



**HAL**  
open science

# Synthesis of new pyrazolo[4,3-a]phenanthridine Pim-1 inhibitors and evaluation of their cytotoxic activity towards the MOLM-13 acute myeloid leukemia cell line

Etienne Auvert, Reidun Aesoy, Francis Giraud, Lars Herfindal, Fabrice Anizon, Pascale Moreau

## ► To cite this version:

Etienne Auvert, Reidun Aesoy, Francis Giraud, Lars Herfindal, Fabrice Anizon, et al.. Synthesis of new pyrazolo[4,3-a]phenanthridine Pim-1 inhibitors and evaluation of their cytotoxic activity towards the MOLM-13 acute myeloid leukemia cell line. *Bioorganic and Medicinal Chemistry Letters*, 2022, 73, pp.128914. 10.1016/j.bmcl.2022.128914 . hal-03764764

**HAL Id: hal-03764764**

**<https://hal.science/hal-03764764v1>**

Submitted on 30 Aug 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Synthesis of new pyrazolo[4,3-*a*]phenanthridine Pim-1 inhibitors and evaluation of their cytotoxic activity towards the MOLM-13 acute myeloid leukemia cell line

Etienne Auvert<sup>a</sup>, Reidun Aesoy<sup>b</sup>, Francis Giraud<sup>a</sup>, Lars Herfindal<sup>b</sup>, Fabrice Anizon<sup>a,\*</sup> and Pascale Moreau<sup>a,\*</sup>

<sup>a</sup> Université Clermont Auvergne, CNRS, Clermont Auvergne INP, ICCF, F-63000 Clermont-Ferrand, France.

<sup>b</sup> Centre for Pharmacy, Department of Clinical Science, University of Bergen, Bergen, Norway.

## Abstract

We synthesized new analogues of the anti-AML agent **VS-II-173**. We studied the effect of the substitution at the 1- and 5-positions of the pyrazolo[4,3-*a*]phenanthridine scaffold on Pim-1 kinase inhibition and cytotoxicity against AML MOLM-13 cells. We found that compounds **20** and **21**, substituted at the 1-position exhibited stronger Pim-1 inhibition together with a high potency toward MOLM-13 cells, associated with apoptosis induction and selectivity over non-cancerous NRK cells.

**Keywords** – Pyrazolo[4,3-*a*]phenanthridine; Pim kinase inhibitors; Antiproliferative activity; Acute myeloid leukemia

\* Author to whom correspondence should be addressed:

Phone number: +33 (0) 4 73 40 53 64 (FA)

Fax number: +33 (0) 4 73 40 77 17.

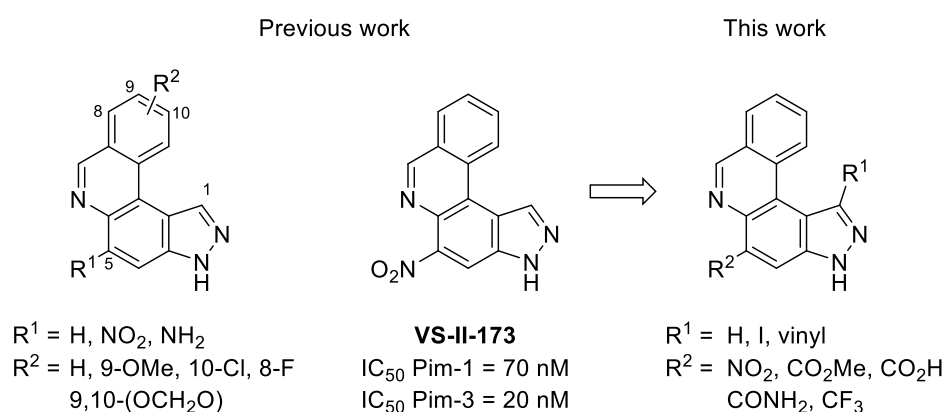
E-mail address: [fabrice.anizon@uca.fr](mailto:fabrice.anizon@uca.fr)

Phone number: +33 (0) 4 73 40 79 63 (PM)

Fax number: +33 (0) 4 73 40 77 17.

