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Report on the manuscript of Mrs Katarzyna OZDARSKA, for the obtention of the Degree of PhD of the Université de Reims Champagne Ardennes and of the Medical University of Warsaw.

The manuscript of Mrs OZDARSKA, entitled « synthesis of HDAC inhibitors and their biological assays (cytotoxicity, hdac inhibition)», deals with her work and results in the field of HDAC inhibitors in particular compounds with new zinc-binding groups and pharmacophores.

The manuscript is divided in 3 writing parts (introduction & theory, objectives, results & discussion) and an experimental part. The manuscript is well written and correctly referenced.
The manuscript begins with a short introduction on the subject of HDACs and CNS, and the 3 chemical series developed in the project. For the introduction, it should have been adequate to transform Figure 1 to include the chemical series of the work.

In the theoretical part, Mrs OZDARSKA nicely presents the context of the research. First, she briefly defines epigenetics and then rapidly enters the subject of post-transcriptional modifications to introduce histone deacetylases. Both the function (and mechanism) as well as the structure of HDAC are then described with appropriate references. Still, presentation of a 3D structure of a ligand-enzyme complex in details (and not a small Figure like Figure 5) would have been a plus (Finnin et al Nature 1999 for the seminal paper in the field or a more recent one like BMCL 2020). Also, a mention that many PDB structures either of the apo-proteins (HDAC or HDAC-like) and liganded-proteins are now available, should have been made. Some marketed HDACI are presented and compounds were sorted regarding their chemical structures and selectivities towards HDACs.

Then the main therapeutic fields for HDACI are detailed. It would have been appropriate to organize this part differently. Indeed, the indications of the marketed HDACI are in oncology. This field should have been developed first. Then CNS applications could have been placed after to make an adequate transition with the part on permeability across BBB.

In this part, titles of Figures are too elusive and no legends are provided. For example Figure 10: The title “epigenetic and alcohol” is not clear and no legend is given (see the size of the legend of the reference figure in Neuropharmacology 122 (2017) 74e84). Figure 10-13 titles are not informative at all. Part 5 of the theoretical part should be entitled differently “Ability of HDACI to be used for CNS applications: BBB permeability properties)” for ex.

Page 62, Mrs OZDARSKA presents important properties that HDACI should comply with so that they are able to infiltrate passively the brain. I do agree with the first 5 properties listed (references should have been included). The properties listed under the point “pharmacokinetics” have nothing to do with BBB permeability. Also, hERG inhibition is not part of pharmacokinetic properties. Then, Mrs OZDARSKA lists all the other mechanisms like using transporters (this should be another subparagraph) and finally the practical way to measure brain exposure (this should be a third subparagraph).
In the last point of the theoretical part, Mrs OZDARSKA presents key data from literature regarding the ability of some HDACI chemical series to cross BBB. It would have been interesting to compile permeability data, and LogP and pKa and other calculated parameters in a table to allow better comparison.

The second part clearly presents the objectives of the medicinal chemistry work : changing the classical hydroxamate moiety into new zinc-binding groups and exploring new caps groups. Three chemical series have been designed: sulfonylhydrazides, catechols and indolopyrazinones, based on previous work.

Sulfonylhydrazide series: A small paragraph reminding of the envisioned structures (like in Figure 23) and listing the compounds numbers would have been appreciated before directly describing the chemical synthesis. Usually the sulfonylation of an amine (or here hydrazide) is straightforward. No

putative explanation of the low yields is given. Other analogues where the central linker was shortened by one methylene moiety (**11g-11i**), or replaced by an alkyl linker (**17a-i**) have been synthesized. Finally, analogues of the biphenyl R' groups were obtained via a Suzuki reaction of the corresponding iodinated precursor to given thienyl-phenyl analogues (**5j,6j,11j and 17j**). In these series, inhibition of HDAC1 was poor to moderate (Best compound **17f** is 77 µM). Interestingly, some compounds in the series were found to be activators. In the context of the use of HDACI for CNS applications, cytotoxicity on brain cells should be absent. 6 compounds were tested on different cell lines and they showed some toxicity. It is not clear to the reader why best HDACI were not selected (like **17f**) and why all compounds were tested at concentrations far below their IC50s on HDAC1.

Catechol series: As for the first series, a short paragraph reminding the overall structures targeted as well as the fact that the compounds are designed to target also the second zinc in the HDACIIa group (last paragraph @page 97) should be placed first.

The compounds were synthesized using different protecting groups and a key step of Wittig reaction. Several decomposition or purification problems were encountered as some compounds turned out to be more polar than expected but in all compounds (about 15 in the series) were obtained in moderate to good yield and explored different cap groups (with or without fluorines, branched or not) on different positions.

A docking study was then performed to explore in the HDAC7 how the compounds synthesized (**41** and **26** and **45**) bind in comparison to reference inhibitor TMP269. It is not clear whether **41** is active (p107) or inactive (p111). Docking study revealed that compounds adopt a shape different from reference inhibitor and cannot bind both zinc ions.

Indolopyridazinone series: For this series, Mrs OZDARSKA got inspiration from Tessier's molecule. The compounds were synthesized using an original cyclisation between C2 indole position and a ketal, during deprotection in acidic conditions. Analogue **72** was docked in HDAC7 to study its putative binding in comparison to Tessier's compound.

In all Mrs OZDARSKA has synthesized 110 precursors and final compounds. All of these are correctly characterized. In particular for final compounds to be assayed in HDAC tests, compounds are characterized by mp, 1H and 13C NMR even 19F NMR if needed, HRMS and elemental analysis for purity and identity. Chemical protocols are well described. Yields are moderate to very good. Biological protocols are sufficiently detailed. Docking protocol should include the reference PDB structures used (mentioned p105). As well, a paragraph on visualization tools should also be added.

In conclusion, the reading of the manuscript allows the rapporteur to notice the quality of Mrs OZDARSKA's design and chemical work. Though most compounds were poorly active, the rapporteur underlines the synthetic efforts that led to original structures and synthetic procedures. The compounds should definitely be incorporated in libraries to be assayed on several other targets. The field of HDAC inhibitors for CNS applications is an emerging field for which all efforts are worthy. Given all this, the rapporteur gives a favorable opinion for the PhD defense.

Lille, the 21st of October 2020,
Pr Rebecca Deprez-Poulain