compounds were selected for computational analysis due to high, medium or weak affinities. The structures can be explored further in the appendix (see appendix 1). The compounds were synthesized and tested in vitro and in vivo in Prof. Gert Lubec's lab.

3.2 Biological testing of the Modafinil Analogues

The human dopamine transporter was expressed in HEK293 cells in order to analyse the reuptake inhibition. The IC50 values resulting from these experiments can be found in the appendix (Appendix 1). Furthermore, the compounds were also tested in vivo on 72 male Sprague Dawley rats in a radial arm maze. After the training sessions the rats were sacrificed, and their brains were removed for further biochemical exploration.
For further details about the methods used for the biological testing of the compounds see Saroja et al. [18].

3.3 Published Analogues

3.3.1 CE-104

In this compound, namely 2-(benzhydrylsulfinylmethyl)-4-methylthiazole (Fig. 6) the amide function of Modafinil is replaced by a thiazole with an adjacent methyl on position 5. The substance was tested regarding its ability to inhibit DAT, SERT and NET. Though an inhibitory effect was determined on DAT (IC_{50}: 27.9 µM compared to Modafinil's IC_{50}: 11.1 µM) and NET, an effect on SERT was not confirmed. Moreover, the substance's ability to improve cognitive abilities was tested on male rats in a radial arm maze (1 and 10mg/kg), where an enhanced spatial working memory was observed in both dosage groups [19].

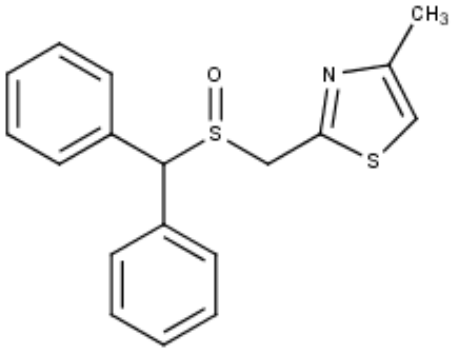


Fig. 6: Compound CE-104

3.3.2 CE-111

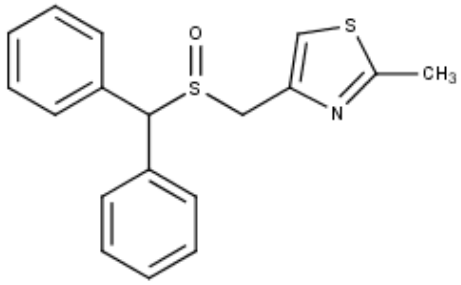


Fig. 7: Compound CE-111

The substance 4-(diphenyl-methanesulfinylmethyl)-2-methyl-thiazole (Fig. 7) showed an IC_{50} value of 3.2 µM when tested for its ability to inhibit the re-uptake of dopamine by the dopamine transporter, which is a better value than shown by Modafinil itself. Similarly to CE-104, Modafinil's amide function is replaced by a thiazole linked to the original structure through the fourth position. It also displays a methyl group, which is attached on position 2. The results of the study demonstrated that the compound CE-111 was able to block DAT specifically and therefore increasing dopamine levels without being a substrate of the transporter. Similarly to Modafinil, the substance was also able to cross the blood-brain barrier in in vitro experiments. In vivo, CE-111 was tested on male Sprague-Dawley rats in a radial arms maze in a dosage of 1 or 10 mg/kg compared to a control group. Subsequently, improvements in memory performance were observed in all groups, although the control group remained stable from the 6th day onwards. The groups administered with the compound CE-111 still showed gradually improving cognitive abilities on the 8th and 9th day [18].

3.4 Structure-activity relationships

The following chapters cover the change of the activity value according to the alteration of the structure of the substance. The original Modafinil structure shows an IC50 value of 11.1 µM. The IC50 values are shown with one digit after the decimal point, the original values can be looked up in the appendix.

3.4.1 Thiophene moiety

Compound CE-146 (Fig. 8) displays a thiophene moiety instead of Modafinil's amide moiety. The IC50 has a value of 1.4 µM and is therefore also the best value of all the tested compounds, which means that this compound has the highest inhibitory effect on the dopamine transporter.

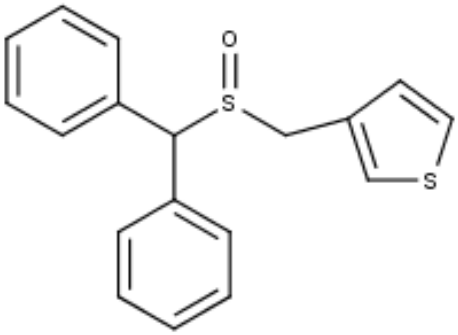


Fig. 8: Compound CE-146

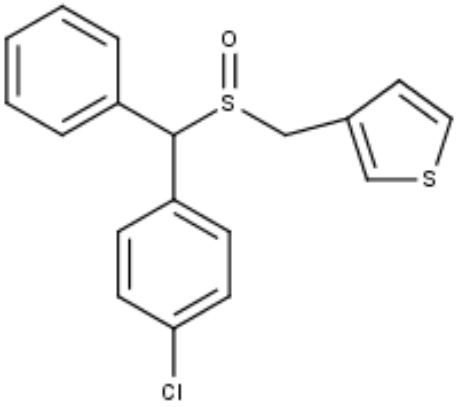


Fig. 9: Compound CE-140

If there is a chlorine atom added in para – position to one of the phenyl groups as depicted in Fig. 9, the IC50 value rises to 3.2 µM.

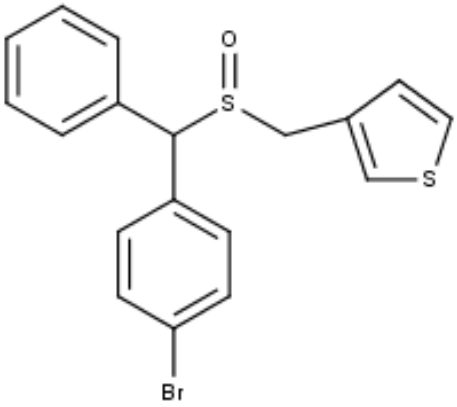


Fig. 10: Compound CE-147

If the chlorine atom is replaced by a bromine atom as shown in Fig. 10, the IC50 rises further to 4.3 µM.

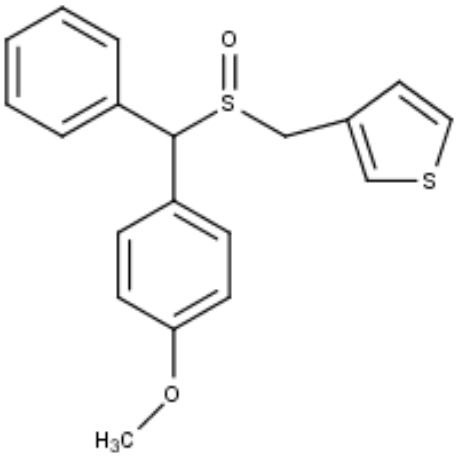


Fig. 11: Compound CE-149

Furthermore, if a methoxy group is added in the way described above as shown in Fig. 11, the value rises slightly higher to 5.7 µM.

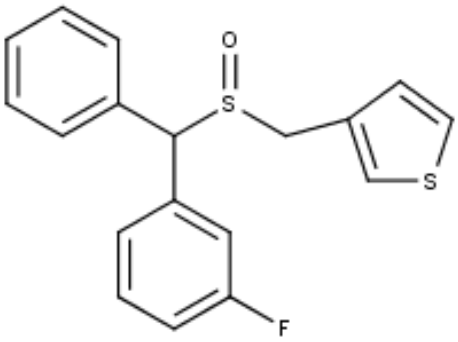


Fig. 12: Compound CE-148

In case another halogen, namely a fluorine atom, is added in meta-position to a phenyl group as shown in Fig. 12, the IC50 value of 5.8 µM does not show a significant difference from the halogens added in para-position.

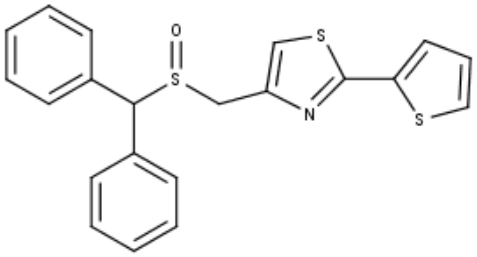


Fig. 13: Compound CE-110

Should a thiazole ring be added in between the Modafinil scaffold and the thiophene ring as shown in Fig. 13, the IC50 climbs even higher to 33.5 µM.

3.4.2 2-Thiazole

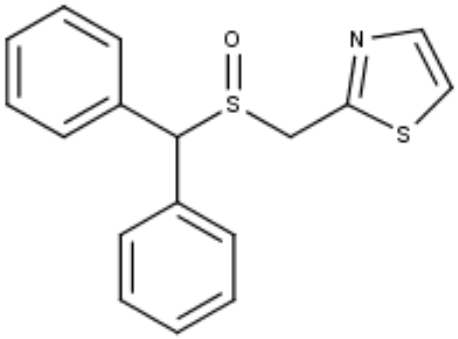


Fig. 14: Compound CE-103

If a thiazole group is connected on its second position to the basic structure of Modafinil instead of the amide group (Fig. 14), the value rises to 14.7 µM, which does not show a significant difference to the value of the original Modafinil structure.

But if the phenyl rings of compound CE-103 are linked by a single bond (Fig. 15) the IC50 value rises dramatically to 663.9 µM, which is the highest value of all the compounds of this set of molecules. Similar outcomes have already been published in connection to the GAT1, which is a GABA transporter and therefore part of the SLC6 family. Tiagabine is a GAT1-Inhibitor, which means that it is responsible for the inhibition of the GABA uptake, which subsequently leads to higher GABA levels in the synaptic cleft. A comparison between derivatives of tiagabine in terms of activity on the binding site depicts a loss of activity linked to a bond between two aromatic structures as well [20].

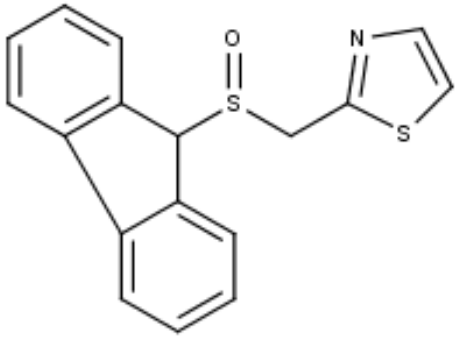


Fig. 15: Compound CE-115

In case a methyl moiety is added on the fourth position of the thiazole ring (Fig. 16), the IC50 value rises to 27.9 µM, which is comparable to the value of 27.5 µM, which is achieved when there are two methyl groups added to the positions four and five (Fig. 17).

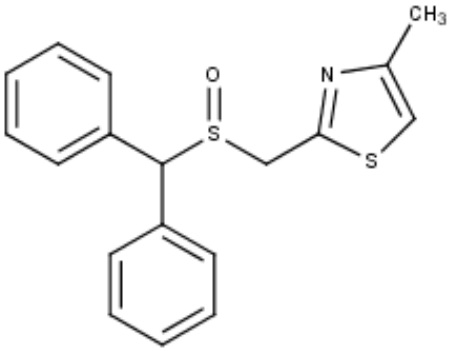


Fig. 16: Compound CE-104

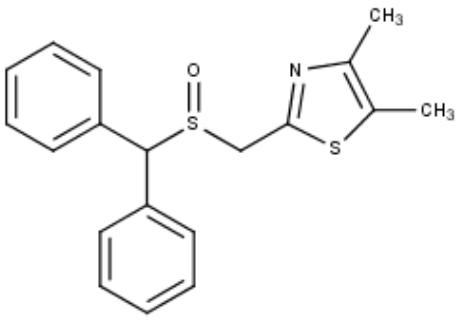


Fig. 17: Compound CE-117

3.4.3 4-Thiazole

Given that a thiazole group is added to the basic structure (Fig. 18), a slightly better IC50 value of 7.3 µM than the original one occurs. But if to this thiazole a thiophene moiety is added (Fig. 13) the value again rises to 33.5 µM.

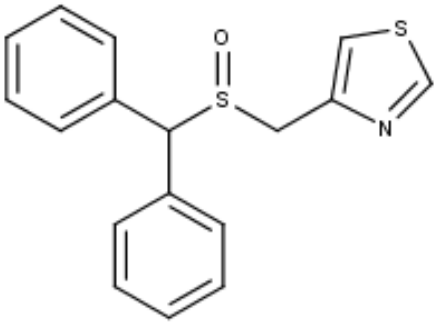


Fig. 18: Compound CE-105

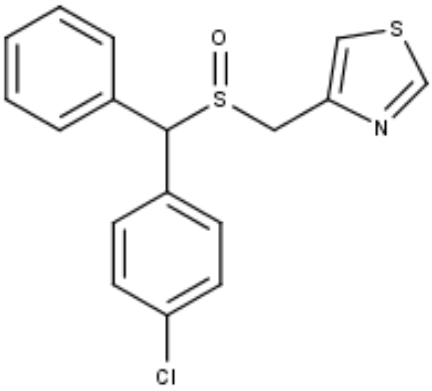


Fig. 19: Compound CE-143

If a chlorine atom is added in para-position to one of the phenyl groups as shown in Fig. 19 the IC50 value drops to 2.9 µM.

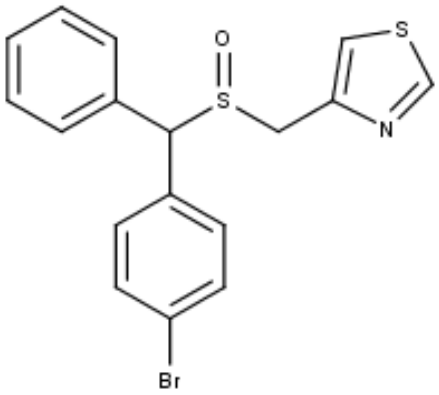


Fig. 20: Compound CE-144

If this chlorine atom is replaced by a bromine atom (Fig. 20) the value drops to 1.9 µM.

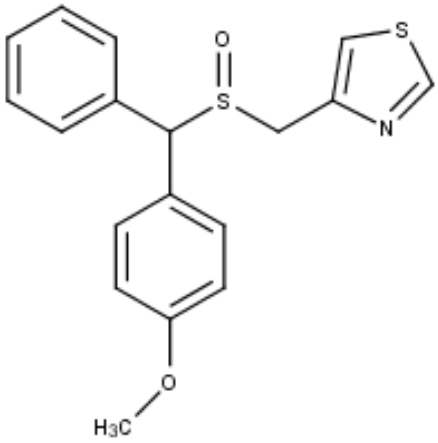


Fig. 21: Compound CE-145

Given that this bromine value again is replaced by a methoxy moiety (Fig. 21), the value rises insignificantly to 4.1 µM.

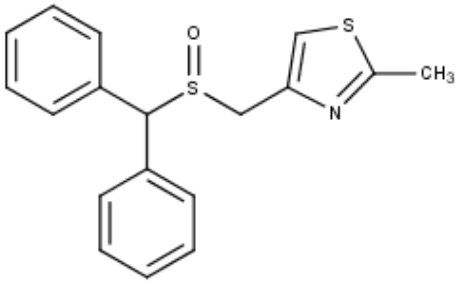


Fig. 22: Compound CE-111

If a methyl structure is added to position 2 of the thiazole ring (Fig. 22) the IC50 value is 3.3 µM, which indicates a stronger activity than a sole thiazole group.

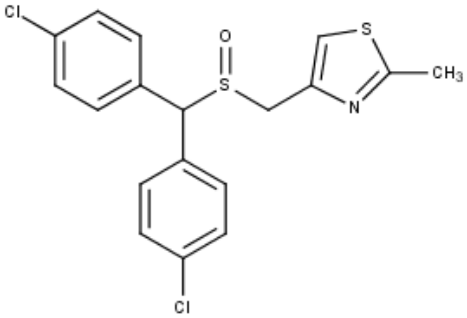
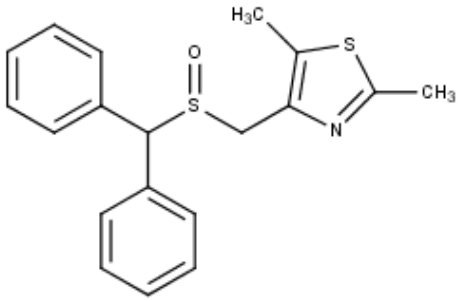


Fig. 23: Compound CE-133

In the case that to compound CE-133 a chlorine atom is added to each phenyl ring (Fig. 23), the IC50 value rises to 16.9 µM.