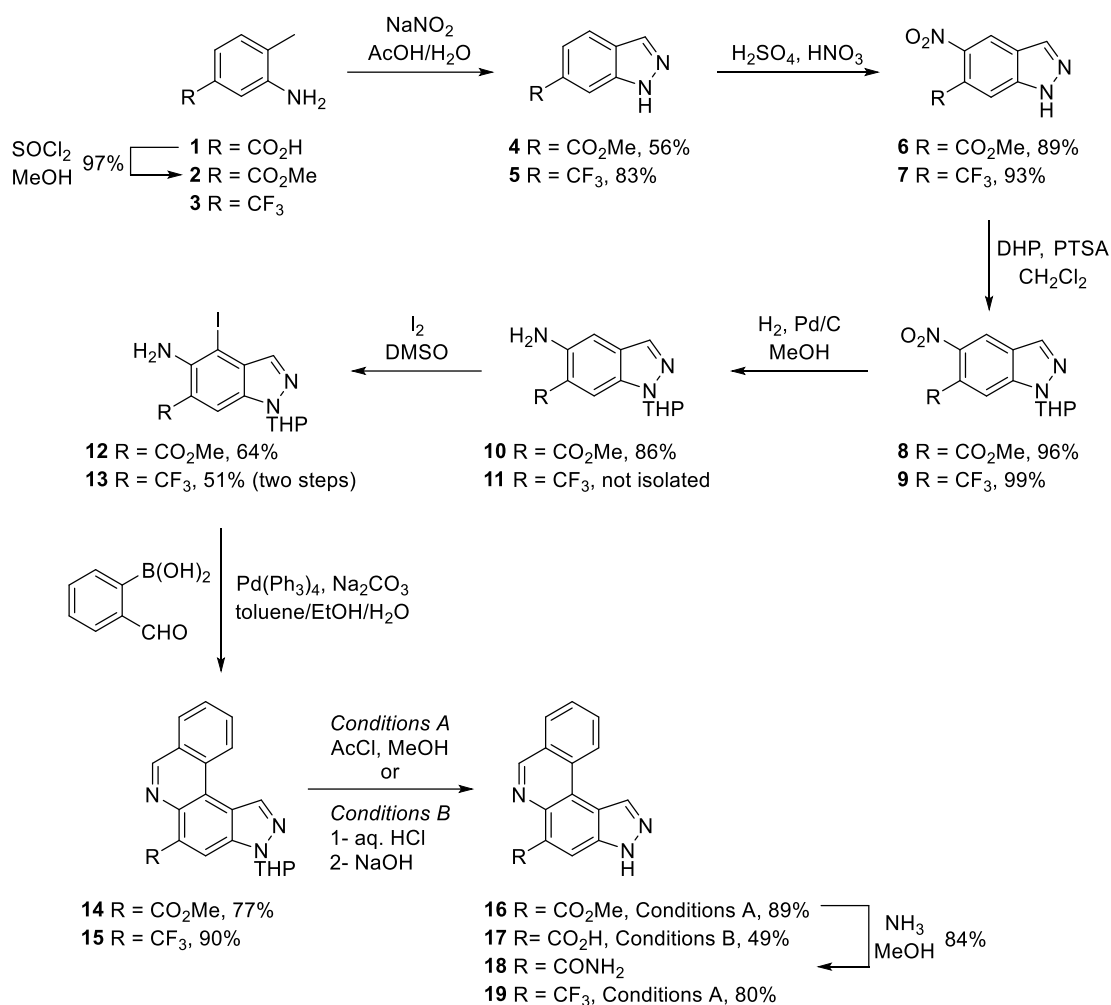
E-mail address: [pascale.moreau@uca.fr](mailto:pascale.moreau@uca.fr)

