EXAMINATION CHEMICAL BIOLOGY 2016-2017

Friday, 23-07-2017

14.00-17.00

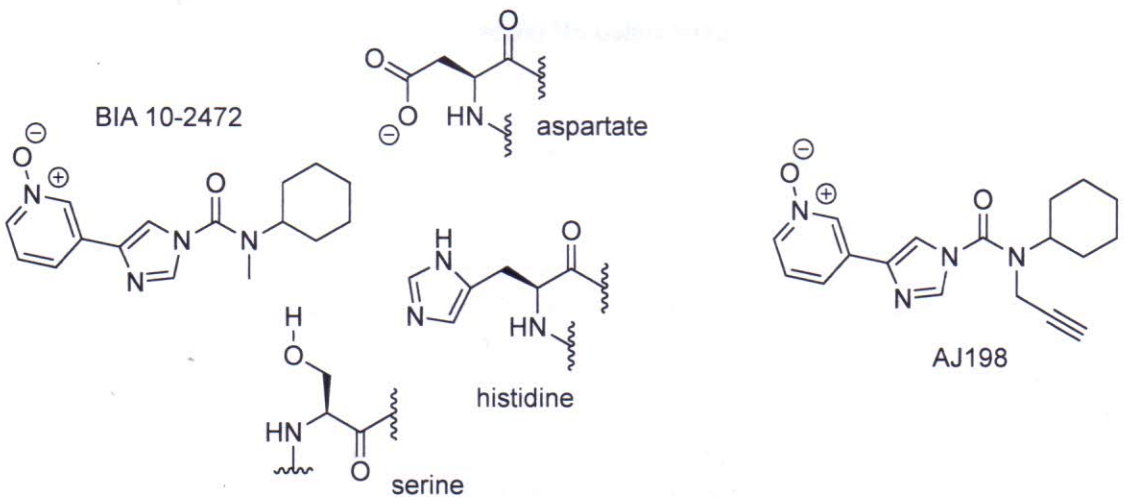
Provided with this examination is a paper on the application of activity-based protein profiling in biomedical research. In answering the questions you may use all papers discussed in the 13 chapters of the course, your notes, and also your imagination: there often is more than one solution to the problem! In answering the questions it is adviced to use chemical structures where appropriate, and written tekst to explain the rationale behind your answers.

As explained during the course, printed manuscripts and hand-written notes are allowed at the examination. Electronic devices are not allowed. Each question carries equal weight in the final score.

Activity-based protein profiling reveals off-target proteins of the FAAH inhibitor BIA 10-2474
Van Esbroeck, Science 2017, 356, 1084-1087

A) Describe the general aim of the paper.

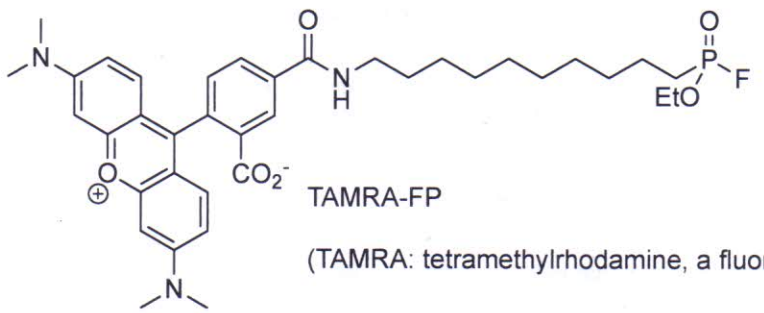
B) The paper predicts that BIA-2474 forms a covalent and irreversible adduct with serine hydrolases that recognise and bind the inhibitor. What would be the structure of this covalent adduct, considering the catalytic amino acids as depicted below are present in many serine hydrolases (in other words, what is the reaction that takes place)? What strategies, other than activity-based protein profiling, can you imagine that would also indicate whether or not BIA-2474 covalently binds to the FAAH active site?



C) AJ198 is a two-step activity-based probe that is derived from BIA 10-2472. Describe how this molecule can be used in copper(I)-catalyzed azide-alkyne click (CuAAC) ligation mediated identification of the targets (FAAH and potential off-targets)? Can you design an alternative bioorthogonally tagged activity-based probe that does not require the addition of copper salts for ligation?

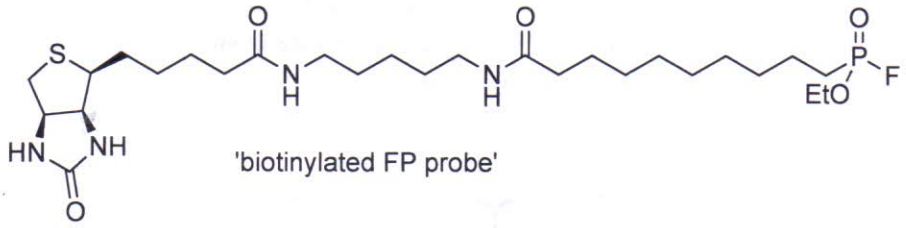
D) If you look at the structure of BIA 10-2474 and that of the more selective compound, PF04457845, is it surprising that the BIA compound has off-targets (given that there are hundreds of serine hydrolases employing essentially the same reaction mechanism on their substrates)? What would be your general strategy to make a BIA 10-2474 derivative that would be a more selective inhibitor (here you may consider either chemical or genetics strategies, even though the latter would of course not yield a medicine).

E) Provide a workflow of the experiments that led to the data as presented in Figure 1C and give an interpretation of the results. The structure of FP-TAMRA is given below. Explain why TAMRA-FP is a suitable activity-based serine hydrolase probe (FP: fluorophosphonate).



TAMRA-FP

(TAMRA: tetramethylrhodamine, a fluorophore)



'biotinylated FP probe'

F) Provide a workflow for the experiments that led to the data as presented in Figure 2AB and give an interpretation of the results. The structure of the biotinylated FP probe is given above.

G) The research described in the paper allows the identification of covalently bound BIA 10-2474 targets only. What strategy would you propose for the identification of potential off-targets that do not form a covalent bond with the molecule?

H) The paper concludes with the statement that '*Regardless, our study highlights the general utility of ABPP as a versatile chemical proteomic method to assess on-target engagement and off-target activity of covalent drugs to guide therapeutic development*'. How, in your opinion, would pharmaceutical industry implement the methodology? Would it also be useful for finding off-targets for competitive enzyme inhibitors (that is, inhibitors that do not form a covalent, irreversible bond)?