Should a second methyl group be added onto the fifth position of the thiazole moiety (Fig. 24) of compound CE-111, the value rises significantly to 55 µM.

Fig. 24: Compound CE-121

If the methyl group is replaced by an ethyl group on position 2 of the thiazole moiety (Fig. 25) the IC50 value rises to 19.2 µM, rising even further to 28.4 µM when replaced with a propyl group (Fig. 26), but dropping again to 19.4 µM when a butyl group is added (Fig. 27).

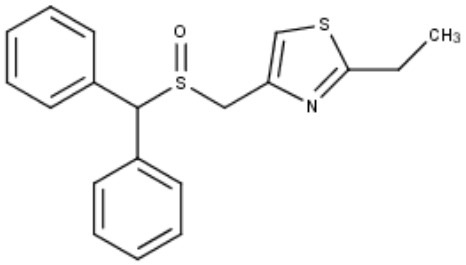


Fig. 25: Compound CE-129

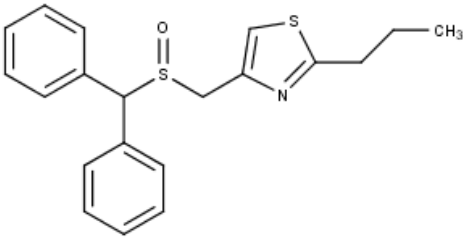


Fig. 26: Compound CE-132

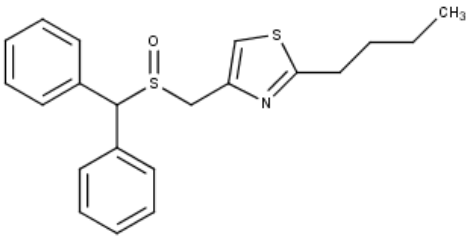


Fig. 27: Compound CE-139

Interestingly there is quite a difference in activity levels when there is an isopropyl group attached to the second position of the thiazole moiety (Fig. 28) to when it is replaced by a

cyclopropyl group (Fig. 29), with the first option depicting a IC50 value of 16.5 µM and the second one a better value with 4.1 µM.

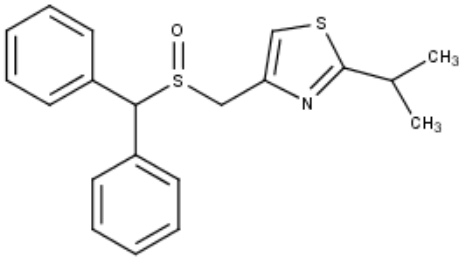


Fig. 28: Compound CE-124

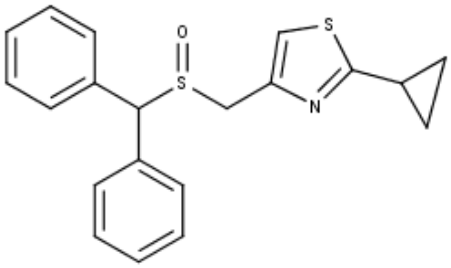


Fig. 29: Compound CE-125

3.4.4 5-Thiazole

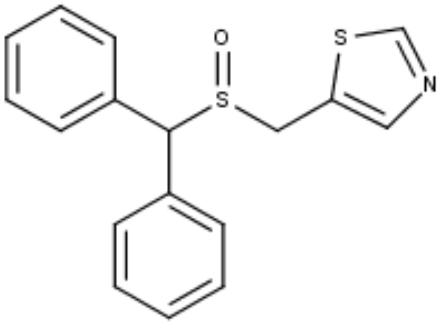


Fig. 30: Compound CE-123

As shown in Fig. 30, a thiazole moiety can also be attached to the basic Modafinil structure through its fifth position, leading to an IC50 value of 4.4 µM.

As previously shown on the other compounds, halogens were added in para-position to one of the phenyl rings of the diphenyl structure here too, namely one bromine-atom (Fig. 31) and one chlorine-atom (Fig. 32), resulting in an IC50 value of 3.4 µM for the first and 4 µM for the second.

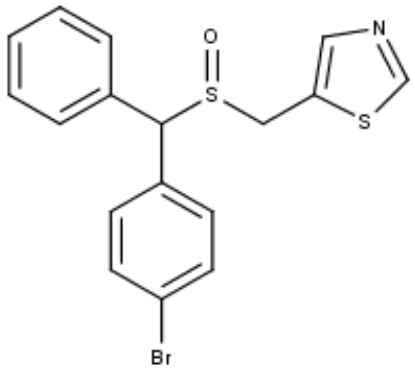


Fig. 31: Compound CE-141

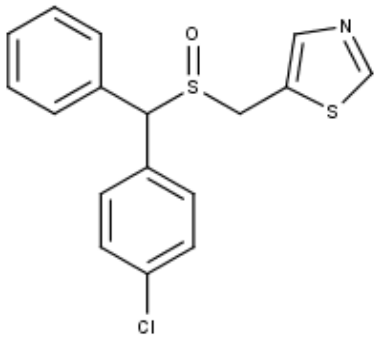


Fig. 32: Compound CE-138

But if the chlorine atom is attached to the thiazole moiety through its second position as shown in Fig. 33, the value rises up to 24.3 µM. A comparable IC50 value, namely 33.9 µM, is achieved when the chlorine-atom is replaced by an isobutyl group (Fig. 34), indicating again that a somewhat bigger structure on this position is not helpful for the compounds' activity level.

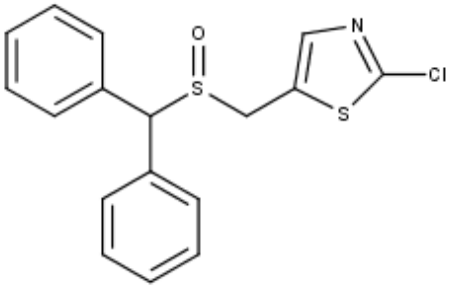


Fig. 33: Compound CE-127

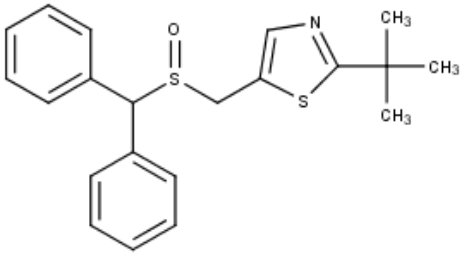


Fig. 34: Compound CE-128

In the case that the same spot from the previous compounds is replaced by a methoxy group as shown in Fig. 35, the IC50 value even reaches 98.5 µM, exceeding all other IC50 values in this group. Though if two methyl groups are attached to the positions 2 and 4 of the thiazole moiety (Fig. 36), a similar value of 87.9 µM is reached.

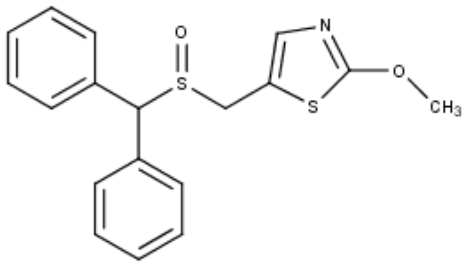


Fig. 35: Compound CE-142

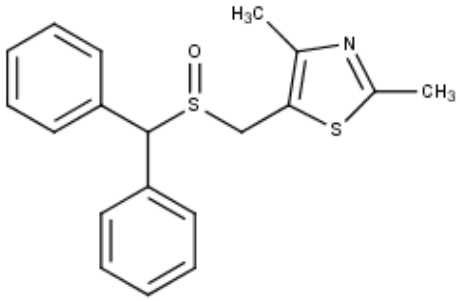


Fig. 36: Compound CE-116

3.4.5 Sulfoxide moiety

Comparing the compound CE-111 (Fig. 37), which is depicting a sulfoxide function in the central area of the molecule, with compound CE-122 (Fig. 38), which lacks this said feature, leads to the discovery of an IC50 value roughly 50 times higher in the second structure. While CE-111 shows a value of 3.3 µM, the value of CE-122 rises up to 148.3 µM.

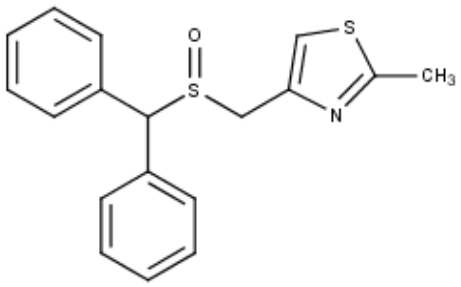


Fig. 37: Compound CE-111

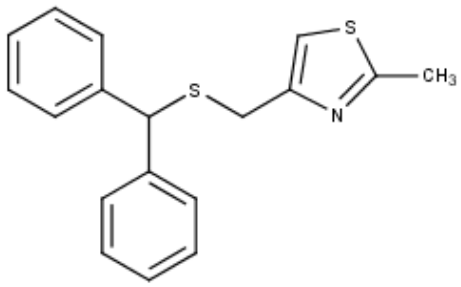


Fig. 38: Compound CE-122

3.4.6 Ethyl instead of methyl linker

The set also displays a compound, which besides the sulfoxide group of the original structure has two C-atoms (Fig. 39) attached instead of one (Fig. 40), building a longer bridge over to the thiazole group. Here too, a significant rise of the IC50 value is evident, in particular from 3.3 µM to 125.1 µM.

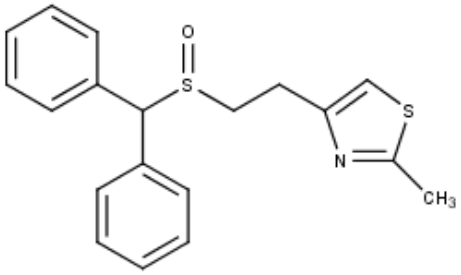


Fig. 39: Compound CE-134

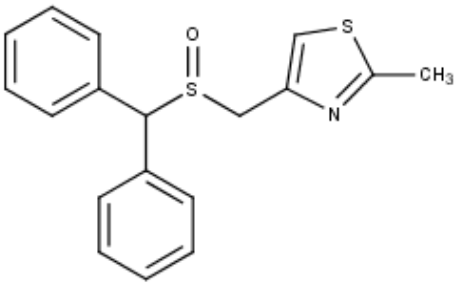


Fig. 40: Compound CE-111

3.4.7 Pyrimidine moiety

This compilation of analogues also has a range of compounds in which the amide structure has been replaced by a pyrimidine moiety. Depending on the way it is linked to the original design and if and how this attached group displays modifications the IC50 value varies.

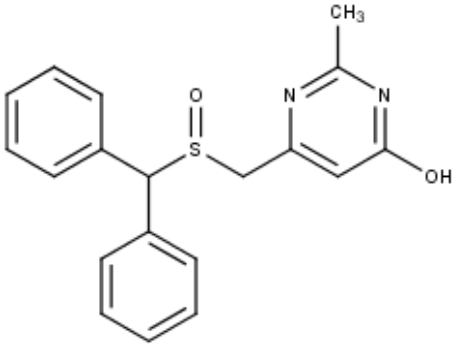


Fig. 41: Compound CE-131

The compound CE-131 (Fig. 41) shows a methyl group on position 2 of the pyrimidine ring and a hydroxyl group on position 4, while being linked to the original structure via position 6. Though this structure is bigger and bulkier than the original amide structure it has better activity levels than Modafinil, as it results in an IC50 value of 3.1 µM.

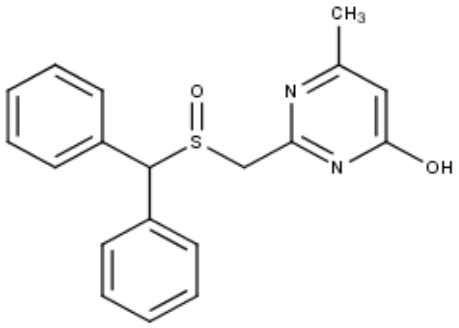


Fig. 42: Compound CE-151

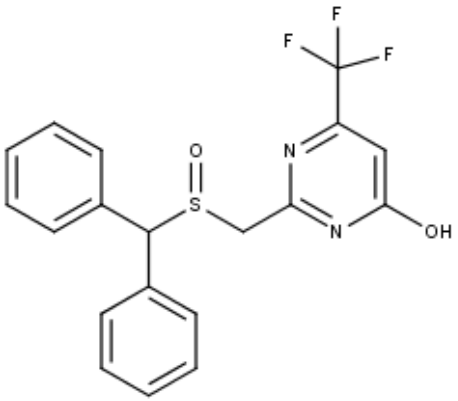


Fig. 43: Compound CE-150

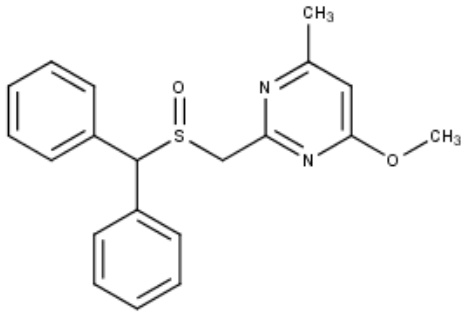


Fig. 44: Compound CE-152

But if the same structure is attached again, though this time through the second position on the pyrimidine ring as shown in Fig. 42, the binding affinity drops again, while the IC50 value rises up to 67.5 µM. If the methyl group on is now replaced by a trifluormethyl-function (Fig. 43) the binding affinity rises again, culminating in an IC50 value of 27.6 µM. In the case that the hydroxyl-group of compound CE-151 is replaced by a methoxy-group as shown by compound CE-152 (Fig. 44), the value rises up to 123.1 µM.

But if all the oxygen-containing groups are left out, the binding affinity drops even lower. Compound CE-153 contains two methyl groups on position 2 and 4 of the pyrimidine ring (Fig. 45) and depicts an IC50 value of 202.1 µM, while compound CE-154, which has just one methyl group on position 5 (Fig. 46) leads to a similar value of 191.8 µM.

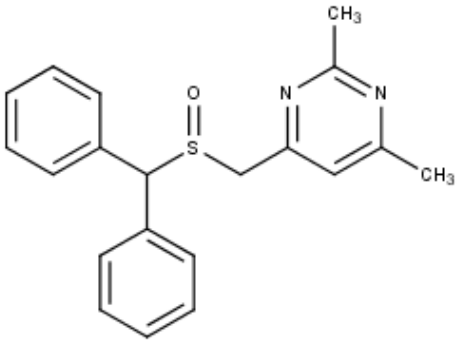


Fig. 45: Compound CE-153

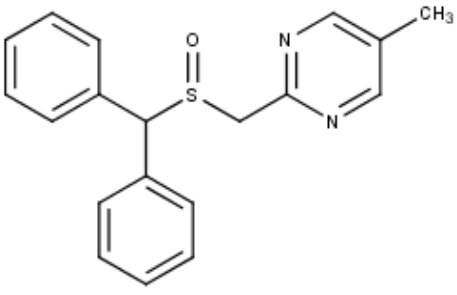


Fig. 46: Compound CE-154

3.5 Structural Alignment

Through structural alignment a comparison between two molecules, in this case two proteins, in terms of their three-dimensional structure and amino acid sequence is possible. Two protein sequences are placed on one another, so that as many amino acids can be matched as possible. Structural alignments can be accomplished by a range of different programs, in this case a program called MOE [15] was used.

3.5.1 MOE (Molecular Operating Environment)

MOE [15] is a computational software which can be used for a large variety of operations concerning molecular modelling, and, among other things, also for structural alignment. These alignments can be altered depending on the operator's intentions, for example they can be specifically coloured, cut out at certain points, or renamed.