Acute myeloid leukemia (AML) is a heterogeneous malignant bone marrow disorder that is usually diagnosed in adults. Intense effort made to identify new drug targets allowed the recent approval of novel AML therapies such as Fms-Like Tyrosine kinase-3 (FLT3) or Isocitrate DeHydrogenase (IDH) inhibitors.<sup>1</sup> However, the discovery of new cellular targets involved in AML development is still useful to expand the therapeutic arsenal, overcome drug resistance and reduce the side effects of current treatments. Pim kinases are serine/threonine kinase members of the CAMK family. The three highly homologous isoforms (Pim-1, Pim-2 and Pim-3) are mainly involved in the regulation of cell cycle progression, apoptosis inhibition, cancerogenesis and tumor escape. These kinases are overexpressed in various tumor types like prostate cancer, lymphoma and leukemia, thus constituting relevant targets for the development of new anticancer drugs.<sup>2,3</sup> As part of our program dedicated to the identification of new heteroaromatic scaffolds with interesting kinase inhibitory profiles, we recently described pyrazolo[4,3-*a*]phenanthridines with nanomolar activities toward Pim kinases.<sup>4</sup> Derivatives substituted at the 5-, 8-, 9- and 10-positions were synthesized<sup>4</sup> and compound **VS-II-173** was identified as a potent and selective cytotoxic agent toward AML patient blasts including some carrying mutations associated with poor diagnosis (Figure 1).<sup>5</sup> Importantly, it was demonstrated that substitution at the 5-position by a nitro group was crucial for potent Pim inhibition. Moreover, molecular modelling studies showed that the nitro group of **VS-II-173** could be involved in hydrogen bonding with the ATP binding site of Pim.<sup>4</sup> Therefore, to complete and enlarge the structure-activity relationship work performed around this interesting scaffold, we decided to introduce other polar groups at the 5-position (carboxylic acid, carboxamide, methoxycarbonyl and trifluoromethyl) (Figure 1). Additionally, the effect of substitution of the 1-position on the desired biological activity was studied (Figure 1).

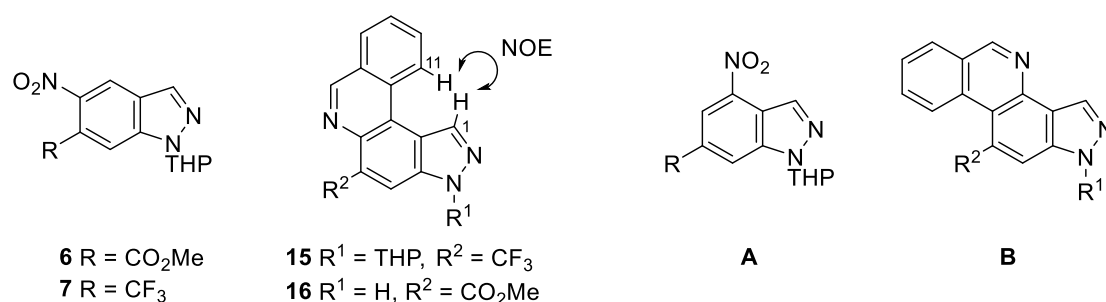


**Figure 1.** Structural changes performed on the pyrazolo[4,3-*a*]phenanthridine scaffold.

Synthesis of compounds **16–19** was performed by construction of the pyrazolophenanthridine scaffold<sup>3</sup> from compounds **2** and **3** (Scheme 1), bearing methoxycarbonyl or trifluoromethyl groups, respectively. Firstly, reaction using sodium nitrite in acetic acid<sup>6</sup> led to the corresponding indazoles **4** and **5**. Then, regioselective nitration gave products **6** and **7**.<sup>7,8</sup> Regioselectivity of this nitration reaction was easily controlled by NMR later in the synthesis using compounds **15** and **16** (see below). The N1 indazole nitrogen of compounds **6** and **7** was protected by a THP group<sup>9</sup> to produce **8** and **9**, and the nitro group was reduced by catalytic hydrogenation leading to **10** and **11**. Iodination using I<sub>2</sub> in DMSO<sup>10</sup> gave compounds **12** and **13**. Pyrazolo[4,3-*a*]phenanthridines **14** and **15** were obtained by Suzuki cross-coupling/cyclization using 2-formylboronic acid.<sup>4,11</sup> Next, cleavage of the THP group in acidic conditions<sup>12</sup> led to **16** and **19**, respectively. Deprotection and subsequent saponification of intermediate **14** afforded carboxylic acid **17**, whereas compound **16** was converted to carboxamide **18** using ammonia in methanol. Analysis of compounds **15** and **16** by NMR spectroscopy (<sup>1</sup>H-<sup>1</sup>H NOESY) showed NOE correlation between <sup>1</sup>H nuclei at the 1-position ( $\delta$  = 9.41 ppm for **15** and 9.27 ppm for **16**) and 11-position ( $\delta$  = 9.24 ppm for **15** and 9.20 ppm for **16**), providing evidence of the regioselectivity of the nitration step affording indazoles **6** and **7** (Figure 2).

