

# War crimes in medicine:

## Unethical malaria medicine lead optimization

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### How are medicines developed?

Theoretical medicine development has three distinct steps:

#### 1, target finding:

First a target must be found, this is the place where the medicine will do something. A target is found by studying the organism you want to develop a medicine for or against and finding critical proteins the organism needs to survive.

#### 2, lead search:

After a target is found the next step is finding a lead which affects the target in some way, this is done by deciding what your medicine must effect and then a molecule that can do that thing is searched for which is the lead.

A lead can affect the protein in multiple ways. An important one for this study is inhibition where the lead takes the place of the molecule that normally binds to this protein (substrate) stopping the protein from working (competitive inhibition). Inhibition can also work by having the lead affect the binding site for the substrate indirectly, for example changing the shape of the protein at the binding site (non-competitive inhibition).

#### 3, lead optimization:

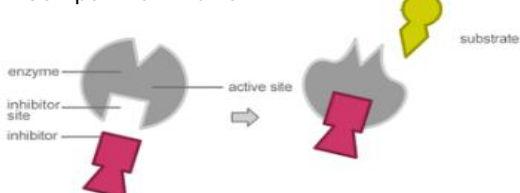
Finally, when a lead is found the last step is to optimize it, this is done by slightly changing the chemistry of the lead molecule and checking how well it bonds to the protein, in this stage the molecule is also heavily checked for having negative side effects.

Afterwards the practical phase begins with preclinical research on cells and animals which lasts about 3 to 5 years. Then clinical research can begin where the medicine is evaluated on people, which lasts about 6 to 7 years. Then after about 2 years of administrative business the medicine can finally be released to the public. However, after the release, the long-term effects of the medicine continue to be monitored.

#### Competitive inhibition



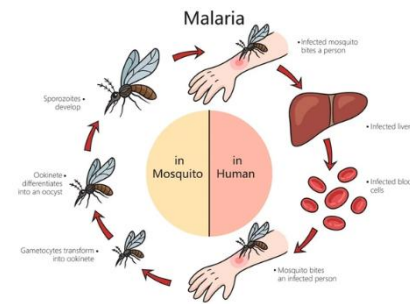
#### Non-Competitive inhibition



### Malaria (how it works and how it can be fought)

Malaria is a fatal disease that is caused by single-cel parasites. Mosquitoes transmit these parasites. There are currently only a couple of medicines against malaria, and the problem is that the parasites are mutating to be resistant to these different medicines. The parasites have distinct stages, and each stage has its own environment.

The cycle “begins” inside the host (which is a human in this case). The host is infected with the malaria parasite, when a mosquito bites the host, the parasites will transfer from the host to the mosquito. There the parasite will then multiply in the intestinal tract and end up in the salivary glands via the blood.



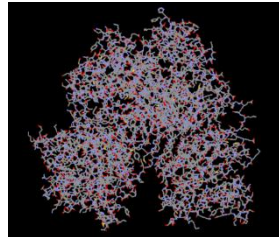
When this mosquito bites his next victim, the parasite will be transferred to their bloodstream via the mosquito's saliva. In a host (human) the parasites will multiply inside the liver and after some time, reenter the bloodstream. Inside the bloodstream they will nest in red blood cells and multiply there, this will be at the cost of the cell itself. The red blood cells will die, and the same applies for the liver cells. This malfunction of the red blood cells and liver will have dire consequences for the host, blood vessels clog and this will result in the malfunction of other organs. In this last stage a mosquito can bite the infected host and quickly infect the next person.

When we look at medicine development, most of them target the last stage of the parasite, in which it multiplies in the red blood cells. This is because this is also the stage where you get sick. There are a couple medicines against malaria:

1. Nivaquine (chloroquine) Pro= preventive, almost no side effects. Con= malaria parasites are becoming resistant to it.
2. Larium (mefloquine). Pro= preventive. Con= You need to start very early, and you need to keep taking it for a very long time
3. Paludrine (proguanil). Pro= preventive. Con= malaria parasites are becoming resistant to it.
4. Malarone (atovaquon, proguanil). Pro= preventive, works very fast, good for emergency treatment. Con= specific method of intake, it is a suspension
5. Daraprim (pyrimethamine). Pro= only a small dose needed, also works on kids (from 10 months old). Con= a lot of side effects

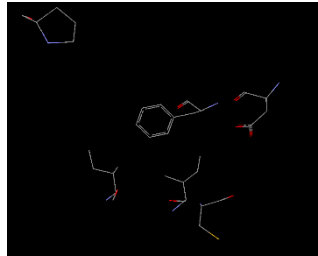
As you can see, each medicine has its own pros and cons, and the parasites are already becoming resistant to two of the medicines. This is why it is important to develop more medicines and research how

parasites are becoming resistant to our medicines. In this report we will focus on the preclinical phase of medicine development. To further specify, the working protein pyrimethamine. Pyrimethamine blocks the DHFR enzyme of the parasite, which is very important for producing a precursor for DNA. In this way, the parasites will die, but humans also have the DHFR enzyme. We do not want pyrimethamine to block this enzyme, because we also need it for our metabolism.