3.5.2 The homology model of the human dopamine transporter

4M48 was used as a template to build the homology model of hDAT published by Saha et al. [21]. The RMSD between the final model (Fig. 47) and the template was 0.15 Å according to the backbone atoms of the transmembrane helices, measured in VMD [22]. The code used for this operation can be looked up in the appendix (see 7.2 Extended Data). The outcome shows that the template is very similar to the human dopamine transporter.

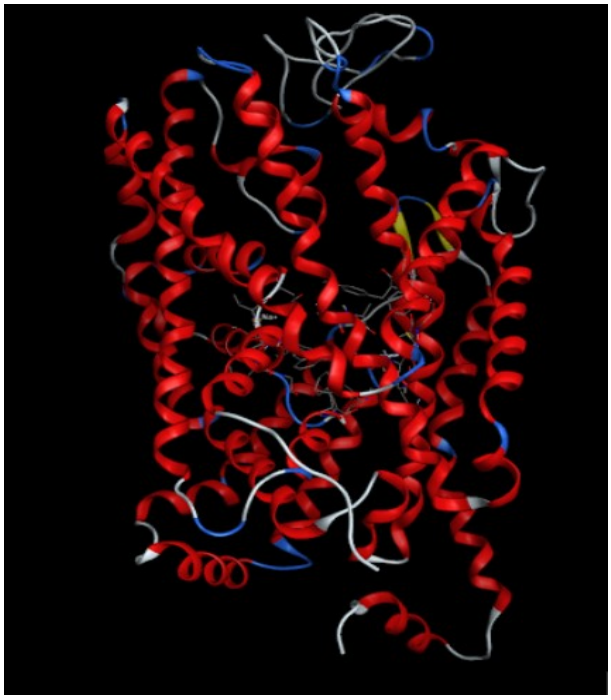


Fig. 47: The homology model of hDAT as seen in MOE [15]

3.5.3 Structural Alignment of dDAT and hDAT

In this case a structural alignment of dDAT, the previously described x-ray crystal structure of the dopamine transporter of Drosophila melanogaster, and hDAT, the human dopamine transporter, was carried out, in order to specify the amino acids of the binding pocket that binds Modafinil in the human analogue. This binding pocket would then later be used for the docking study of the Modafinil analogues. Thus, the three-dimensional structure of dDAT was derived from the "RCSB Protein Data Bank" which can be found under the following link: http://www.rcsb.org/. The chosen template is called "4M48" and is a crystal structure of the dDAT in complex with nortriptyline (2.95 Å). After the alignment of this model and a homology model of the hDAT, the data shown in Table 1 was obtained, whereby the amino acids of the hDAT's binding pocket were defined.

	dDAT (4M48)	**hDAT**
Subsite A	Asp46	Asp79
	Phe43	Phe76
	Gly322	Gly323
	Ser421	Ser422
Subsite B	Phe325	Phe326
	Val120	Val152
	Ala117	Ser149
	Asp121	Gly153
	Gly425	Gly426
	Ser422	Ala423
	Tyr124	Tyr156
Subsite C	Phe319	Phe320
	Asp475	Asp476
	Ala479	Ala480

Table 1: Structural Alignment of the binding pockets of dDAT and hDAT

3.6 Docking study

After the amino acids of the binding pocket of the human dopamine transporter were clarified by a structural alignment, several Modafinil analogues of the previously mentioned set were

chosen for docking studies. Selection was based on affinity differences and the SAR observed. Due to the higher activity and stability of R-Modafinil, only the R-enantiomers of the analogues were used for the study. Furthermore, the binding site was coloured in MOE [15], to distinguish between the subsites – subsite A being turquoise, subsite B yellow and subsite C pink. (Fig. 48)

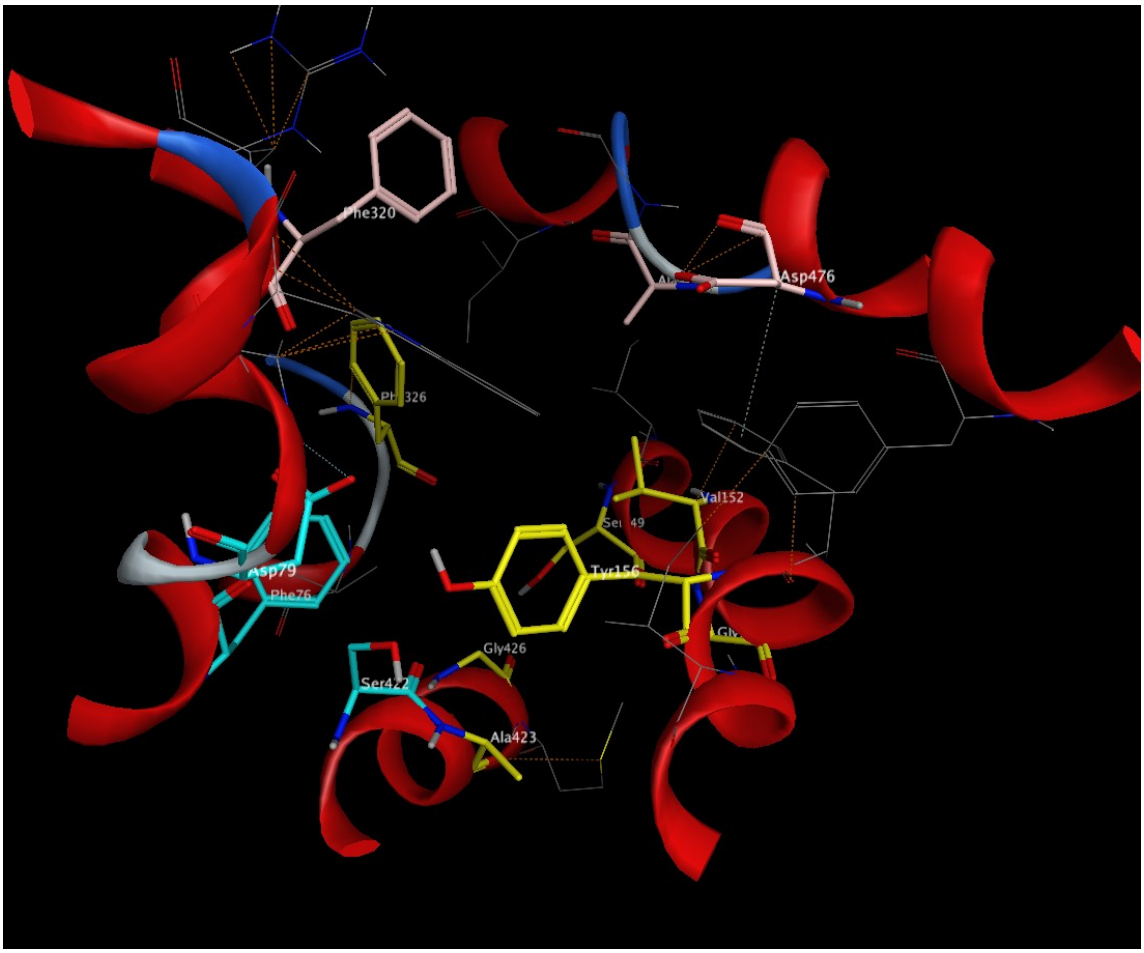


Fig. 48: The binding pocket of the human dopamine transporter as shown in MOE [15]. Subsite A is coloured in turquoise, subsite B in yellow and subsite C in pink

The handpicked compounds, which were used for the docking study are listed in Table 2. The corresponding structures can be looked up in the appendix (Appendix 2).

CE-101	CE-140
CE-103	CE-142
CE-109	CE-144
CE-111	CE-146
CE-112	CE-151
CE-115	CE-153
CE-116	CE-154
CE-133	

Table 2: The handpicked compounds used for the docking study

The next step was the determination of the docking settings. For this purpose, Modafinil underwent the docking procedure with all the possible settings, resulting in different score values (Table 3), which represent the binding affinity of the molecule for the binding site. Given the fact that, the more negative the value, the better the binding affinity, the setting combination "Alpha PMI" and "Affinity dG" was used throughout the subsequent docking study.

Alpha PMI	London dG	-6,6945
Alpha PMI	Affinity dG	-6,7141
Alpha PMI	Alpha HB	-6,6891
Alpha Triangle	Affinity dG	-6,7022
Alpha Triangle	London dG	-6,3305
Alpha Triangle	Alpha HB	-6,6713
Triangle Matcher	London dG	-6,4423
Triangle Matcher	Affinity dG	-6,4111
Triangle Matcher	Alpha HB	-6,4430

Table 3: MOE [15] docking settings – the most negative value is accomplished by the settings "Alpha PMI" and "Affinity dG"

Furthermore, the derived pose was compared with the already published Modafinil pose by Schmitt et al. [7] and shows undeniable similarities. Here too, the diphenyl structure is facing the yellow subsite B, which contains the in the paper [7] mentioned amino acids Val152, Gly153

and Tyr156. Additionally, in this model the sulfinylacetamide chain is also facing the amino acids Phe76, Asp79 and Phe320, which are parts of the remaining subsites A and C (Fig. 49). After selecting the settings, the 15 handpicked compounds went through the docking procedure, resulting in 30 poses per ligand.

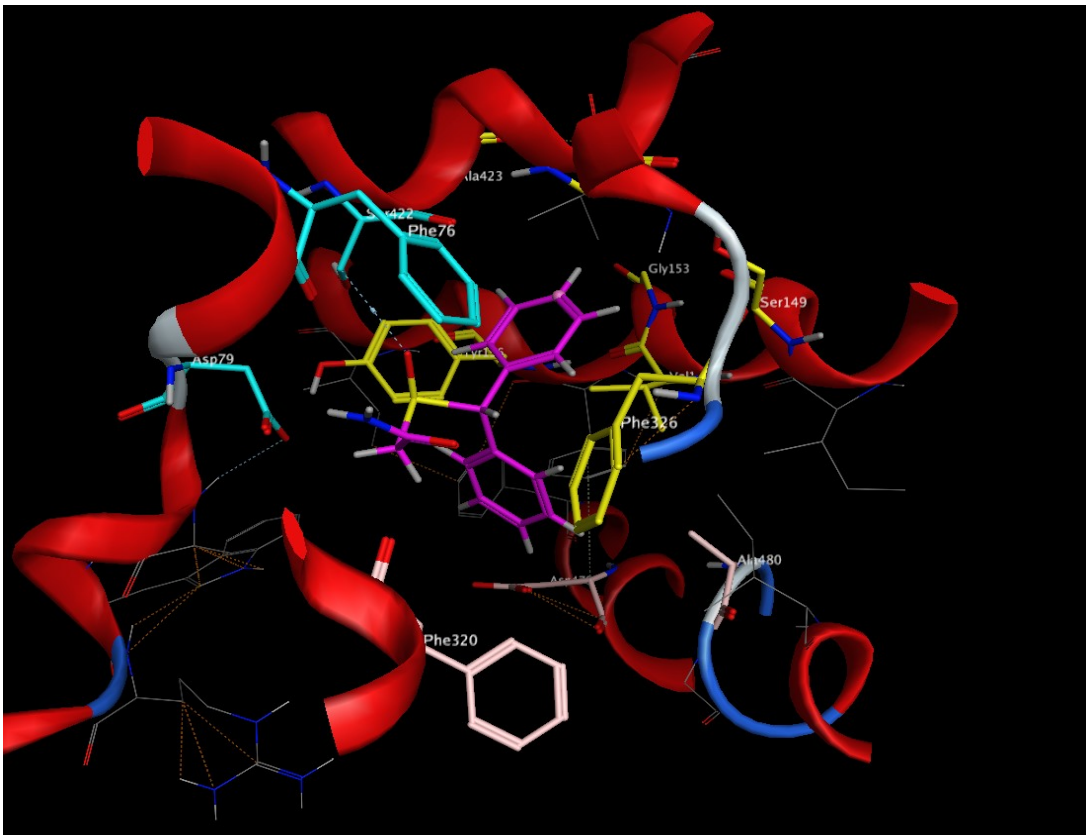


Fig. 49: Modafinil in complex with the hDAT as seen on MOE [15]. Modafinil is coloured in bright pink, whereas subsite A is shown in turquoise, subsite B in yellow and subsite C in light pink

3.7 Common Scaffold Clustering

With the help of this method the various poses of the Modafinil analogues derived by the docking method were split into a series of clusters, all depicting similar positions in the binding spot. Four in-house scripts were used in combination with MOE [15] and the R software [23] to complete this task. The common scaffold used for the procedure is shown in Fig. 50 and described by the SMILES code [#16]([#8])(C)C(c1ccccc1)c1ccccc1.

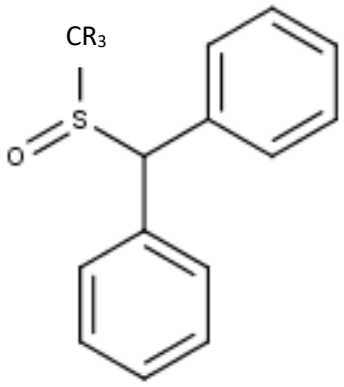


Fig. 50: The common scaffold used for Clustering

4 Results and discussion

This chapter displays the various results gained by the use of the methods described above. The results due to analysis of the structure-activity relationship show how the slightest changes of the original scaffold can alter the biological activity. The docking scores demonstrate how the individual poses of the compounds interact with the binding site and can be compared to the corresponding IC50 values obtained by biological testing. Finally, the interpretation of the results from the Common Scaffold Clustering shows which poses of which compounds are the most similar to the published Modafinil pose [7].

4.1 Results from structure-activity relationship studies

4.1.1 Thiophene moiety added to the scaffold

- Under the circumstances described above, it can be assumed that a thiophene structure increases the activity of the basic structure if it replaces the amide group, as long as there is nothing else added to it, though the values stay on a similar level.
- Given that halogens and a methoxy group are added in para-position to one of the phenyl rings, the following order in binding affinity is notable: Cl>Br>methoxy.
- The combination of a thiazole group and a thiophen group makes the affinity worse.

4.1.2 Thiazole moiety added to the scaffold

4.1.2.1 Linked via position 2

- Adding a mere thiazole to the scaffold doesn't lead to much difference from the original structure.
- However, a link between the phenyl moieties leads to a dramatic loss of binding affinity. This shows that the rotational flexibility of the phenyl rings is very important for the inhibitory effect on the dopamine transporter.
- Furthermore, apparently the number of methyl groups attached to the thiazole structure is not important, as the IC50 value stays roughly the same when adding one or two of them.

4.1.2.2 Linked via position 4

- Notably the binding affinity of a thiazole group added via position 4 has a better affinity than through the second position.
- Contrary to the thiophene moiety, the thiazole gets a better value when a halogen or methoxy group is connected in para-position to the system, with the order being Br>Cl>methoxy. The same also happens when a methyl group is added to position 2 of the thiazole group
- In the case of an ethyl group being added on position 2 of the thiazole group the binding affinity gets lower, dropping further when replaced by a propyl group. However, when a butyl group is added, the activity level rises again.
- Nevertheless, there is a difference between adding an isopropyl group or a cyclopropyl group, because apparently the latter one fits better into the binding pocket, resulting in a low IC50 value.

4.1.2.3 Linked via position 5

- This structure provides the best value of the thiazole group, when no extra functions are further added to the structure.
- The previous mentioned value does not change significantly if a halogen is attached in para-position to a phenyl ring.

4.1.3 Sulfoxide function

Apparently, the sulfoxide function in the central area of the original Modafinil structure plays a pre-eminent role in the binding process, since its loss leads to a dramatic drop concerning activity levels.

4.1.4 Extended linker

As previously shown the IC50 value rises extraordinarily high if the linker in the middle of the molecule is extended with a further C-atom. This previously mentioned point about the sulfoxide function and the dramatic loss of activity due to a link between the phenyl rings show, that the best binding activities can be reached by maintaining the diphenyl structure and the sulfoxide function in the original state.

