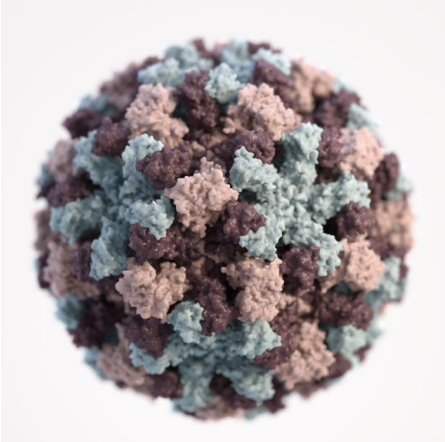
NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Stomp the Stomach Bug: Designing Norovirus Inhibitors



by
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Suzie opened her email to see another message from the director of the student health center. The subject of the message read, "Health alert: winter vomiting disease found on campus." The message was to inform the students that a large number of norovirus cases had been confirmed on campus and that students should be extra vigilant about washing hands and to stay in their rooms if they felt sick.

"Great," she thought. "This place is like a petri dish. I can't afford to get sick with exams right around the corner."

Just then, her phone buzzed to alert her to a text message. It was her boyfriend Daniel. The text read, "Up all night throwing up. Can I get your notes in Med Chem later?"

Suzie googled *norovirus* and found that human noroviruses are a common cause of "stomach bugs." According to the Centers for Disease Control and Prevention, they cause more than 50% of all food-borne disease outbreaks and are the most common cause of diarrhea in adults. People become infected with the virus when they eat something contaminated by the virus or touch their mouth, nose or eyes after touching a contaminated surface or object. She also found that because noroviruses are very hardy, highly contagious, and can be resistant to some sanitary measures, they are common on cruise ships, in day care centers, and on college campuses where people are often in close contact. One of the strains of the virus was even responsible for shutting down Hampden-Sydney College for a couple of days in early 2015. In addition, there is no vaccine or norovirus-specific antiviral therapy or prophylactic.

Questions

1. Given that most people who fall ill with norovirus have a very unpleasant, but basically short-lived bout with vomiting and diarrhea, is it worth researching a specific treatment or cure for the illness?

In Suzie's medicinal chemistry course, she had been studying antiviral medications and wondered if a norovirus specific treatment could be possible.

2. What are the general features of targets for possible antiviral drugs?

Upon further reading, Suzie learned that noroviruses contain a single strand of positive sense RNA. Upon replication, a large polypeptide is produced, which is processed into individual proteins by the virus' 3CLPro protease. This protease is a chymotrypsin-like cysteine protease that cleaves glutamine-glycine bonds and has been identified as a potential target for anti-norovirus medicines.

3. What is the general job of a protease?

4. What general characteristics do competitive protease inhibitors need to possess?

5. What does it mean to be a chymotrypsin-like cysteine protease?

Suzie found an article (Kankanamalage *et al*., 2015, <https://doi.org/10.1021/jm5019934>) that reported the results of researchers who analyzed more than 30 potential dipeptidyl inhibitors of the noroviruse protease.

6. Why are peptide analogs often used for the inhibition of proteases?

Upon looking at the general structure of these peptide analogs, Suzie wondered why the researchers chose this particular scaffold.

7. Explain the potential significance of each portion of the general structure (Figure 1).

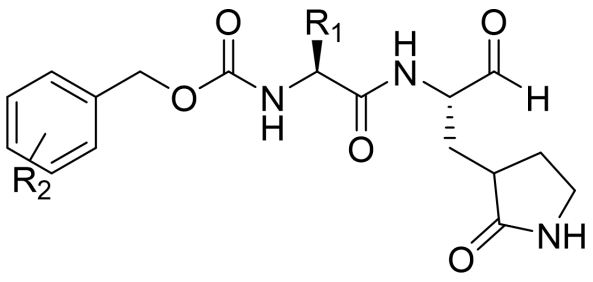
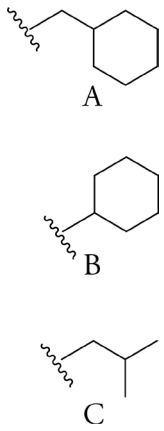


Figure 1. General structure of dipeptidyl 3CLPro inhibitors.

In her medicinal chemistry course, Suzie had just finished learning about structure-activity relationships (SAR) where the structure of the lead compound can be varied in a semi-systematic way to determine the best possible structure for optimal activity, selectivity, and bioavailability. She noted with interest how the different functional groups in the lead compound were changed in Table 1 (next page) and wondered why. She also saw two different values for reporting how good the inhibitor was, which confused her.

Table 1. Activity against norovirus 3CLPro protease and norovirus (data from Kankanamalage *et al.*, 2015).

Compound	R_1	R_2 *(see right)*	IC_{50} *(μM)*	ED_{50} *(μM)*
1	*o*-Cl	A	0.8	0.08
2	*m*-Cl	A	0.1	0.02
3	*m*-Cl	B	5.1	0.15
4	*m*-Cl	C	0.9	0.1
5	*p*-Cl	A	0.71	0.08
6	*o*-F	A	0.9	0.08
7	*m*-F	A	1.2	0.09
8	*m*-Br	A	0.3	0.03
9	*m*-I	A	0.35	0.05
10	*o*-OCH_3	A	>10	0.1
11	*m*-OCH_3	A	1.5	0.15



8. Explain to Suzie the difference between IC_{50} and ED_{50} in Table 1. Why might these values be different?

9. In order to help Suzie understand the SAR study, look at the inhibitor data shown in Table 1. What conclusions can you draw about the structure of the best inhibitors?

In her other classes, Suzie had learned about a lot of other proteases, including digestive enzymes that help break down food for energy and enzymes that help in the blood clotting response. She knew that drugs that inhibit the Norovirus protease might also be able to inhibit these important enzymes and that could lead to serious side effects. She decided to look more into the specificity of these potential drugs.

10. Why would specificity for the norovirus 3CLPro protease be important for this class of inhibitors?

Table 2. Selectivity of compounds 2 and 8 against other proteases (data from Kankanamalage *et al.*, 2015).

		Compound (% inhibition)		
Enzyme	*[I]/[E]*	*2*	*8*	*[I] (μM)*
Human neutrophil elastase	50	15	26.5	15.4
Chymostrypsin	250	0	0	2.5
Trypsin	250	5	0	125
Thrombin	250	0	0	2.75
Factor Xa	250	1	0	1.23
Plasmin	250	0	7	2.5
Carboxypeptidase A	250	12	11.6	43

11. Look at the specificity data in Table 2 and draw conclusions about which compound would be the best inhibitor tested.

12. What additional information would the researchers need to consider before moving forward with these inhibitors as a potential drug?

References

Burlingham, B.T., and T.S. Widlanski. 2003. An intuitive look at the relationship of K_I and IC_{50}: a more general use for the Dixon plot. *Journal of Chemical Education* 80: 214–8. DOI: 10.1021/ed080p214.

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Kankanamalage, A.C.G. *et al.* 2015. Structure-guided design and optimization of dipeptidyl inhibitors of norovirus 3CL protease. Structure–activity relationships and biochemical, X-ray crystallographic, cell-based, and *in vivo* studies. *Journal of Medicinal Chemistry* 58, 3144–55. DOI: 10.1021/jm5019934.