Dolutegravir
- An HIV Integrase Inhibitor

An elaboration within the project DaMocles
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01.07.2020
1 Introduction

Over 36,9 million people [1] all over the world are diagnosed with HIV. Due to this illness being incurable those people live with the certainty that there lives will never be fully normal again as the virus will never completely leave the infected body [2].

To help these people medicine like Dolutegravir is needed. Unable to destroy the virus, Dolutegravir prevents the virus from multiplying in the human body and therefore reduces the speed in which the infection influences the person.

2 Chemical Structure

![Dolutegravir 3D structure](image1)

![Lewis structure](image2)

Figure 1: (a)3D structure of Dolutegravir. (b)Lewis structure.
The following picture shows the structure of dolutegravir. The red circle marks oxygen atoms that complex bivalent metal cations in the active center of the HIV-1 integrase. The blue circle is the area of the molecule that is able to absorb the positive charge after the complexation of the metal ions. The purple circle is a flexible compound, whereas the green circle shows the hydrophobic region of the molecule.

![Figure 2: Structure of Dolutegravir. [3]](image)

Furthermore, Dolutegravir is a tetracyclic compound that has a peptide bond, which is represented by the NH-CO bond. It should be added that two or three ketone groups can be identified, which can enter a complex with magnesium. The compound is an enantiomer, since it behaves like a mirrored image and cannot be merged into one another by rotation or other symmetry operators. Two chiral C-atoms can be recognized, which can point backwards by mirroring at the yz-plane.

The fluorine atom of the aroma exerts a -I effect on the ring. Thus, the fluorine draws the electrons of the ring, as it has a high electronegativity. Nevertheless, it simultaneously exerts a positive mesomeric effect, thus the electron density of the ring is increased, since the free electron pair of the fluorine can enter the ring. Therefore an electrophilic aromatic substitution at the ring would be conceivable.

The first step to synthesise Dolutegravir is the condensation reaction of 4-methoxyacetoacetate 1 and 1.6 equiv dimethylformamide dimethylacetal 2 (DMF-DMA). If the reactants are streamed through a T-mixer at 85°C with an residence time of 10 minutes the reaction results in a full conversion of 4-methoxyacetoacetate 1 to dimethyl vinylogous amide 4. By adding aminoacetaldehyde dimethylacetal 5 directly into the output of reactor 1 the second T-mixer can also be directly connected to output. In the second T-mixer the reactants combine to vinylogous amide 6.

In the third step of the synthesis the product 6 of reactor 2 is premixed with dimethyl oxalate 7 in an interposed T-mixer. For dimethyl oxalate 7 being a solid, a solvent is required. For this solvent is also needed to solve NaOMe, which is added after the premixing methanol is being used. The mixture including the NaOMe is lead into another T-mixer where the pyridone 8 is formed. To utilise this step of the synthesis several back pressure regulators (BPR) had to be implemented.

The next step is to form amide 9 from pyridone 8 by adding difluorobenzylamine 10. For this reaction the pyridone 8 is solved in acetic acid with a bit additional PhCH₃ and optimally reacts with the difluorobenzylamine 10 at 200°C and a residence time of 124 min.

In the next step p-Toluenesulfonic acid (tosylic acid (TsOH)) is added to the output of step 4 to deprotect amide 9 and therefore allow a reaction with amino alcohol 11. For the PhCH₃ added in the
fourth step inhibiting these steps the concentration of the reactants in the solution must not be too low.

Figure 6: Three-step telescoped synthesis of Methoxydolutegravir 12. [4]

After purifying the product of the sixth step 12 the last step of the synthesis, a demethylation, can be conducted. Using LiBr, H₂O and tetrahydrofuran leads to a small amount of byproducts and is therefore particularly suited. This step cannot be telescoped to the previous steps due to clogging issues preventing the system from running more than 10 hours at a time.

Figure 7: Flow synthesis of Dolutegravir. [4]

The whole synthesis of Dolutegravir as described above consists of 7 steps which can be merged into three flow operations. The overall yield in this specific synthesis is 24%.

4 Medical Effect

The Dolutegravir belongs to the second generation of active ingredients known as INSTI (Integrase-Strand-Transfer-Inhibitor). This type of integrase-inhibitors are virustatic acting agents, meaning a substance which inhibits the propagation of viruses.
In general, the medicine is administered in form of its sodium salt and has a half-life of 13-15 hours. The plasma concentration remains for more than 30 hours above the IC90-value, which means the virus replication in this period is inhibited to 90%. [3] [6]

4.1 Mechanism of the Virus reproduction

![Human Immunodeficiency Virus (HIV)](image)

**Figure 8**: (a)HI-Virus. [7] (b)HIV Life Cycle. [8]

After the fusion of the virus with the host cell, enzymatically active proteins are responsible for the transcription in the reverse direction of RNA into DNA.