4.1.5 Pyrimidine

- Is a pyrimidine structure be linked onto the original Modafinil scaffold via its sixth position, a better binding activity than the original drug is reached, as long as there is a methyl group on position 2 and a hydroxyl group on position 4. If the hydroxyl group is replaced by another methyl group a decline concerning the affinity can be noticed. This leads to the assumption that regarding pyrimidine functions, it is better to have on it also a hydrophilic group, to enhance the interaction with the binding pocket.
- Furthermore, if the pyrimidine structure is linked to the scaffold through its second position, the value gets worse than Modafinil, but here also the affinity rises again if the methyl group is replaced by a trifluormethyl-function.

4.2 Docking results

Using the MOE [15] software package to perform the structure-based study, the previously mentioned handpicked Modafinil analogues underwent a docking procedure, while the amino acids defined by the structural alignment served as binding pocket.

Compound	Docking Score	IC50 value
CE-109	-7.3921	439.7
CE-153	-7.3693	202.1
CE-116	-7.0332	87.97
CE-142	-6.9743	98.45
CE-154	-6.9502	191.8
CE-146	-6.9147	1.352
CE-133	-6.8662	16.96
CE-140	-6.8594	3.179
CE-111	-6.8280	3.250.
CE-151	-6.7421	67.45
CE-101 (Modafinil)	-6.7021	11.11
CE-103	-6.6393	14.73
CE-112	-6.5759	90.99
CE-144	-6.4296	1.853
CE-115	-5.9755	663.9

Table 4: Results of the docking study using MOE [15] and the setting "Alpha PMI" and "Affinity dG". The values highlighted in blue have a high IC50 value, whereas the values highlighted in red have quite low IC50. CE-115, which is highlighted in green, has the highest IC50 of the whole set of compounds.

The results (Table 4) show, that the IC50 values, depicting the activity levels of the individual structures do not correlate with the corresponding docking score. The highest docking score of each compound, and thus the most fitting pose was used. The blue highlighted compounds, namely CE-109, CE-153 and CE-154, which show the highest IC50 values, and therefore the lowest activity rates, mark the top of the docking score, whereas the compounds, which depict the highest affinity rates, namely CE-146, CE-140, CE-111 and CE-144 dominate the lower half of the docking scores. Nevertheless, there is one compound, which does show the expected score, which is CE-115, the compound with the link between the diphenyl-rings. Highlighted in green in the table, it completes the list on the bottom end, and therefore indicates the worst docking score, while also showing the lowest activity level in the biological assay.

4.3 Cluster Analysis

Thereupon, the resulting poses were split into various clusters, combining those with similar orientations within the binding pocket. The result were 30 different clusters, but only the ones with at least ten poses were used for the following analysis and compared to the published Modafinil pose. Furthermore, here again the compounds with the highest binding affinities shown in the biological assay were highlighted in blue, whereas the ones with the lowest in red. Moreover, the sulfinylacetamide function of Modafinil is highlighted in yellow in the pictures, whereas the same function is highlighted in red in the cluster. As can be seen, the number besides the compound's name indicates how often the same compound is represented in the cluster, with the corresponding scores being shown in the same table.

4.3.1 Cluster 1

This cluster consists of 42 poses. Only one of them shows a hydrogen bond, but 22 of them show clashes with the protein. The docking scores are shown in table 5. Altogether the average IC50 value here is 124.3 µM, while the average score is -6.0212 (range: -7.3921 to 4.2877).

#	Compound	IC50	Docking score
1	CE-109 (8)	439.7	-7.3921, -7.0481, -6.8504, -6.6424, -6.1240, -5.3093, -4.7306,-4.2877
2	CE-116 (3)	87.97	-7.0332, -6.2662, -5.4285
3	CE-142 (4)	98.45	-6.9743, -6.7380, -6.6459, -6.3294
4	CE-153 (2)	202.1	-6.7836, -6.2798
5	CE-111 (5)	3.250	-6.6521, -6.3468, -6.0312,-5.9192
6	CE-154 (1)	191.8	-6.5822
7	CE-112 (2)	90.99	-6.5641, -6.0843
8	CE-151 (3)	67.45	-6.5056, -6.3128, -6.0031
9	CE-146 (2)	1.352	-6.3532, -6.2805
10	CE-133 (3)	16.96	-6.3031, -5.8236, -5.5475
11	CE-103 (5)	14.73	-6.2655, -6.2144, -6.0975, -5.3781, -5.2161
12	CE-140 (1)	3.179	-6.1568
13	CE-144 (3)	1.853	-6.1075, -5.7149, -5.5683

Table 5: The results of cluster 1

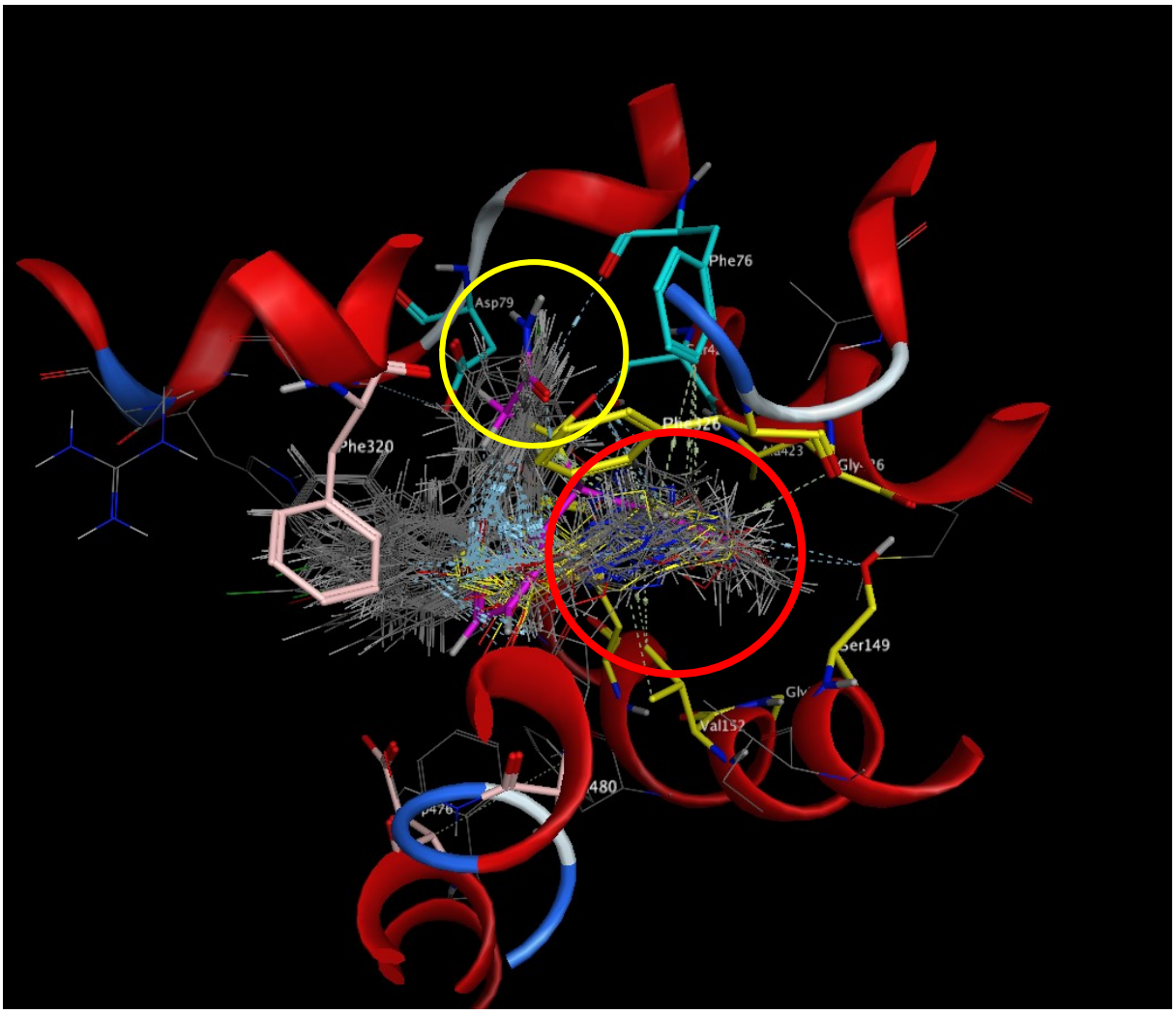


Fig. 51: Cluster 1 in complex with the human dopamine transporter in comparison with Modafinil

As shown in Fig. 51, this cluster does not correspond with the Modafinil pose, as the diphenyl structure faces subsite A (turquoise) and in between subsites A and C (pink), whereas the rest of the structure faces subsite B (yellow). This lies in total contrast to Modafinil placing its diphenyl structure in subsites B and C and the sulfinylacetamide function in subsite A.

4.3.2 Cluster 2

This cluster contains only 11 poses, depicting no hydrogen bonds, but 12 clashes. The docking scores are shown in table 6, the average IC50 value is 175.5 µM, while the average score is -6.6727 (range: -7.3693 to -5.4658).

#	Compound	IC50	Docking score
1	CE-153 (5)	202.1	-7.3693, -7.0318, -6.9147, -6.7836, -6.4703
2	CE-109 (2)	439.7	-7.0671, -6.9103
3	CE-154 (1)	191.8	-6.9502
4	CE-133 (3)	16.96	-6.4098, -6.0275, -5.4658

Table 6: The results of cluster 2

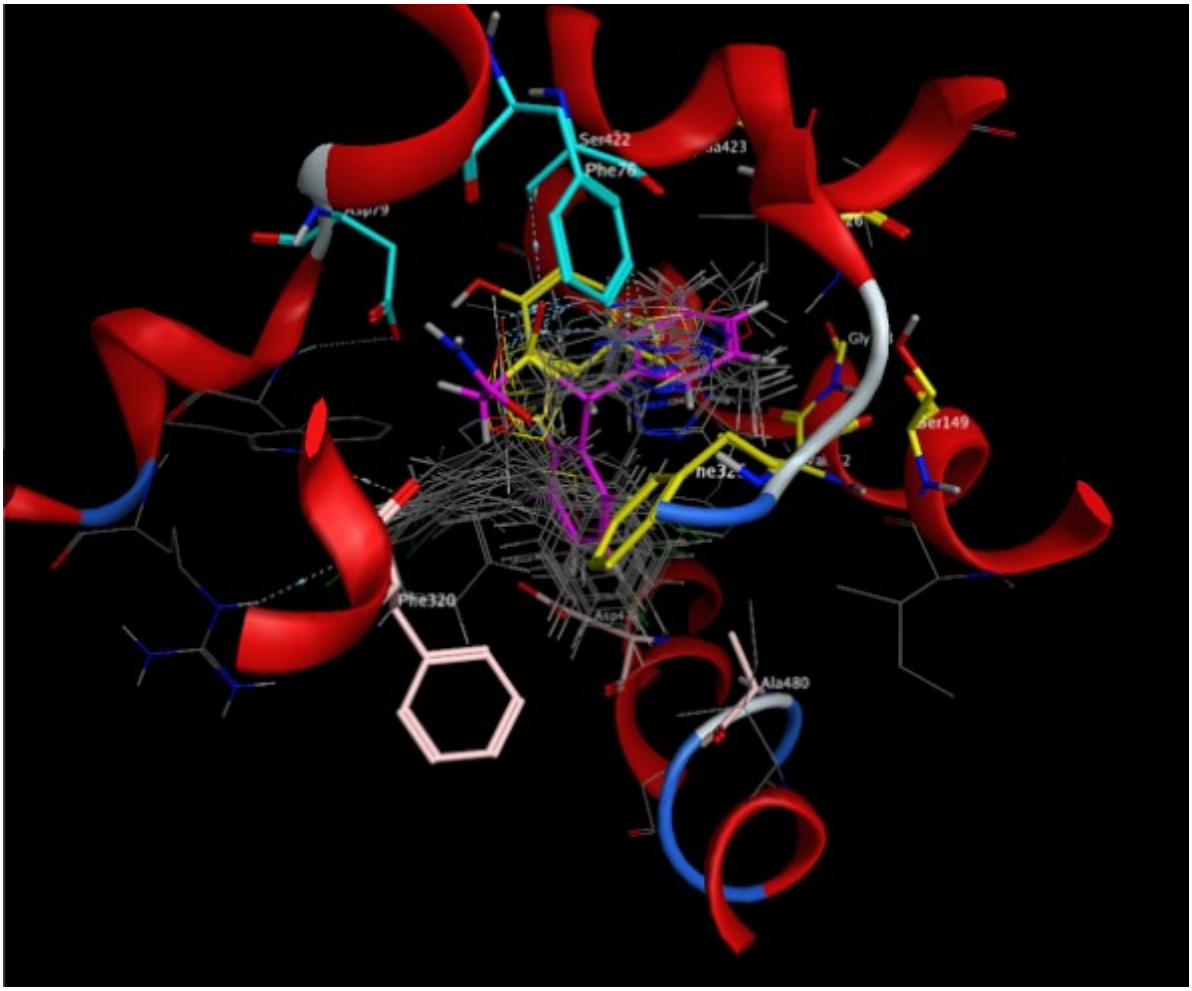


Fig. 52: Cluster 2 in complex with the human dopamine transporter in comparison with Modafinil

Fig. 52 shows how this cluster does not correspond with the Modafinil pose, as the diphenyl structure faces subsite C (pink), whereas the rest of the structure faces in between subsite A (turquoise) and B (yellow). This pose is also contrary to Modafinil placing its diphenyl structure in subsites B and C and the sulfinylacetamide function in subsite A.

4.3.3 Cluster 3

Consisting of 23 poses, this cluster shows 2 hydrogen bonds and 11 clashes. The average IC50 is 61 µM and the average score -5.9775 (range: -7.1406 to -4.6666), also the docking scores are shown in table 7.

#	Compound	IC50	Docking score
1	CE-109 (2)	439.7	-7.1406, -6.7115
2	CE-116 (3)	87.97	-6.9294, -6.8662, -5.1477
3	CE-146 (3)	1.352	-6.8808, -6.3429, -4.8801
4	CE-140 (2)	3.179	-6.8594, -6.3836
5	CE-111 (3)	3.250	-6.8280, -6.5938, -5.1983
6	CE-103 (3)	14.73	-6.5980, -6.2137, -4.6666
7	CE-112 (1)	90.99	-6.4470
8	CE-144 (2)	1.853	-6.3839, -6.2190
9	CE-101 (3)	11.11	-6.2957, -6.2497, -5.6478
10	CE-151 (1)	67.45	-5.2106

Table 7: The results of cluster 3

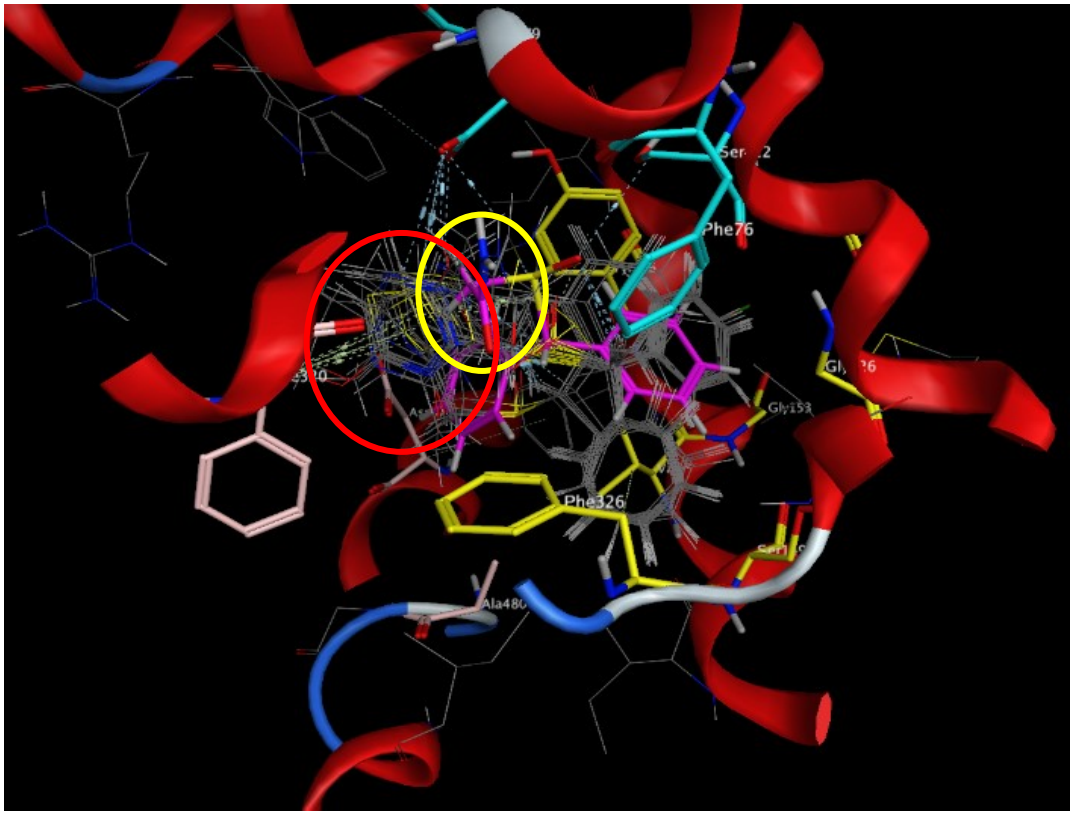


Fig. 53: Cluster 3 in complex with the human dopamine transporter in comparison with Modafinil

Again, this cluster does not correspond with the Modafinil pose as can be seen in Fig. 53. Here the diphenyl structure faces subsite B (yellow), while the sulfinylacetamide function faces in between subsite A (turquoise) and C (pink). This is also contrary to Modafinil placing its diphenyl structure in subsites B and C and the sulfinylacetamide function in subsite A.