DHFR

So, we want a medicine that binds well with our target (the (mutated) DHFR) that also leaves the human DHFR alone as much as possible. To do this we will be taking the existing medicine pyrimethamine and altering it slightly so it will hopefully function better and look at another medicine proguanil. With the help of a program called ArgusLab we could quickly evaluate the binding strength of the hypothetical medicines by finding the best position for a medicine to bind to the protein in a procedure called docking.



Binding site of DHFR (1j3i)

## Hypothesis

With an analysis of the site where molecules normally dock to DHFR we find a couple of opportunities for strong bonds:

1. **Isoleucine** is very apolar which allows for an apolar bond.
2. **Cysteine** has a sulfur atom that can form sulfur bonds.

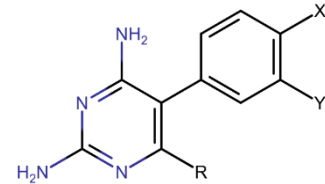
3. **Phenylalanine** has a benzene ring which is hydrophobic, making hydrophobic groups sit next to it.
4. **Aspartic acid** allows for electrostatic interaction and can also form hydrogen bonds.

We intend our variants to make use of at least one or more of these opportunities. We hypothesize that taking advantage of these opportunities will produce far more effective medicine.

Our goal is also to be as unethical as possible in finding our lead variations and the chemical structure of the lead variations themselves.

## Method and results

For every lead variant we have evaluated, we measured their performance by the bond strength of the lead variant with the target protein in Kcal/mol.



We started by docking the already well-known malaria medicines pyrimethamine and the active metabolite of proguanil (cycloguanil) to the wildtype protein DHFR found in malaria parasites (code: 1j3i), three mutated variants of the protein (1j3j, 3um5 and 1j3k) and finally the human variant (3s7a). Then we docked seventeen variants of pyrimethamine to the target with differing side groups at the X, Y and R sites. Finally, we docked a cycloguanil analog modified with the side groups from the best performing pyrimethamine variant. The results are shown in Table 1 below.

Lead variants	Wildtype <b>1j3i</b> (Kcal/mol)	double mutant <b>1j3j</b> (Kcal/mol)	double mutant <b>3um5</b> (Kcal/mol)	quadruple mutant <b>1j3k</b> (Kcal/mol)	human DHFR <b>3s7a</b> (Kcal/mol)	Performance	X	Y	R
<b>Pyrimethamine</b>	-10.374	-10.6791	-11.1003	-10.1264	-10.326	0.244	Cl	H	CH <sub>2</sub> CH <sub>3</sub>
<b>Cycloguanil</b> (proguanil)	-9.568	-8.9903	-9.44806	-9.24005	-9.81851	-0.507	Cl	H	(CH <sub>3</sub> ) <sub>2</sub>
Pyrimethamine variant 1 <b>(CC83)</b>	-9.7061	-10.2121	-9.66074	-9.86595	-9.25968	0.602	H	O- CH <sub>3</sub>	CH <sub>2</sub> CH <sub>3</sub>
Pyrimethamine variant 2 <b>(S03)</b>	-10.4471	-10.4041	-10.62	-10.6749	-9.83701	0.700	H	Cl	CH <sub>2</sub> CH <sub>3</sub>
Pyrimethamine variant 3 <b>(AR6)</b>	-9.7478	-10.96	-9.87767	-10.872	-10.2256	0.139	H	CF <sub>3</sub>	CH <sub>2</sub> CH <sub>3</sub>
Pyrimethamine variant 4 <b>(AR4)</b>	-10.1969	-10.1221	-10.1135	-10.2544	-9.24898	0.923	H	F	CH <sub>2</sub> CH <sub>3</sub>
Pyrimethamine variant 5 <b>(AR3)</b>	-10.495	-9.86068	-10.3757	-10.7022	-10.2126	0.146	H	Br	CH <sub>2</sub> CH <sub>3</sub>
Pyrimethamine variant 6 <b>(Lawrenced)</b>	-10.4661	-9.75178	-9.52807	-10.7355	-10.0808	0.040	Lr	Cl	CH <sub>2</sub> CH <sub>3</sub>
Pyrimethamine variant 7 <b>(OH HA!)</b>	-9.8756	-9.8577	-9.48121	-10.7265	-9.95	0.035	OH	Cl	CH <sub>2</sub> CH <sub>2</sub> S
Pyrimethamine variant 8 <b>(Sulfurious)</b>	-10.2	-10.02	-10.1568	-11.0811	-9.9	0.464	H	Cl	CH <sub>2</sub> CH <sub>2</sub> S
Pyrimethamine variant 9 <b>(Tentacle man)</b>	-11.96666	-11.7137	-12.0617	-11.56	-10.6956	1.130	H	Cl	C(CH <sub>2</sub> CH <sub>3</sub> ) <sub>3</sub>
Pyrimethamine variant 10 <b>(Fluoride man)</b>	-11.6016	-11.262	-11.4618	-10.7961	-10.9294	0.351	H	F	C(CH <sub>2</sub> CH <sub>3</sub> ) <sub>3</sub>
Pyrimethamine variant 11 <b>(Musty ruff)</b>	-11.1007	-10.7778	-10.2163	-11.5426	-11.0532	-0.144	Cl	CH <sub>2</sub> NF <sub>3</sub>	C(CH <sub>2</sub> CH <sub>3</sub> ) <sub>3</sub>
Pyrimethamine variant 12 <b>(Oxidator)</b>	-12.1378	-9.84626	-12.0374	-12.6792	-11.3246	0.351	Cl	CH <sub>2</sub> ClF <sub>3</sub>	C(CH <sub>2</sub> CH <sub>3</sub> ) <sub>3</sub>