The enzyme from the retrovirus (viruses whose genetic information is available in the form of ribonucleic acid) builds viral DNA into the chromosomes of the host cell. This is also known as the work of an integrase, which is able to cut the human chromosomes and insert the newly formed viral DNA. [8]

4.2 Mechanism of the integrase-inhibitor

The active substance binds to the active centre of the integrase, thus inhibiting the strand transfer and the integration of the retroviral deoxyribonucleic acid. Viruses are completely dependent on host cells because they do not have their own metabolism and need those of the host cells to realize their genetic material and replication. The enzyme of the virus is no further able to integrate any more viral DNA into the chromosomes of the host cell. As a result, the replication cycle is blocked. [9]
4.3 Side effects

One of the studies which has hit a big wave was the Tsepamo study. The study revealed that neural tube defects have been observed by women who have taken Dolutegravir during the time of conception. In Botswana, where the study was conducted in 2018, 4 of 426 cases of pregnant women have shown such results. Nevertheless, the WHO (World Health Organization) came to the conclusion that the rest of neural tube defects were significantly lower than previously assumed. The WHO therefore recommends Dolutegravir for all population groups because it is more effective and easier to absorb. [10] [11]

Another side effect besides the common ones, including headache, nausea, vomiting, was the increase of the infected person's weight. This was seen in a study by Menard et al. (2017) in which 517 patients receiving dolutegravir-containing antiretroviral therapy were observed. On average the body weight increased by 3 kg.

<table>
<thead>
<tr>
<th>Group</th>
<th>Observed change/increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among 27%</td>
<td>4%-10%</td>
</tr>
<tr>
<td>Among 20%</td>
<td>More than 20%</td>
</tr>
<tr>
<td>Among women</td>
<td>about 5Kg</td>
</tr>
</tbody>
</table>

Table 1: Observed body weight increase.

The researchers recognized that the body weight increases parallel with the lowering of the HI-Virus load and an immune reconstruction. [12]

Besides these effects the newly recognized problem is, certain HIV integrase inhibitors can reduce the function of CD4-T helper cells and thus having a potential harmful effect on the activity of immune cells. Within the group of integrase inhibitors (INSTI), the scientists detected differences: The active substances Elvitegravir and Dolutegravir had a significant influence on cell function, while Raltegravir did not. In order to understand the cause of the reduced function, growth and cell division of CD4-T cells, the researchers investigated a possible influence of the active substances on the mitochondria. They found Elvitegravir and Dolutegravir interfering with the electron transport chain of the mitochondria and impaired their respiratory capacity, thereby slowing down cell activity overall. Nevertheless according to them, in perspective of the widespread use of INSTI, further studies are needed to determine the clinical impact of the results. [13]

4.4 Social benefits of Dolutegravir

When HIV had its massive outbreak during the mid 1980s it had a huge impact on society. Since its outbreak around 35,000,000 people have died from AIDS. The life expectancy of untreated AIDS patients is around eleven years. Thus, medical aid is crucial for infected patients. With proper medical treatment they may have a normal life expectancy. Today, more than 70,000,000 people are still infected and depend on working anti-viral drugs. Dolutegravir, being such a drug, is a hope for many patients as 47% AIDS-patients do not have access to medical treatment. Especially because Dolutegravir is recommended by the WHO as preferred drug in all populations, it could raise the life expectancy in developing countries even further. [1] [14] [15] [11]
5 Hope in a Pandemic

5.1 Using already know drugs for a new disease:

Due to the rapid spread of SARS-CoV-2 a treatment against it is strongly needed. While some companies try synthesizing a new drug, others focus on testing viral drugs that already exist. This has the advantage that those existing drugs were already tested on short- and long-term side effects. Thus, saving money and valuable time in the testing process in the fight against the disease. [16] [17]

5.2 The advantage of AI based drug research

One way to find existing, effective medicaments is to use artificial intelligence (AI). Deep learning-based programs are able to precisely predict binding affinities between given drugs and the target molecules, using amino acid sequences and chemical sequences of target proteins. Furthermore, the AI does not need structural information of the protein. This is especially useful since finding drugs targeting an uncharacterized protein with conventional 3D structure-based programs generally is a problem that delays progress. [16] [18] [17] [19]
5.3 Dolutegravir and SARS-CoV-2:

Multiple artificial intelligence based researches proposed (amongst other molecules) Atazanavir, Efavirenz, Ritonavir, Raltegravir, Paritaprevir, Bictegravir and Dolutegravir as possibly drugs against SARS-CoV-2. While some of the listed drugs were only proposed once (depending on the used program), Dolutegravir was listed in a majority of the studies.

![Image of Dolutegravir interaction with nCoV 2'-OMTase]

Figure 10: The predicted interaction of Dolutegravir (Top) (DB08930) and Bictegravir (Bottom) (DB11799) with the nCoV 2'-OMTase. [16]

Artificial intelligence predicted Dolutegravir being able to interact with the nCoV 2'-OMTase. The 2'-OMTase is responsible for providing the viral mRNA which is necessary for the virus to hide from the host cell. Not being detected by the host cell the infected body does not activate its immune system. This is ability is crucial for a successful viral infection and thus making Dolutegravir a very promising candidate for a potential cure. Unfortunately, no further research was done towards Dolutegravir as other antiviral molecules showed better results in further research. As of June 2020, the research targeting a COVID-19 cure is still ongoing. [16] [19] [18] [20]

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