4.3.4 Cluster 4

Cluster 4 contains 37 poses, 2 hydrogen bonds and 10 clashes. Table 8 shows all the docking scores, the average IC50 is 43.4 µM and the average docking score is -6.0676 (range: -6.9602 to -2.5472)

#	Compound	IC50	Docking score
1	CE-153 (1)	202.1	-6.9602
2	CE-140 (3)	3.179	-6.8520, -6.7846, -5.8874
3	CE-116 (3)	87.97	-6.8114, -6.7917, -6.7304
4	CE-103 (5)	14.73	-6.6393, -6.3873, -6.1812, -5.9063, -5.7033
5	CE-109 (1)	439.7	-6.5851
6	CE-112 (4)	90.99	-6.5759, -6.3131, -6.2987, -5.7952
7	CE-133 (3)	16.96	-6.4483, -6.0563, -5.2512
8	CE-111 (3)	3.250	-6.3965, -5.9437, -5.8507
9	CE-146 (4)	1.352	-6.3960, -6.2006, -5.7928, -2.5472
10	CE-151 (2)	67.45	-6.3683, -4.6525
11	CE-144 (4)	1.853	-6.3559, -6.2343, -5.6123, -5.5071
12	CE-101 (4)	11.11	-6.2574, -6.0842, -5.9609, -5.3853

Table 8: The results of cluster 4

Contrary to the Modafinil pose, the compounds in this cluster are facing subsites A (turquoise) and B (yellow) with the phenyl structures as can be seen in Fig. 54. The sulfinylacetamide function faces subsite C (pink), while in the Modafinil structure it faces subsite A, while the other two are occupied by the phenyl structures.

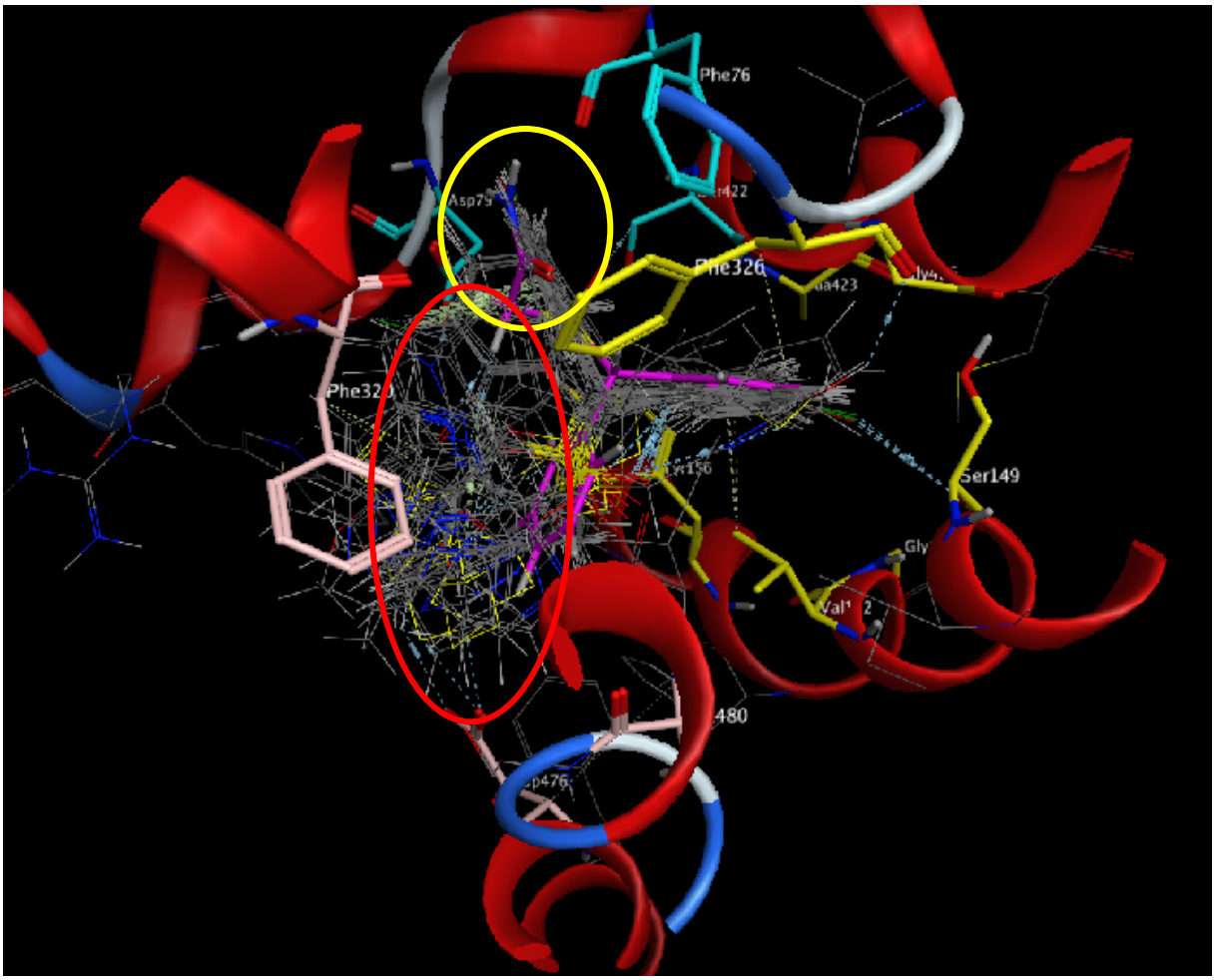


Fig. 54 Cluster 4 in complex with the human dopamine transporter in comparison with Modafinil

4.3.5 Cluster 5

Although this cluster consists of just 10 poses, these are similar to the Modafinil pose. The docking scores are shown in table 9, the average score is -5.85786 (range: -6.8662 to -4.3316) and the average IC50 value is 79.1 µM. The number of clashes in this cluster is 8, while there is only one hydrogen bond.

#	Compound	IC50	Docking score
1	CE-133 (1)	16.96	-6.8662
2	CE-151 (1)	67.45	-6.6139
3	CE-109 (1)	439.7	-6.1990
4	CE-144 (2)	1.853	-6.61285, -6.1013
5	CE-140 (1)	3.179	-6.1194
6	CE-146 (1)	1.3520	-5.9220
7	CE-112 (2)	90.99	-5.1713, -5.1254
8	CE-101 (1)	11.11	-4.3316

Table 9: The results of cluster 5

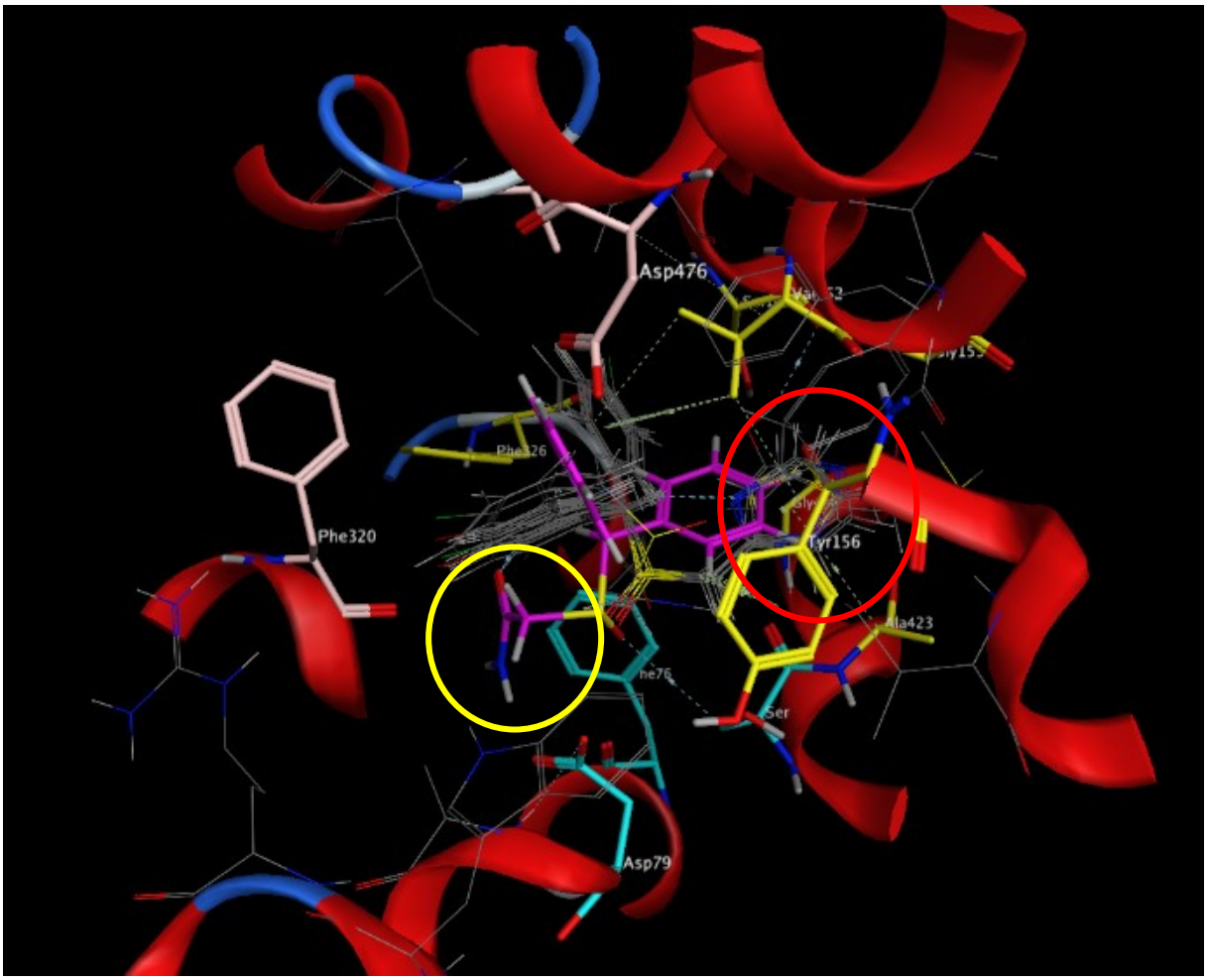


Fig. 55: Cluster 5 in complex with the human dopamine transporter in comparison with Modafinil

Fig. 55 shows how the poses in cluster 5 are quite similar to the published Modafinil pose, as the functional groups are facing almost in the same directions.

4.3.6 Cluster 7

Just as cluster 5, cluster 7 consists of only 10 poses, but shows quite a similarity to the published Modafinil pose. Table 10 shows the docking scores, the average IC50 is 91.3 µM and the average docking score is -5.90128 (range: -6.7021 to -3.3345). There are also 2 hydrogen bonds and 8 clashes.

#	Compound	IC50	Docking score
1	CE-101 (2)	11.11	-6.7021, -6.3707
2	CE-142 (2)	98.45	-6.6725, -6.4266
3	CE-151 (3)	67.45	-6.5893, -6.1339, -3.3345
4	CE-116 (1)	87.97	-6.2059
5	CE-112 (1)	90.99	-5.6800
6	CE-154 (1)	191.8	-4.897

Table 10: The results of cluster 7

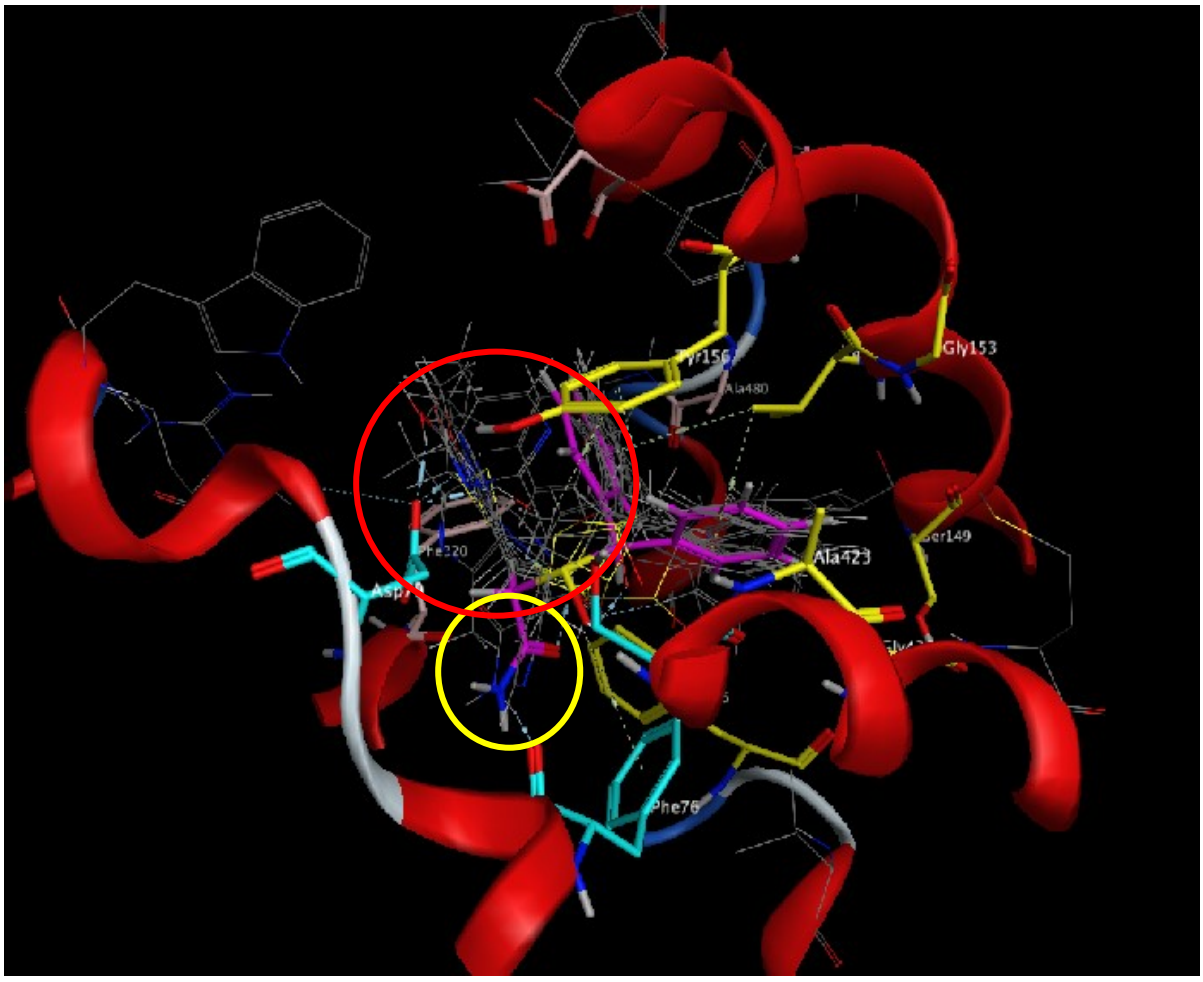


Fig. 56: Cluster 7 in complex with the human dopamine transporter in comparison with Modafinil

As can be seen in Fig. 56, the phenyl structures face subsite B (yellow) and C (pink), which is in correlation to the original Modafinil pose. Concerning the sulfinylacetamide function, while in the published pose it faces the subsite A (turquoise), here the poses in cluster 7 partly do the same, and partly face in between subsites A and C.