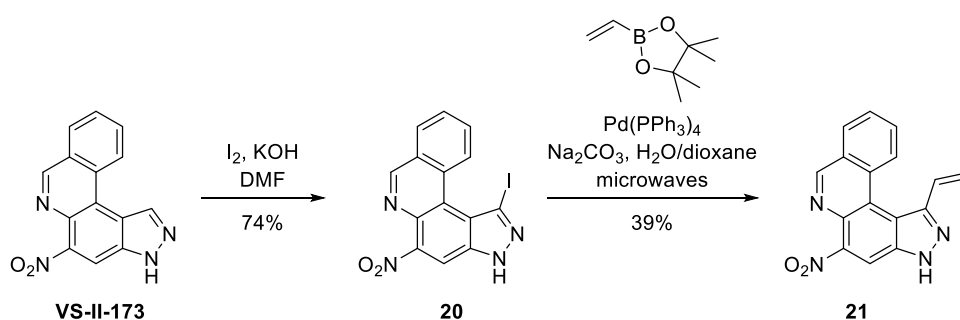


**Scheme 1.** Synthesis of compounds **16–19**.



**Figure 2.** <sup>1</sup>H-<sup>1</sup>H NOESY NMR experiment on compounds **15** and **16** provided evidence of the position of the nitro group in compounds **6** and **7**. Nitro regioisomer **A** would have afforded pyrazolo[3,4-*c*]phenanthridine structure **B** inconsistent with observed NOE correlation.

Two other new derivatives were also prepared from **VS-II-173** (Scheme 2). The first one was 1-iodo analogue **20**, prepared to evaluate the feasibility of further functionalization at this position using palladium cross-couplings. Unfortunately, we experienced difficulties to performed reactions such as Sonogashira or Suzuki couplings, probably due to steric hindrance of the terminal benzo ring of the phenanthridine moiety. Thus, compound **21** was obtained by Suzuki cross-coupling with vinylboronic acid pinacol ester<sup>13</sup> under microwave irradiation, with a low yield incompatible with further vinyl functionalization. Given these disappointing preliminary results, we did not deepen the functionalization at this position.



**Scheme 2.** Synthesis of compounds **20** and **21**.

**VS-II-173** was previously reported<sup>4</sup> as a potent Pim kinase inhibitor. Thus, to get an insight into the kinase inhibitory potency in this new series, compounds **16–21** were evaluated toward Pim-1 (International Center for Kinase Profiling, ICKP, Dundee, Scotland)<sup>14</sup>. Pyrazolophenanthridines **16–19**, in which the nitro group of **VS-II-173** was substituted by a methyl ester, carboxylic acid, carboxamide or trifluoromethyl functions respectively, were less potent than the parent compound, even if the potency remained in the submicromolar range for **16**, **18** and **19** (Table 1). Among compounds **16–19**, the best inhibitor was carboxamide **18** with an  $IC_{50}$  value of 125 nM. We previously showed by molecular modelling that the nitro group of **VI-II-173** could be involved in a H-bond with the side chain of Pim-1 Lys67 residue, and that the absence of the nitro group led to a strong decrease of kinase inhibitory potency.<sup>4</sup> Our results confirmed that the substitution at the 5-position of the scaffold is critical for the inhibitory activity. In addition, whereas the nitro group at the 5-position was conserved in compounds **20** and **21**, the substitution at the 1-position led to strong Pim-1 inhibitory potency with  $IC_{50}$  values of 50 nM and 31 nM for **20** and **21**, respectively.

**Table 1.** Pim-1 inhibitory potencies of **VS-II-173** and compounds **16–21**.

Cpds	% of Pim-1 residual activities (IC <sub>50</sub> , nM)	
	10 μM	1 μM
<b>VS-II-173</b> <sup>4</sup>	2.2 ± 0.0	16 ± 1
	(70 ± 20)	
<b>16</b>	21 ± 2	38 ± 6
<b>17</b>	29 ± 3	77 ± 5
<b>18</b>	7 ± 4	23 ± 2
	(125 ± 29)	
<b>19</b>	18 ± 7	43 ± 1
<b>20</b>	1.4 ± 0.1	8.6 ± 6.3
	(50 ± 5)	
<b>21</b>	1.5 ± 0.5	5.3 ± 4.2
	(31 ± 4)	

% of residual activities at 10 μM and 1 μM compound concentration and IC<sub>50</sub> in brackets (nM) (mean of two measurements ± SD). [ $\gamma$ -<sup>33</sup>P]ATP was used at 20 μM and RSRHSSYPAGT (300 μM) was used as kinase substrate.<sup>14</sup>

