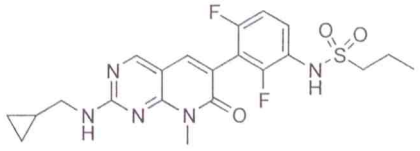
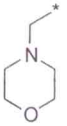
1



2



3

In February 2012 the European Medicine Agency (EMEA) has approved vemurafenib (structure **1**) as a new anti-cancer drug for the treatment of metastatic melanomas. Vemurafenib is an ATP-competitive inhibitor of the mutated kinase BRAF in which valine at codon 600 is replaced by a glutamate. The crystal structure of vemurafenib with $BRAF^{V600E}$ has been elucidated. It has been shown that vemurafenib has a similar bindingsmode as PLX4720 (See Tsai et al., Proc. Natl. Acad. Sci, 2008).

In general, there are two types of kinase inhibitors (type I and II), which stabilize different conformations of the protein.

1a. What type of kinase inhibitor is vemurafenib (**1**) and what conformation is preferentially stabilized by (**1**)? Explain your choice. (50 p.)

Recently, other $BRAF^{V600E}$ – inhibitors have been disclosed. For example, compound **2** was also shown to inhibit $BRAF^{V600E}$ with an IC50 of 3 nM and was effective in a xenograft model.

1b. Describe (and draw) the main molecular interactions (H-bonds, hydrophobic interactions) of compound **2** with important amino acids of the ATP binding pocket of $BRAF^{V600E}$ (50 p.)

Vemurafenib potently inhibits $BRAF^{V600E}$ with an IC50 of ± 30 nM, however, the dose required to obtain sufficient plasma exposure is rather high (960 mg, twice a day). This is due to extremely low water solubility and high serum protein binding of the compound. To find a novel drug for the treatment of metastatic melanomas, which can be given at lower dose than vemurafenib, a back-up program has been initiated to synthesize selective $BRAF^{V600E}$ inhibitors in which *solubilizers* (water-soluble functional groups) will be introduced to vemurafenib.

2a. At what position in vemurafenib (**1**) would you introduce a morpholine (**3**) as a *solubilizer* (asterisk indicates the attachment point)? Choose your position in such a way that the morpholine group is unlikely to affect potency and/or selectivity of your drug. Explain your choice. (50p.)

One of the side effects of vemurafenib is to induce another type of cancer (low grade squamous cell carcinomas) in some patients. It is thought that this effect may be mediated by the activation of wild-type BRAF by vemurafenib.

2b. Give a plausible explanation how an ATP-competitive kinase inhibitor can paradoxically activate a kinase? (50 p.)

EXAM CHEMICAL GENETICS
OCTOBER 25, 2012

GUBBENS

Questions antibiotics in prokaryotes

1. In virtual all cases a gene cluster coding for the synthesis of an antibiotic also contains genes that do not code for synthesis activity.

   a) (20 pt) Which other two types of genes are found in such a cluster and why are they always present?
   b) (40 pt) Explain why these genes are typically not identified after filtering in a proteomining experiment?

   In the PRiSM approach, NRPS/PKS-derived peptides containing a phosphopantetheine modification are detected by MS/MS analysis. This is not trivial due to the presence of thousands of unmodified peptides, rendering this approach a search for the proverbial 'needle in a haystack'.

   a) (40 pt) Explain how a combination of the OASIS and PRiSM approaches might simplify this search?