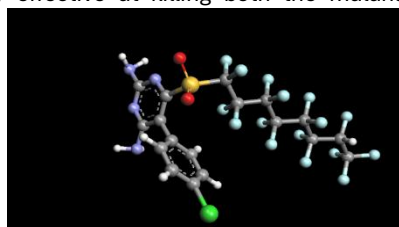
Pyrimethamine variant 13 ( <b>Tentacle man 2.0</b> )	-12.4999	-11.6229	-11.5633	-12.3445	-9.7106	2.297	Cl	H	C(CH <sub>2</sub> CH <sub>3</sub> ) <sub>3</sub>
Pyrimethamine variant 14 ( <b>Passed gas</b> )	-14.5254	-13.752	-13.2231	-14.0281	-12.9524	0.930	Cl	S(CH <sub>2</sub> CH <sub>2</sub> Cl) <sub>2</sub>	C(CH <sub>2</sub> CH <sub>3</sub> ) <sub>3</sub>
Pyrimethamine variant 15 ( <b>Forever medicine</b> )	-10.48	-11.8436	-10.2293	-10.4756	-10.8327	-0.076	Cl	H	C <sub>8</sub> F <sub>17</sub> O <sub>2</sub> S
Pyrimethamine variant 16 ( <b>Cocainum</b> )	-7.87162	-8.4699	-9.16662	-3.88694	-10.269	-2.920	Cl	H	C <sub>17</sub> H <sub>20</sub> NO <sub>4</sub>
Pyrimethamine variant 17 ( <b>Urane</b> )	-11.9997	-11.3914	-11.2456	-11.9569	-9.77051	1.878	Cl	U	C(CH <sub>2</sub> CH <sub>3</sub> ) <sub>3</sub>
Cycloguanil variant 1 ( <b>Cyclotentacle man</b> )	-10.9669	-10.0946	-9.46476	-10.7971	-11.4753	-1.144	Cl	H	HC(CH <sub>2</sub> CH <sub>3</sub> ) <sub>3</sub>

As for the colors, yellow has been set as the calibration for which we used pyrimethamine, green means the variant performs very well in that category, and red means the variant performs very badly for that category. Performance is a measure of how good the variant does overall and is calculated with the following formula:

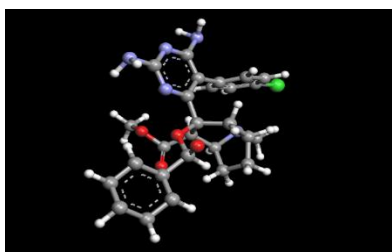
$$\frac{E_{1j3i} + E_{1j3j} + E_{3um5} + E_{1j3k}}{4} + E_{3s7a}$$

Where E is the bond energy in Kcal/mol, this formula gives extra weight to the performance of the variant with the human protein because that is a very important measure and we wouldn't want the medicine to be very effective at killing both the malaria parasite and humans.