4.3.7 Cluster 9

Consisting of 10 poses, this cluster depicts no hydrogen bonds, but 3 clashes. The average IC50 value is 100.8 µM and the average docking score is -5.89482 (range: -6.5137 to -2.6846). The docking scores are shown in table 11.

#	Compound	IC50	Docking score
1	CE-151 (1)	67.45	-6.5137
2	CE-144 (3)	1.8530	-6.4296, -6.2898, -5.9757
3	CE-133 (1)	16.96	-6.3989
4	CE-109 (1)	439.7	-6.3623
5	CE-112 (1)	90.99	-6.1710
6	CE-142 (1)	98.45	-6.0694
7	CE-116 (1)	87.97	-6.0532
8	CE-111 (1)	3.25	-2.6846

Table 11: The results of cluster 9

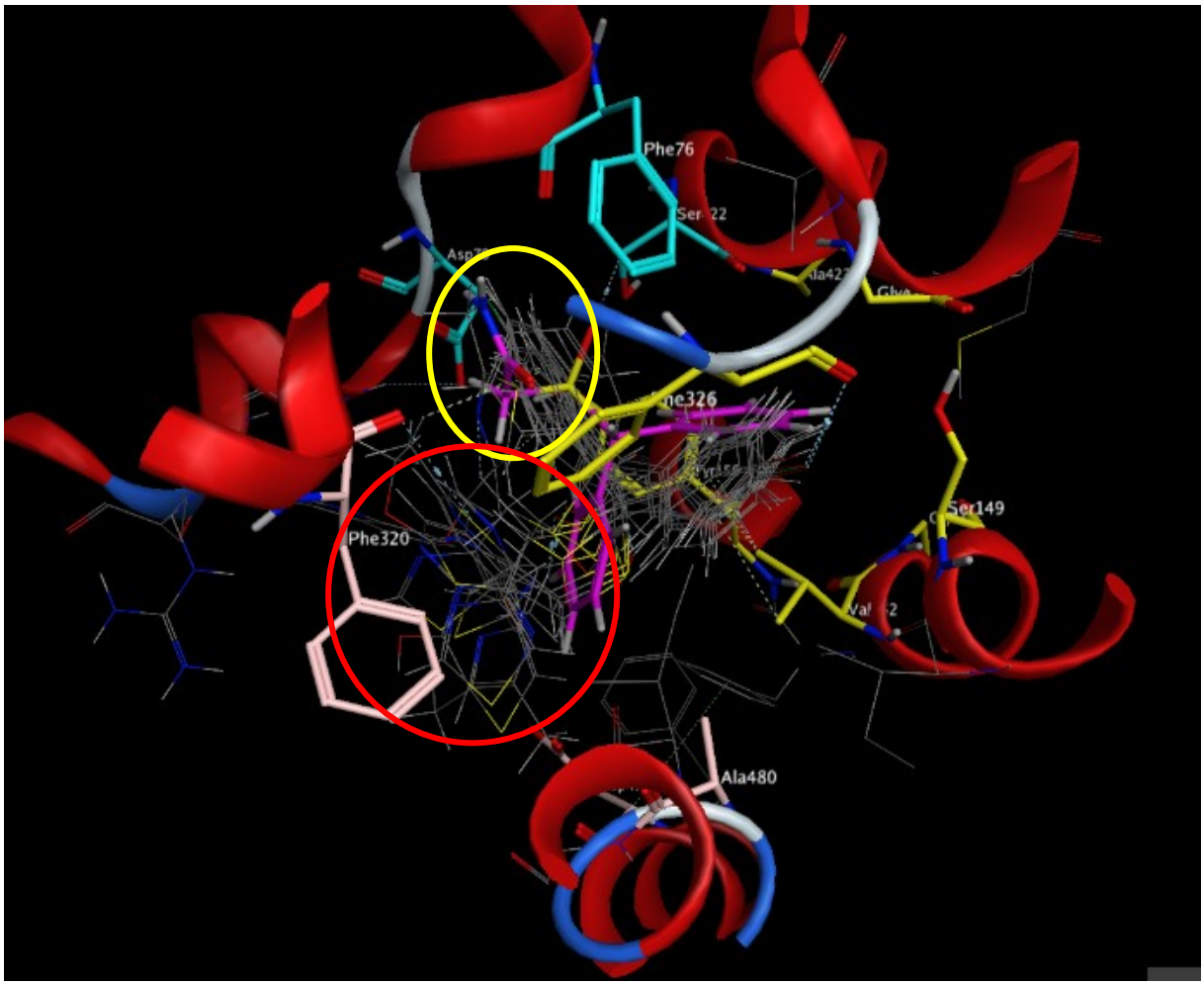


Fig. 57: Cluster 9 in complex with the human dopamine transporter in comparison with Modafinil

Fig. 57 shows how the phenyl rings of the structures of cluster 9 face in the directions of subsite B (yellow) and A (turquoise), while the residue faces subsite C (pink). This pose does not correlate with the published Modafinil pose.

4.3.8 Cluster 12

Also made up of 10 poses, cluster 12 shows no hydrogen bonds, but 3 clashes. The average IC50 value is 39.8 µM and the average docking score is -5.839 (range: -6.5030 to -5.2638). The docking scores are shown in table 12.

#	Compound	IC50	Docking score
1	CE-116 (2)	87.97	-6.5030, -5.7219
2	CE-133 (1)	16.96	-6.4877
3	CE-142 (2)	98.45	-6.1859, -6.1531
4	CE-111 (1)	3.25	-5.9656
5	CE-112 (1)	90.99	-5.4026
6	CE-146 (1)	1.352	-5.3687
7	CE-103 (1)	14.73	-5.3374
8	CE-144 (1)	1.8530	-5.2638

Table 12: The results of cluster 12

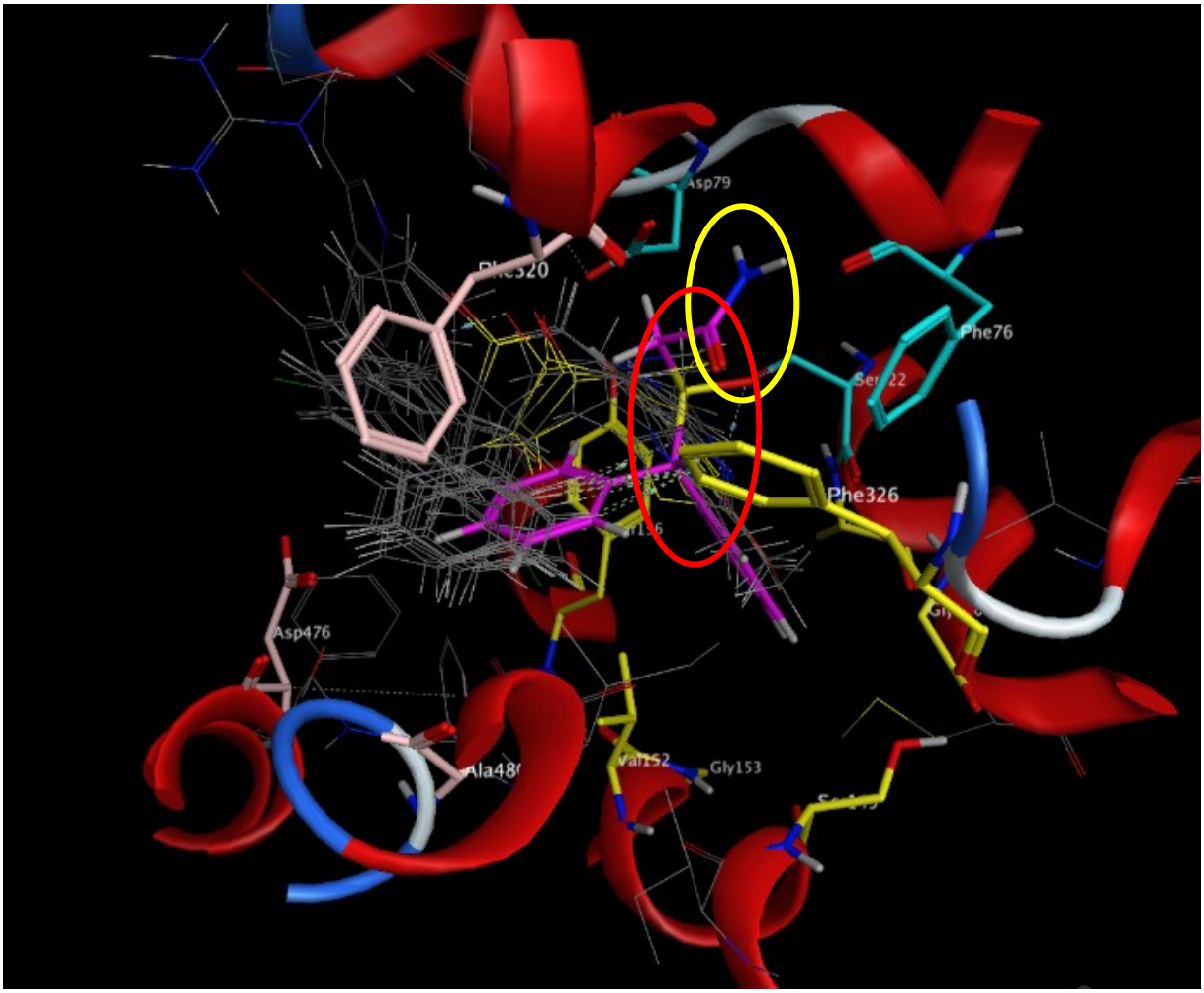


Fig. 58: Cluster 12 in complex with the human dopamine transporter in comparison with Modafinil

The poses of the compounds of cluster 12 also do not match with the published Modafinil pose as can be seen in Fig. 58. While Modafinil places its phenyl structures in subsites B (yellow) and C (pink) and the residue in subsite A (turquoise), the poses here face the phenyl structures in subsite C and in between C and A, while the sulfinylacetamide function faces in between subsites A and B.

4.3.9 Cluster 14

Cluster 14 consists of 17 poses of which the docking scores are shown in table 13. The average IC50 is 19 μM and the average docking score is -5.03608 (range: -6.2527 to -3.0633). Furthermore, there are 26 clashes and 1 hydrogen bond.

#	Compound	IC50	Docking score
1	CE-101 (4)	11.11	-6.2527, -6.2498, -6.1959, -5.0202
2	CE-133 (3)	16.96	-6.1952, -4.2348, -3.7664
3	CE-111 (3)	3.250	-5.8638, -5.8529, -3.6115
4	CE-154 (1)	191.8	-5.7393
5	CE-140 (2)	3.179	-5.6994, -3.2040
6	CE-144 (2)	1.853	-5.6617, -3.5094
7	CE-103 (1)	14.73	-5.4931
8	CE-146 (1)	1.352	-3.0633

Table 13: The results of cluster 14

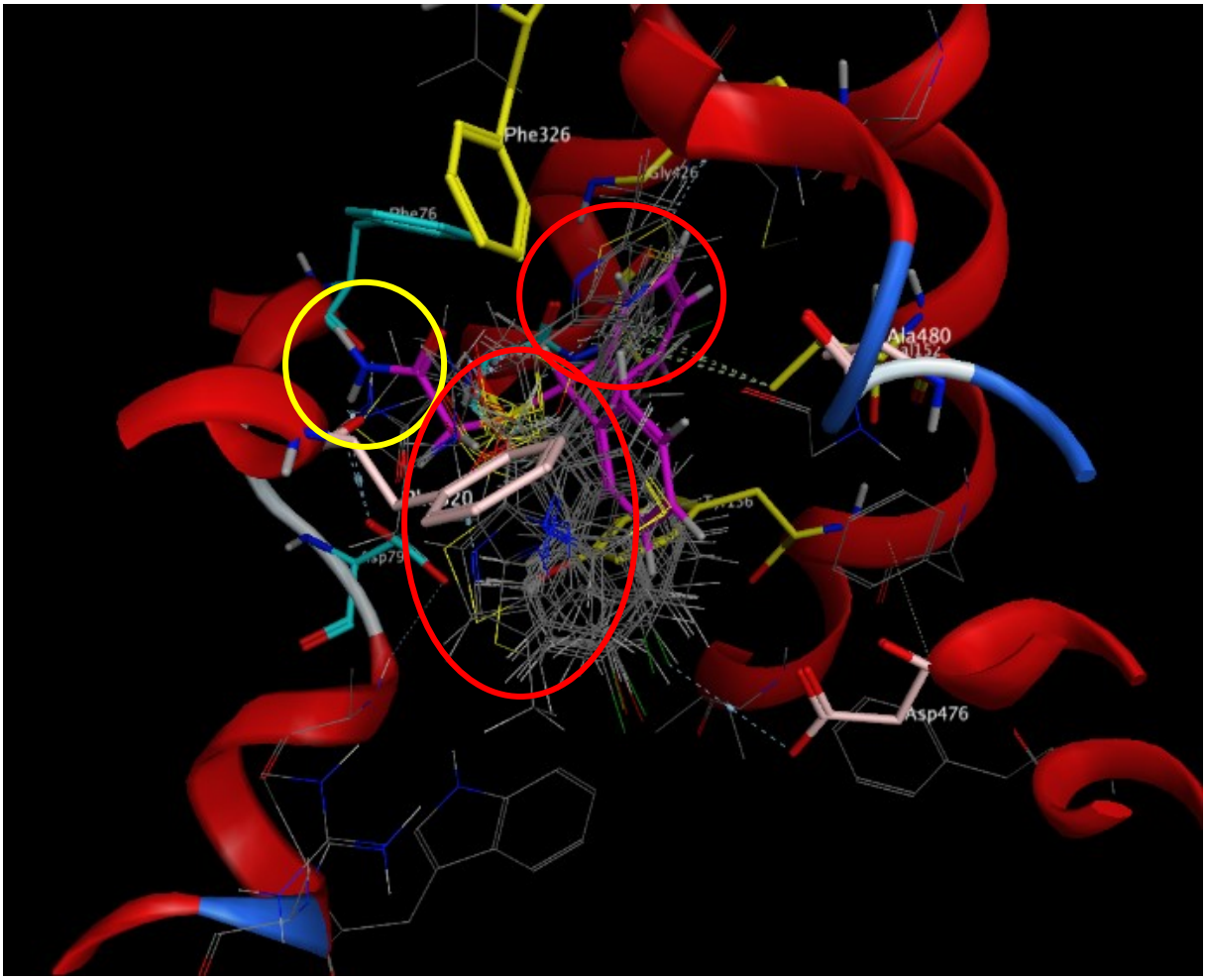


Fig. 59: Cluster 14 in complex with the human dopamine transporter in comparison with Modafinil

As can be seen in Fig. 59 the poses in cluster 14 partly correlate with the published Modafinil pose, as the diphenyl structure is facing subsites B (yellow) and C (pink). But the residue does not show a consistent direction as some of the poses face into subside A (turquoise), some in between A and C and some in between B and C.

4.3.10 Cluster 24

Consisting of 18 poses, cluster 24 shows 23 clashes and 2 hydrogen bonds. The average IC50 value is 87.1 µM and the average docking score is -4.79076 (range: -5.5447 to -3.5288). The docking scores are enlisted in table 14.