The cytotoxic potency of the compounds was evaluated using the human AML MOLM-13 cells and finding the half maximal effective concentration (EC<sub>50</sub>) after 24 or 72 hours of incubation (Table 2, Figure 3). At 24 hours of incubation, the most potent compounds were the two 5-nitro derivatives substituted at the 1-position, *i.e.* compounds **20** and **21**, with EC<sub>50</sub> at 3.5 ± 0.69 μM and 2.9 ± 0.32 μM, respectively. These results were in line with the high Pim-1 inhibitory potency of these two compounds. Compounds **16** and **17** had very low activity towards the MOLM-13 cells at 24 hours of incubation, with a biphasic dose-response curve, which indicates growth inhibition, rather than cell death. After 72 hours of treatment, compounds **20** and **21** were still among the most potent ones; however, compounds **18** and **19** also showed EC<sub>50</sub> in the micromolar range, **19** having the lowest EC<sub>50</sub> in the series, even though not being as cytotoxic as the parent compound **VS-II-173**.

To be able to distinguish between pro-apoptotic and growth-inhibitory activity, we studied the nuclear morphology of Hoechst 33342-stained MOLM-13 cells. At 72 hours of incubation, this can reveal whether the reduced metabolic activity is due to cell death or reduced proliferation, since the cells have undergone 3-4 cell divisions at normal conditions. For compounds **18**, and **19**, there was a large difference in the EC<sub>50</sub> values determined by metabolic activity and presence of apoptotic nuclei at 72 hours of incubation (Table 2). In line with this, the cell density was much lower in the treated wells compared to the control wells, even if apoptosis was not present. For compound **19**, EC<sub>50</sub> was around 1.2 μM calculated from the WST-1 data, while there was less than 50% apoptotic nuclei even at the highest concentrations tested (250 μM). On the other hand, compound **20** and **21** showed less discrepancy between EC<sub>50</sub> values determined by metabolic conversion of WST-1 and by identification of apoptotic nuclei (Table 2, Figure 3).

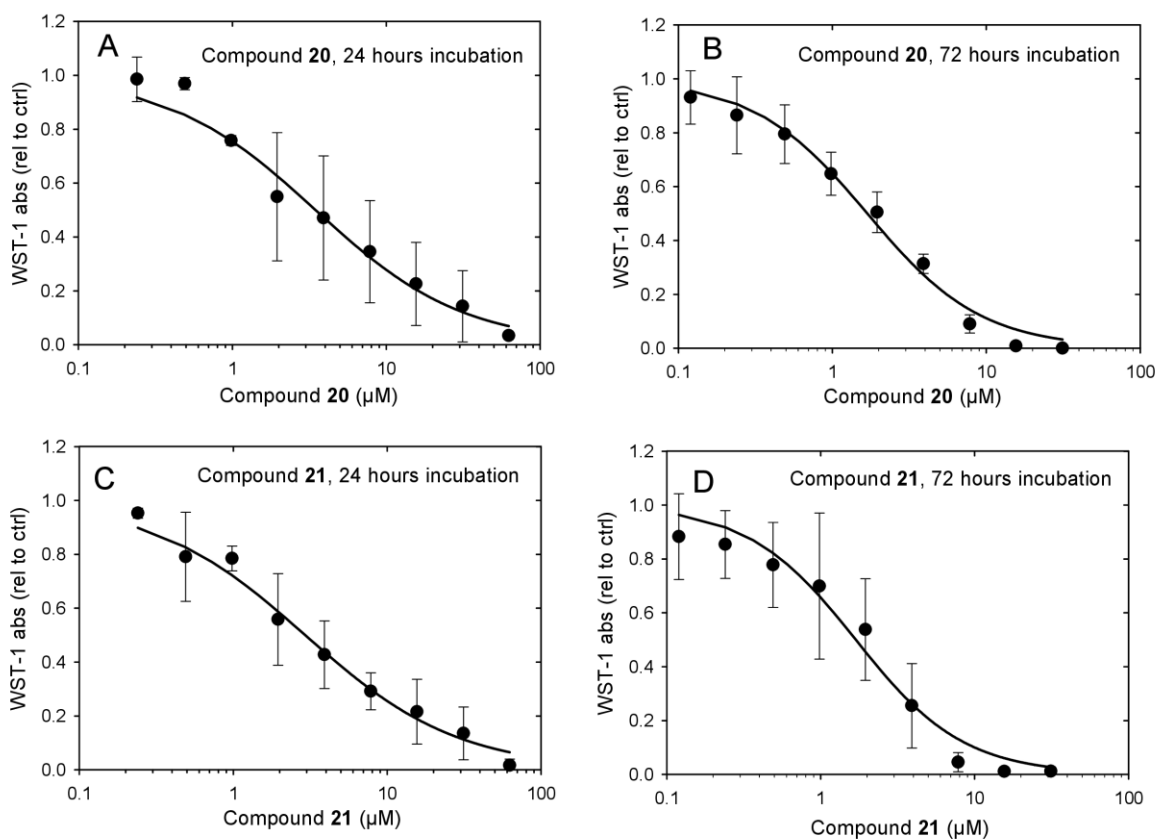
Compounds **20** and **21** showed high activity towards MOLM-13 both at 24 and 72 hours and were tested for cytotoxic activity towards the non-cancerous Normal Rat Kidney (NRK) epithelial cells (Table 2). The NRK cells are derived from normal kidneys, and will give an indication to whether our most potent compounds are able to distinguish between cancer cells and non-cancerous cells. If compounds are active towards NRK cells at equal concentrations as those needed to kill cancer cells, there is a high chance for toxic effects also in *in vivo* studies. At 72 hours of incubation, we found that the EC<sub>50</sub> values were around 15 times higher for NRK-cells compared with the EC<sub>50</sub> values for MOLM-13 cells. This lower cytotoxicity toward NRK cells was also found for VS-II-173<sup>5</sup>, confirming our findings that pyrazolo[4,3-*a*]phenanthridines represent promising drug candidates for AML therapy, with a selectivity profile in favor of MOLM-13 cells.