Having said that, the pyrimethamine variants 6, 11, 12, 14, 15, 16 and 17 are of note. Variant 6 includes an atom of Lawrencium meaning the medicine would only be in this state for approximately 4.2 seconds before the lawrencium decays, variant 11 or "Musty ruff" contains nitrogen trifluoride which is toxic and variant 12 contains chlorine trifluoride which is an oxidizer for rocket fuels. Variant 14 or "Passed gas" contains mustard gas as a side group at position Y, variant 15 contains Perfluorooctanoic acid or PFOA which is a PFAS and is carcinogenic. Variant 16 has a side group at location R which is cocaine and variant 17 in a much comparable way to variant 6 has a uranium atom.



Variant 15 "Forever medicine"



Variant 16 "Cocainum"

Looking at the performance of all variants, a couple of things become clear:

1. Proguanil is inferior to pyrimethamine.
2. A sulfur side group is not very effective.

3. Chlorine at position X is very beneficial to the effectiveness of the variant.
4. The variant with mustard gas has the strongest bonds overall but also has a very strong bond to the human protein resulting in lower effectiveness.
5. The C(CH<sub>2</sub>CH<sub>3</sub>)<sub>3</sub> side group is very effective in producing a stronger bond.
6. Cocaine is not a good medicine.
7. However, a uranium atom side group is not horrible.

Of course, there also needs to be in vitro research as these results may not entirely match what happens in the real world. With in vitro research, the medicine is tested in living cells/tissue (not whole organisms), and it happens in a lab. We have found some results of in vitro research about all the different variants of the DHFR enzyme and the distinct types of pyrimethamine. The values you see below are the Ki values, the higher the Ki value, the more active DHFR is, so the less the medicine works.

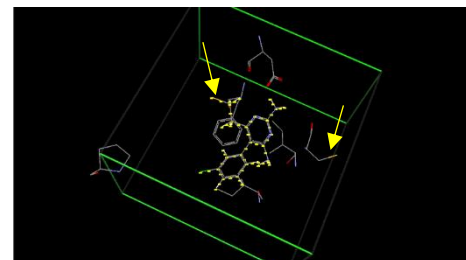
**Table 1** Inhibition of DHFR activity by *meta*-pyrimethamine analogs of recombinant wild type (WT) and mutant DHFR domains of bifunctional DHFR-TS from *P. falciparum* assayed as described in the experimental section

Compound	pDHFR (Ki/nM)				hDHFR (Ki, nM)
	Mutant				
	WT	A16V + S108T	C59R + S108N	N511 + C59R + S108N + I64L	
1 Pyrimethamine	1.5 ± 0.2 <sup>a</sup>	2.4 ± 0.2 <sup>a</sup>	72 ± 3 <sup>a</sup>	859 ± 117 <sup>a</sup>	58 ± 3.3
Cycloguanil	2.6 ± 0.3 <sup>a</sup>	1.314 ± 165 <sup>a</sup>	82 ± 4 <sup>a</sup>	730 ± 19 <sup>a</sup>	85 ± 14.5
WR99210	0.9 ± 0.16	3.2 ± 0.5	0.8 ± 0.1	0.9 ± 0.1	8.1 ± 0.8
2 CC83( <i>m</i> -OMe)	1.07 ± 0.10 <sup>b</sup>	nd	14.0 ± 2.6 <sup>b</sup>	nd	nd
3 S03 ( <i>m</i> -Cl)	0.3 ± 0.03 <sup>b</sup>	nd	2.4 ± 0.26 <sup>b</sup>	nd	nd
4 AR6 ( <i>m</i> -CF <sub>3</sub> )	3.9 ± 0.1	3.6 ± 0.2	3.3 ± 0.5	12 ± 0.7	17 ± 1.2
5 AR4 ( <i>m</i> -F)	2.0 ± 0.5	3.7 ± 0.5	3.6 ± 0.6	28.8 ± 2.6	21 ± 4.0
6 AR3 ( <i>m</i> -Br)	0.7 ± 0.1	2.5 ± 0.2	1.1 ± 0.1	5.1 ± 0.4	12 ± 1.0

<sup>a</sup>Data from ref. 10. <sup>b</sup>Data from ref. 15. nd = Not determined.