#	Compound	IC50	Docking score
1	CE-109 (1)	439.7	-5.5447
2	CE-153 (4)	202.1	-5.4968, -5.1026, -4.9955, -4.4978
3	CE-111 (2)	3.250	-5.4034, -4.4569
4	CE-116 (1)	87.97	-5.0967
5	CE-151 (1)	67.45	-4.9600
6	CE-144 (1)	1.853	-4.8931
7	CE-101 (1)	11.11	-4.8894
8	CE-133 (2)	16.96	-4.7888, -4.5497
9	CE-112 (1)	90.99	-4.7775
10	CE-146 (2)	1.352	-4.5283, -4.4085
11	CE-103 (1)	14.73	-4.3152
12	CE-140 (1)	3.179	-3.5288

Table 14: The results of cluster 24

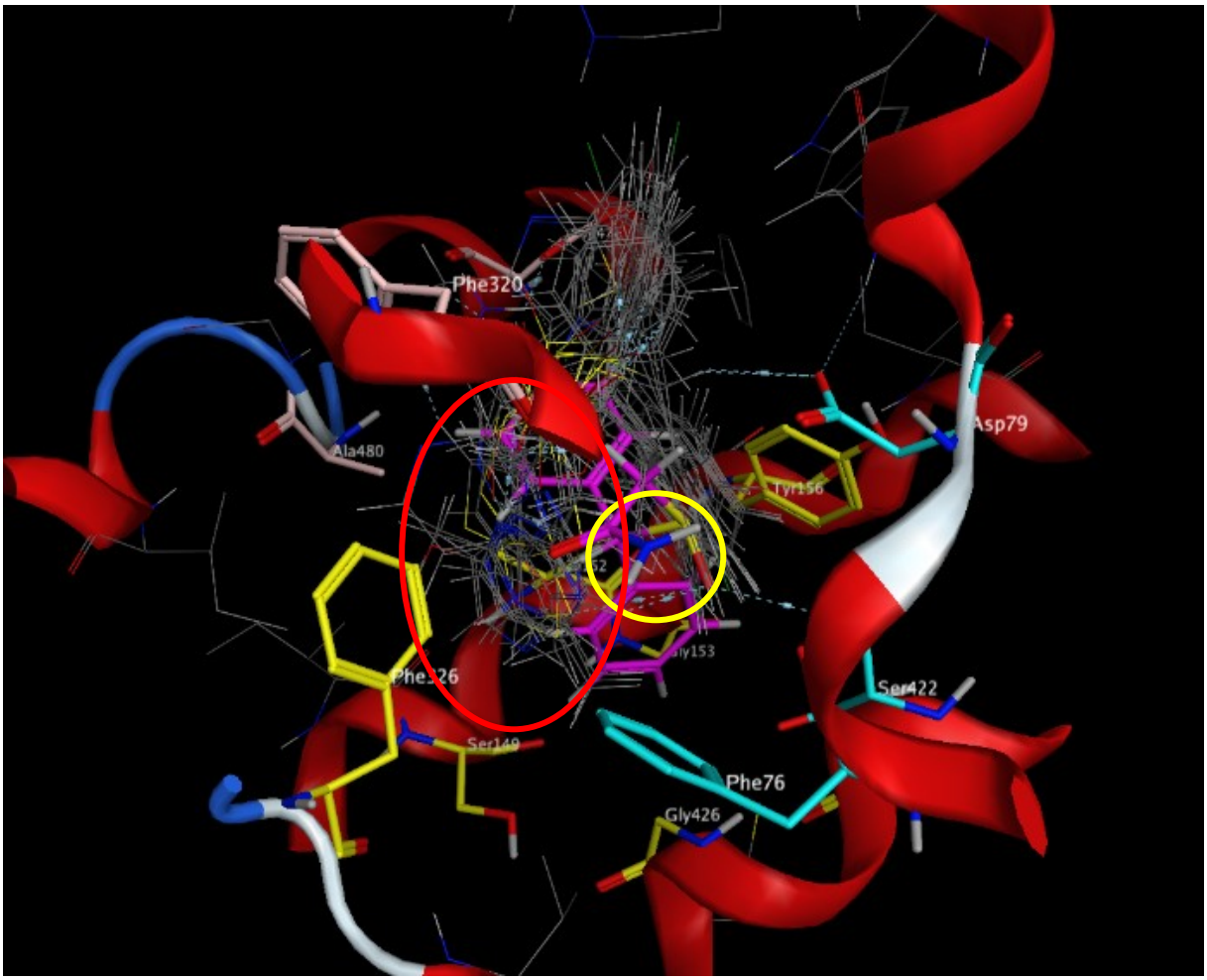


Fig. 60 Cluster 24 in complex with the human dopamine transporter in comparison with Modafinil

Fig. 60 shows how this cluster does not have anything in common with the original Modafinil pose. The polar structure is pointing in between B (yellow) and C (pink), while the phenyl structures are not facing any particular subsite at all.

4.4 Discussion

As can be seen in the analysis shown above, none of the clusters shows an identical orientation as the published Modafinil pose [7]. The clusters 5, 7 and 14 at least show some correlations:

- Cluster 5 contains poses, which show a similarity to the published pose, because the functional groups are pointing in similar directions

- Some of the poses in cluster 7 are facing subsite A with their sulfinylacetamide function in accordance to the Modafinil pose, but some are just facing in between subsites A and C.
- In cluster 14 the poses partly correlate with the published pose, because the diphenyl structure is facing the subsites B and C. But the residue is not showing any constant orientation, which can be compared to Modafinil.

Moreover, these clusters don't show any equivalence amongst themselves, as they vary in terms of the number of their poses, IC50 values, docking scores or even the compounds composing the cluster.
Most of the other clusters do face the subsites at least, but the moieties are placed in different subsites than shown by Modafinil.
Cluster 24 is the only one which does not seem to show any similarity to the original Modafinil pose, because the polar moiety is placed somewhere in between subsites B & C, whereas the diphenyl structure does not show into any subsite at all but points towards the extracellular pathway.
All in all, these approaches did not lead to clusters reflecting the published Modafinil pose, although it was possible to reproduce the said pose with the software MOE [15]. However, different software packages can have different results on the same protein. In the future, this dataset could be tested with other docking algorithms, for example GOLD [24] with flexible sidechains or Schrödinger [25] using induced fit docking.

5 Conclusions and Outlook

The human dopamine transporter is a prominent target in today's research as it is linked to a lot of processes concerning the nervous system and thus the human well-being. As part of this research Modafinil is a very important compound, whose properties could be expanded by changing its structural features.

As a basis of this computational study, a set of Modafinil analogues synthesized and tested for its biological activity on the human dopamine transporter was provided to us. Through interpretation of the structure-activity relationship a few compounds with a better activity than the original Modafinil structure stood out, while others were outranked due to their bad activity levels.

Furthermore, a docking study on a handpicked set of compounds was conducted in order to put the biological activity and the docking scores into correlation. As can be seen above this

attempt did not succeed as the highest rankings are occupied by compounds showing the lowest biological activity apart from one distinctive exception.

Lastly, common scaffold clustering was carried out to put similar poses into individual clusters in order to compare them to the published Modafinil pose in the human dopamine transporter. No cluster showed the exact same orientation, but cluster 5, 7 and 14 show at least a similarity.

All in all, the computational methods used in this study could not explain the biological activities measured in-vitro. Nonetheless, the knowledge derived from this study can be used in order to prove the biological qualities of these compounds using other software packages and methods. Docking methods with flexible side chains in the binding side or even allowing slight backbone movements of the protein might lead to more accurate results. Furthermore, a molecular dynamics simulation could show which poses of the compounds would stay stable over time or binding free energy calculations of the complexes could provide clearer insights into the molecular drug-transporter interactions and broaden our understanding of these molecules.

6 References

[1] N.-H. Chen, M. E. A. Reith, und M. W. Quick, „Synaptic uptake and beyond: the sodium- and chloride-dependent neurotransmitter transporter family SLC6“, *Pflüg. Arch. Eur. J. Physiol.*, Bd. 447, Nr. 5, S. 519–531, Feb. 2004.

[2] Pramod, J. Foster, L. Carvelli, und L. K. Henry, „SLC6 Transporters: Structure, Function, Regulation, Disease Association and Therapeutics“, *Mol. Aspects Med.*, Bd. 34, Nr. 2–3, S. 197–219, Apr. 2013.

[3] A. S. Kristensen *u. a.*, „SLC6 Neurotransmitter Transporters: Structure, Function, and Regulation“, *Pharmacol. Rev.*, Bd. 63, Nr. 3, S. 585–640, Jan. 2011.

[4] C. J. Loland, L. Norregaard, T. Litman, und U. Gether, „Generation of an activating Zn2+ switch in the dopamine transporter: Mutation of an intracellular tyrosine constitutively alters the conformational equilibrium of the transport cycle“, *Proc. Natl. Acad. Sci. U. S. A.*, Bd. 99, Nr. 3, S. 1683–1688, Feb. 2002.

[5] A. Penmatsa, K. H. Wang, und E. Gouaux, „X-ray structure of the dopamine transporter in complex with tricyclic antidepressant“, *Nature*, Bd. 503, Nr. 7474, S. 85–90, Nov. 2013.

[6] M. Mereu, A. Bonci, A. H. Newman, und G. Tanda, „The neurobiology of modafinil as an enhancer of cognitive performance and a potential treatment for substance use disorders“, *Psychopharmacology (Berl.)*, Bd. 229, Nr. 3, S. 415–434, Okt. 2013.

[7] K. C. Schmitt und M. E. A. Reith, „The Atypical Stimulant and Nootropic Modafinil Interacts with the Dopamine Transporter in a Different Manner than Classical Cocaine-Like Inhibitors“, *PLoS ONE*, Bd. 6, Nr. 10, Okt. 2011.

[8] D. M. Batéjat und D. P. Lagarde, „Naps and modafinil as countermeasures for the effects of sleep deprivation on cognitive performance“, *Aviat. Space Environ. Med.*, Bd. 70, Nr. 5, S. 493–498, Mai 1999.

[9] F. B. Taylor und J. Russo, „Efficacy of Modafinil Compared to Dextroamphetamine for the Treatment of Attention Deficit Hyperactivity Disorder in Adults“, *J. Child Adolesc. Psychopharmacol.*, Bd. 10, Nr. 4, S. 311–320, Jan. 2000.

[10] V. Cakic, „Smart drugs for cognitive enhancement: ethical and pragmatic considerations in the era of cosmetic neurology -- Cakic 35 (10): 611 -- Journal of Medical Ethics“. [Online]. Verfügbar unter: http://jme.bmj.com/content/35/10/611.short. [Zugegriffen: 04-Mai-2016].

[11] Y. Karabacak *u. a.*, „The effect of modafinil on the rat dopamine transporter and dopamine receptors D1–D3 paralleling cognitive enhancement in the radial arm maze“, *Front. Behav. Neurosci.*, Bd. 9, Aug. 2015.

[12] T. M. Jay, „Dopamine: a potential substrate for synaptic plasticity and memory mechanisms“, *Prog. Neurobiol.*, Bd. 69, Nr. 6, S. 375–390, Apr. 2003.

[13] J. P. Wisor, S. Nishino, I. Sora, G. H. Uhl, E. Mignot, und D. M. Edgar, „Dopaminergic Role in Stimulant-Induced Wakefulness“, *J. Neurosci.*, Bd. 21, Nr. 5, S. 1787–1794, Jan. 2001.

[14] J. Cao *u. a.*, „Structure-Activity Relationships at the Monoamine Transporters for a Novel Series of Modafinil (2-[(diphenylmethyl)sulfinyl]acetamide) Analogues“, *ACS Med. Chem. Lett.*, Bd. 2, Nr. 1, S. 48–52, Okt. 2010.

[15] *Molecular Operating Environment (MOE), 2013.08; Chemical Computing Group ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2017.* .

[16] „UniProt: the universal protein knowledgebase“, *Nucleic Acids Res.*, Bd. 45, Nr. D1, S. D158–D169, Jan. 2017.

[17] A. H. Newman, A. C. Allen, S. Izenwasser, und J. L. Katz, „Novel 3 alpha-(diphenylmethoxy)tropane analogs: potent dopamine uptake inhibitors without cocaine-like behavioral profiles“, *J. Med. Chem.*, Bd. 37, Nr. 15, S. 2258–2261, Juli 1994.

[18] S. R. Saroja *u. a.*, „A novel heterocyclic compound targeting the dopamine transporter improves performance in the radial arm maze and modulates dopamine receptors D1-D3“, *Behav. Brain Res.*, Bd. 312, S. 127–137, Okt. 2016.

[19] Y. D. Aher *u. a.*, „A Novel Heterocyclic Compound CE-104 Enhances Spatial Working Memory in the Radial Arm Maze in Rats and Modulates the Dopaminergic System“, *Front. Behav. Neurosci.*, Bd. 10, Feb. 2016.

[20] A. Jurik, B. Zdrazil, M. Holy, T. Stockner, H. H. Sitte, und G. F. Ecker, „A Binding Mode Hypothesis of Tiagabine Confirms Liothyronine Effect on γ-Aminobutyric Acid Transporter 1 (GAT1)“, *J. Med. Chem.*, Bd. 58, Nr. 5, S. 2149–2158, März 2015.

[21] K. Saha *u. a.*, „‘Second-Generation’ Mephedrone Analogs, 4-MEC and 4-MePPP, Differentially Affect Monoamine Transporter Function“, *Neuropsychopharmacology*, Bd. 40, Nr. 6, S. 1321–1331, Mai 2015.

[22] *Humphrey, W., Dalke, A. and Schulten, K., `VMD - Visual Info) Molecular Dynamics', J. Molec. Graphics 1996, 14.1, 33-38.* .

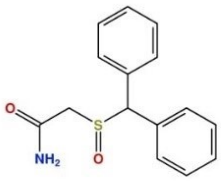
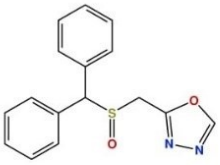
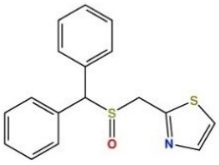
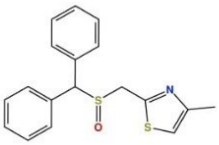
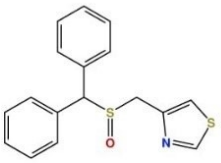
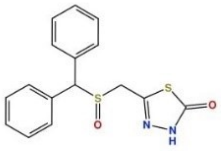
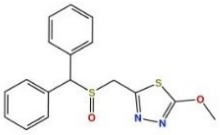
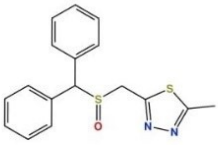
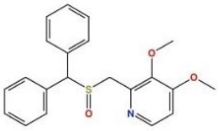
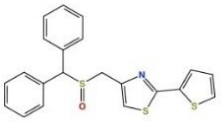
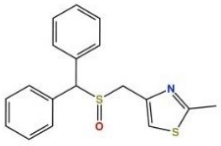
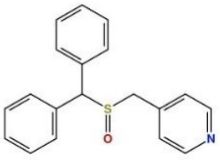
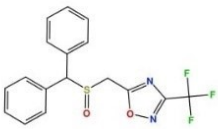
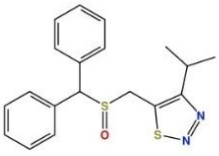
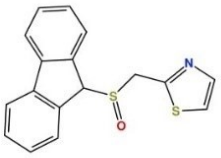
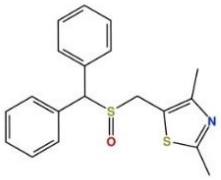
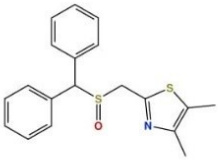
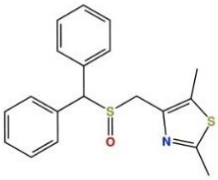
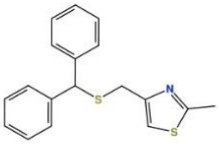
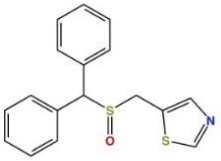
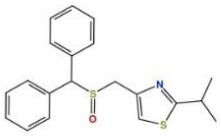
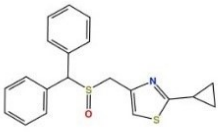
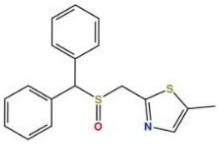
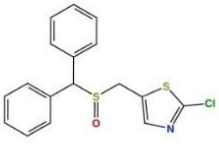
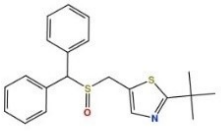
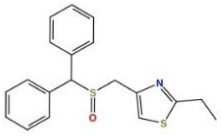
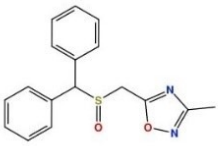
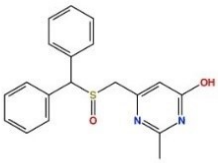
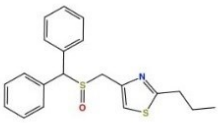
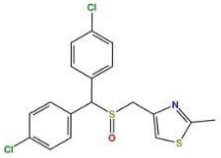
[23] *R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.* .