2. Peptide-based antibiotics are almost always produced by non-ribosomal peptide synthases (NRPS).

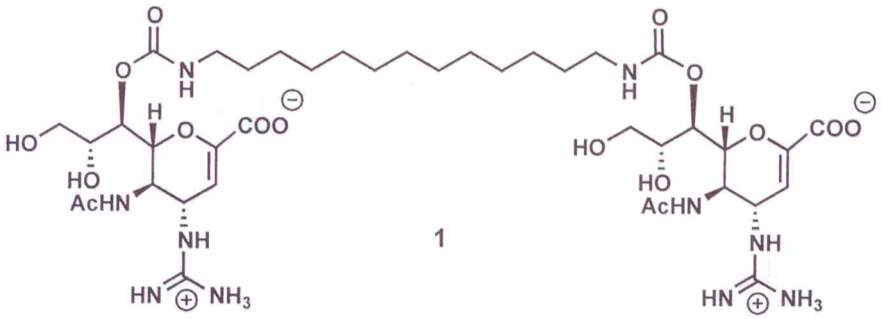
   a. (30 pt) Why are most peptide antibiotics not produced by the ribosome?
   b. (35 pt) Although not synthesized by the ribosome one can still deduce the sequence of peptide antibiotics by examining the gene sequence of NRPS. Explain how this can be done?

   From the sequence of an NRPS it is deduced that one of the incorporated amino acids should be proline.

   c. (35 pt) How can this fact be used to find the final product?

CG-Question 1 (40 + 40 + 40 points)

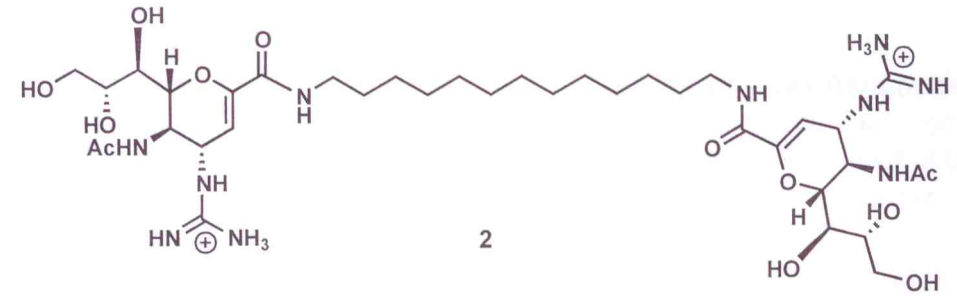
One of the future directions for the development of more potent influenza inhibitors entails the development of multimeric Zanamivir constructs such as compound **1** depicted below.



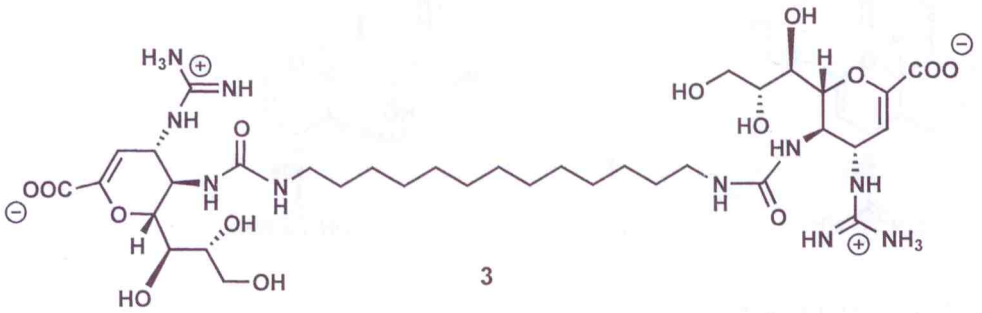
1

In an isolated enzyme essay, the activity of this compound is reduced to that of the parent Zanamivir, while the effect of the dimer on the replication of (whole) viruses greatly exceeded the activity of the monomeric Zanamivir (also when corrected for the two copies of the Zanamivir present).

A) Explain why the exploitation of dimeric or multivalent Zanamivir constructs can lead to more potent inhibitors.
B) Provide an explanation why the compound shown is less active in the isolated enzyme essay but more potent in inhibition of virus replication.
C) In the dimeric construct shown the two copies of Zanamivir are joined together via a spacer at the C7-hydorxyl. Is this a logical place for the spacer? Could the two copies of Zanamivir also be welded together via a spacer connecting the acid functions or acetyl groups as in the dimers **2** and **3** depicted below?



2



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CG-Question 2 (80 points)

A) Based on the mechanism of action of retaining glycosidases the compound depicted below has been designed as a potential glucosidase inhibitor. Describe the structural features that make this compound an attractive lead compound.