## Conclusion

Coming back to our hypothesis we find that the sulfur hydrogen bonds were not actually that important compared to the hydrophobic benzene ring of Phenylalanine, the



The sulfur not working (variant 7)

addition of a very large hydrophobic group for variant 9 or “Tentacle man” greatly improved bond strength. With variant 14 we observed both hydrophobic groups ‘huddling’ around the benzene ring resulting in very strong bonds. We also found the chlorine at position X to be critical in preventing bonding to human DHFR.

If we compare our results of table 1 and the  $K_i$  values of the in vitro studies in table 2, we find that some parts are similar. It corresponds, but there are some big differences. If we look at the binding energy of regular pyrimethamine with the double and/or quadruple mutant, we see that in table 1 it binds fairly good. But if we look at table 2, the  $K_i$  value is high, so it does not work all that well according to in vitro research. We also see that the differences in binding energy are not particularly big between the different types of pyrimethamine and DHFR variants. But if we look at table 2, the values differentiate a lot. Then there is the problem that the values can have a big margin, it is all very roughly described. They may have high starting values but can also be very low because of the big margin. So, to conclude, it is very difficult to compare the two tables.

### **But in the end how unethical was this study?**

We docked very many variants, and all the docking was performed in ArgusLab which is an older program that is not optimized for modern systems. All of this resulted in a significant power draw releasing a larger amount of  $\text{CO}_2$  than traditional methods like pen and paper or more modern programs.

We have also evaluated many unethical side groups, and a uranium atom turned out not to be entirely horrible, if this medicine were administered to people cancer and radiation poisoning rates would significantly increase due to the alpha radiation of the uranium. The highly effective  $\text{C}(\text{CH}_2\text{CH}_3)_3$  side group would probably also require oil derived chemicals to produce resulting in more environmental pollution.

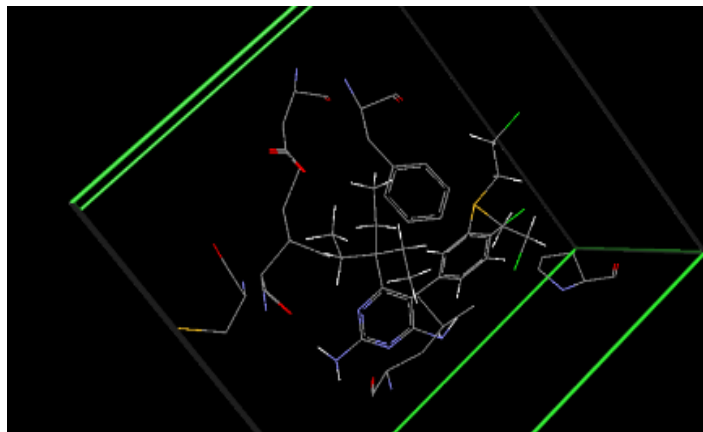
Thus, our research was very unethical overall.

## Discussion

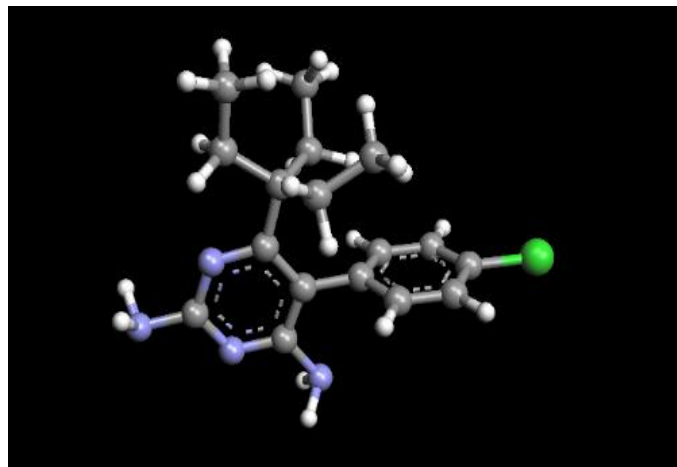
There are more opportunities for being unethical, there are many more dangerous and carcinogenic molecules that have not been tested as variants and the process could be automated to dock even more variants for a larger power draw.

For improving the medicine more an interesting option that could be explored more is hydrophobic groups that have trouble docking with human DHFR. More proguanil variants can also be explored however as its base performance is worse than pyrimethamine this is less effective than looking for better pyrimethamine variants.

An important note is the fact that the variants we docked are in their active state, however we simplified our paper to not include decomposition of the molecule through biochemical processes and we also didn't include what the passive state of a variant would look like and how it would be activated.



*'Hydrophobic huddling' with "Passed gas."*



*Variant 13 "Tentacle man 2.0"*