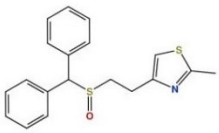
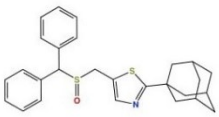
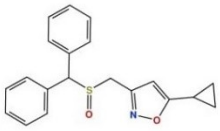
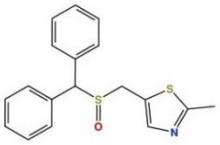
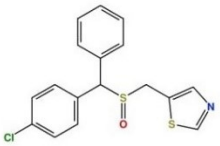
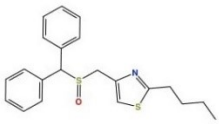
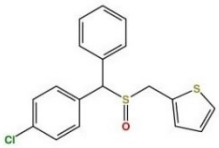
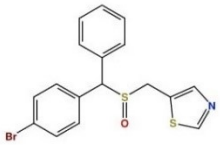
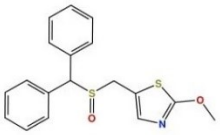
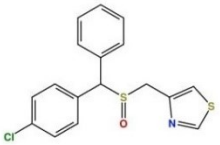
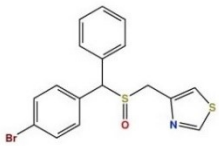
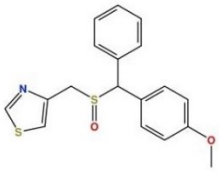
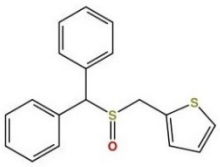
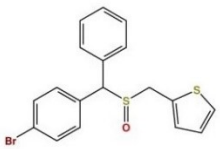
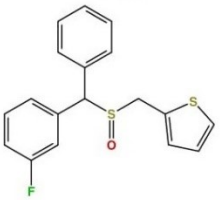
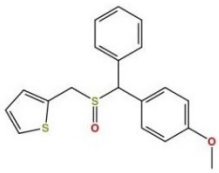
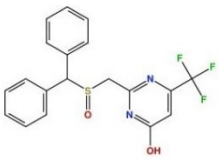
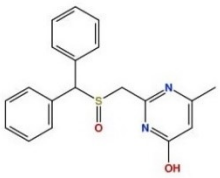
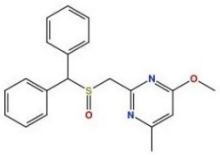
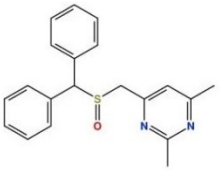
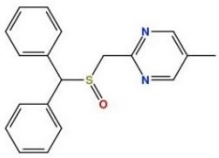
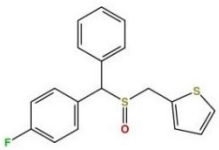
[24] *Development and Validation of a Genetic Algorithm for Flexible Docking G. Jones, P. Willett, R. C. Glen, A. R. Leach and R. Taylor, J. Mol. Biol., 267, 727-748, 1997.* .

[25] *Small-Molecule Drug Discovery Suite 2017-4, Schrödinger, LLC, New York, NY, 2017.* .

7 Appendix

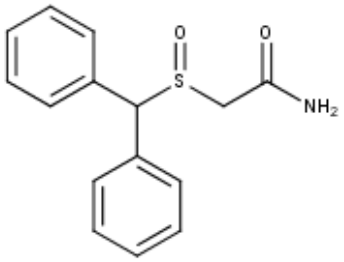
7.1 Supplemental Material

CE-101  11.1100	**CE-102**  54.4100	**CE-103**  14.7300	**CE-104**  27.8800	**CE-105**  7.3180
CE-106  41.8700	**CE-107**  109.1000	**CE-108**  116.3000	**CE-109**  439.7000	**CE-110**  33.5000
CE-111  3.2500	**CE-112**  90.9900	**CE-113**  26.4100	**CE-114**  64.5800	**CE-115**  663.9000
CE-116  87.9700	**CE-117**  27.4500	**CE-121**  55.0300	**CE-122**  148.3000	**CE-123**  4.3890
CE-124  16.5300	**CE-125**  4.1330	**CE-126**  49.7100	**CE-127**  24.3200	**CE-128**  33.9000
CE-129  19.2100	**CE-130**  56.7200	**CE-131**  3.0650	**CE-132**  28.4300	**CE-133**  16.9600

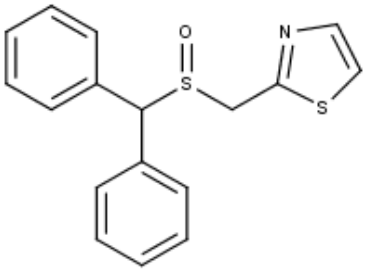
CE-134  125.1000	**CE-135** 	**CE-136** 	**CE-137**  66.7900	**CE-138**  4.0420
CE-139  19.4000	**CE-140**  3.1790	**CE-141**  3.4330	**CE-142**  98.4500	**CE-143**  2.8570
CE-144  1.8530	**CE-145**  4.1370	**CE-146**  1.3520	**CE-147**  4.2350	**CE-148**  5.7980
CE-149  5.6760	**CE-150**  27.5600	**CE-151**  67.4500	**CE-152**  123.1000	**CE-153**  202.1000
CE-154  191.8000	**CE-155** 			

Appendix 1: The Modafinil analogues synthesized and tested in vitro and in vivo in Dr. Gert Lubec's lab. The IC50 values are displayed under each compound as far as they were measured.

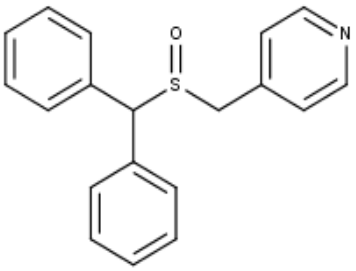
CE-101 (Modafinil)



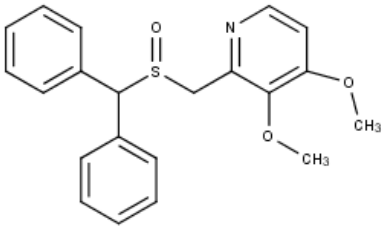
CE-103



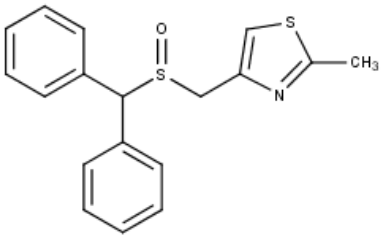
CE-112



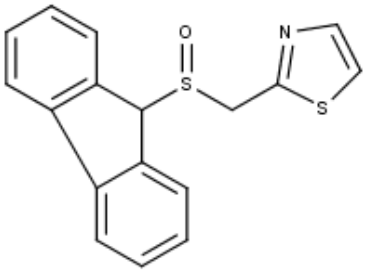
CE-109



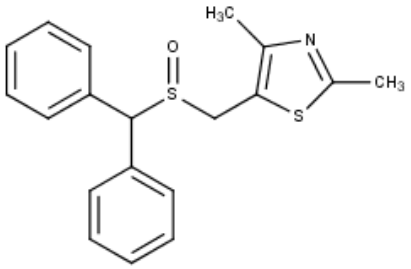
CE-111



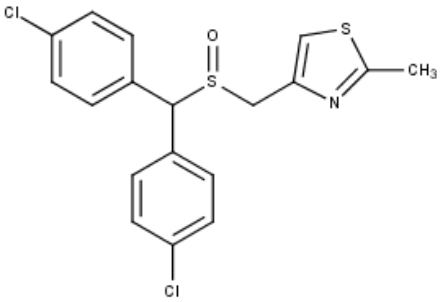
CE-115



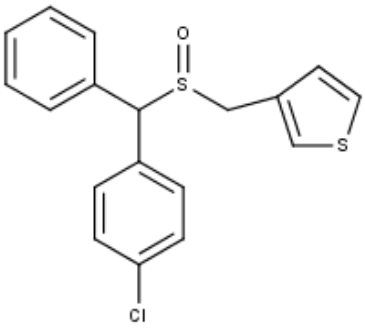
CE-116



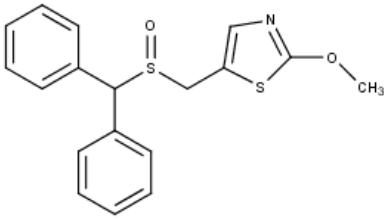
CE-133



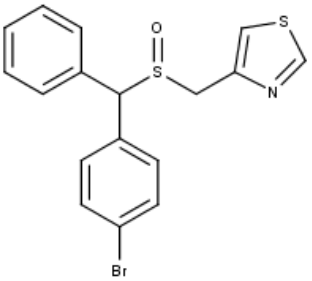
CE-140



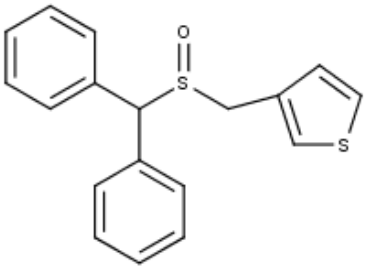
CE-142



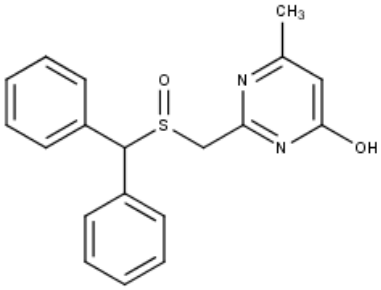
CE-144



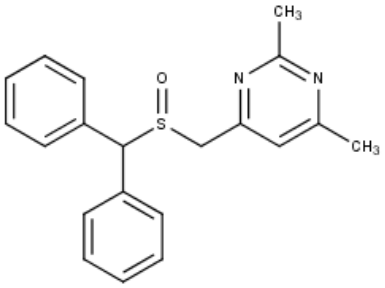
CE-146



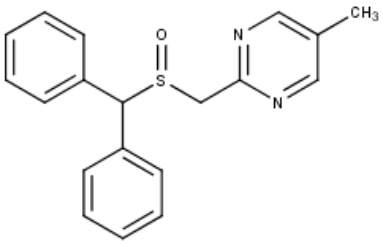
CE-151



CE-153



CE-154



Appendix 2 - The handpicked compounds used for the docking study

7.2 Extended Data

The code used for the structural alignment in VMD [22]:

```
# atomselect 0 = 4M48
# atomselect 1 = hDAT model

set xray_TM_bb [atomselect 0 "backbone and (resid 32 to 59 or resid 63 to 92 or resid 107 to
137 or resid 236 to 255 or resid 257 to 285 or resid 308 to 333 or resid 340 to 374 or resid
402 to 436 or resid 444 to 464 or resid 466 to 497 or resid 516 to 541 or resid 553 to 581)"]

set model_TM_bb [atomselect 1 "backbone and (resid 65 to 92 or resid 96 to 125 or resid 139 to
169 or resid 237 to 256 or resid 258 to 286 or resid 309 to 334 or resid 341 to 375 or resid
403 to 437 or resid 445 to 465 or resid 467 to 498 or resid 517 to 542 or resid 554 to 582)"]

set trmat1 [measure fit $model_TM_bb $xray_TM_bb]set mosel1 [atomselect 1 "all"]
$mosel1 move $trmat1

measure rmsd $model_TM_bb $xray_TM_bb
```

7.3 Abstract

The knowledge about the human dopamine transporter and its function on the human nervous system is growing steadily and so is the desire for a broader insight of the mechanism of action of substances that have an effect on it. Among them, Modafinil has become a popular target in contemporary research as it can improve neurological disorders and enhance cognitive abilities of healthy individuals by blocking the human dopamine transporter.

This project's aim was to analyse a set of chemically synthesized Modafinil analogues regarding their structure-activity relationships and to bring the outcomes of a biological assay, which was conducted previously, in correlation with a docking study.

First, the analysis of the structure-activity relationship showed how the biological activity could be altered by changing the original Modafinil structure. Then a structural alignment of dDAT and hDAT allowed us to highlight the binding pocket of our homology model of the human dopamine transporter, which was subsequently used for a docking study, performed on handpicked compounds from the previously mentioned set. This study was conducted with the software package MOE (Molecular Operating Environment) [15]. The outcoming values of this docking study were compared with the IC50 values derived from the biological assay, but the ranking did not correlate with the activity levels. Poses which showed a good docking score did not necessarily have good IC50 values.

Finally, a common scaffold clustering was performed using the software packages MOE [15] and R [23], which resulted in 30 clusters, of which 10 were used for further examination. The poses in each cluster were compared to a published Modafinil pose, but unfortunately this comparison did not lead to any significant connection to any particular compounds concerning their binding affinity.

7.4 Zusammenfassung

Das Wissen über den humanen Dopamintransporter und dessen Wirkung auf das menschliche Nervensystem wächst stetig, und damit verbunden auch der Wunsch danach, einen tieferen Einblick in den Wirkmechanismus von Substanzen, die einen Effekt auf ihn haben, zu erlangen. In diesem Zusammenhang ist Modafinil ein beliebtes Forschungsziel in der heutigen Zeit geworden, da es neurologische Störungen verbessern und die kognitiven Fähigkeiten gesunder Individuen durch Blockade des Dopamintransporters steigern kann.

Das Ziel dieses Projekts war es, eine Reihe chemisch synthetisierter Modafinil-Analoga im Hinblick auf ihre Struktur-Wirkungs Beziehungen zu analysieren und die Ergebnisse eines biologischen Assays, der zuvor durchgeführt worden war, mit einer Dockingstudie zu vergleichen.

Zunächst zeigte die Struktur-Wirkungs Beziehung wie die biologische Aktivität durch das Variieren der ursprünglichen Modafinilstruktur verändert werden konnte. Danach konnten wir durch ein Strukturalignment von dDAT und hDAT die Bindungstasche in unserem Homologiemodell des humanen Dopamintransporters analysieren und anschließend für eine Dockingstudie verwenden, die an ausgewählten Verbindungen der zuvor erwähnten Reihe durchgeführt wurde. Diese Studie wurde mithilfe der Computersoftware MOE (Molecular Operating Environment) [15] ausgeführt. Die Ergebnisse der Dockingstudie wurden mit den Werten des biologischen Assays verglichen, doch die Reihung zeigte keinerlei Korrelation mit den Aktivitätswerten. Posen für Substanzen die einen guten Docking Score aufwiesen, hatten nicht gezwungenermaßen gute IC50 Werte.

Zuletzt wurde ein Common Scaffold Clustering mithilfe der Programme MOE [15] und R [23] durchgeführt, dessen Ergebnis 30 Cluster waren, von denen 10 weiter analysiert wurden. Die Positionen in jedem Cluster wurden hinsichtlich ihrer Ähnlichkeit zur veröffentlichten Modafinil Position verglichen, lieferten jedoch ebenfalls keinen Zusammenhang zu bestimmten Molekülen hinsichtlich deren Bindungsaffinität.