**Table 2.** EC<sub>50</sub> values of compounds **VS-II-173** and **16–21** on MOLM-13 AML cells and NRK kidney epithelial cells.

Compound	MOLM-13 cells			NRK cells
	EC <sub>50</sub> (μM), metabolic activity <sup>1</sup>		EC <sub>50</sub> (μM), apoptotic nuclei	EC <sub>50</sub> (μM), metabolic activity
	24 hours incubation	72 hours incubation	72 hours incubation	72 hours incubation
<b>VS-II-173</b>	0.8 ± 0.10	0.2 ± 0.01	3.7 ± 0.1	>30
<b>16</b>	50 ± 21	30 ± 3	61 – 125	n.t.
<b>17</b>	>250	62 ± 176	> 250	n.t.
<b>18</b>	n.d.	2.5 ± 3.16	> 250	n.t.
<b>19</b>	n.d.	1.2 ± 0.13	> 250	n.t.
<b>20</b>	3.5 ± 0.69	1.7 ± 0.13	6.6 ± 0.3	28 ± 4.0
<b>21</b>	2.9 ± 0.32	1.7 ± 0.23	4.6 ± 0.5	24 ± 2.0

<sup>1</sup> The EC<sub>50</sub> values are calculated by non-linear regression analyses of data from 3-5 separate experiments. n.d. = not determined, due to no cytotoxicity at the highest concentration tested (250 μM). n.t. = not tested. >250 or >30 indicates that some cytotoxic activity was observed, but not sufficient to determine EC<sub>50</sub> value.



**Figure 3.** Dose-response curves showing the cytotoxic potency of compounds **20** (A and B) and **21** (C and D) at 24 and 72 hours of incubation before assessment of viability using the WST-1 assay for metabolic activity. The data are average and standard deviation from 3–5 separate experiments, and the regression lines are from four-parameter non-linear regression analysis.

In summary, we synthesized novel analogues of **VS-II-173**, a pyrazolo[4,3-*a*]phenanthridine recently identified as a promising cytotoxic agent against AML. We confirmed that the substitution at the 5-position was critical for Pim-1 inhibition, the nitro group being preferred over methoxycarbonyl, carboxyl, carboxamide, or trifluoromethyl groups. However, compounds bearing carboxamide (**18**) or a trifluoromethyl (**19**) groups were cytotoxic toward AML MOLM-13 cells, through an anti-proliferative effect rather than apoptosis induction. Compounds **20** and **21** substituted at the 1-position showed improved Pim-1 inhibitory potency and induced apoptosis in AML MOLM-13 cells, however without reaching a cytotoxicity as potent as that of **VS-II-173**. This could be due to the efficient targeting by **VS-II-173** of other cellular targets (**VS-II-173** is a multi-kinase inhibitor<sup>5</sup>) and/or to a different

cellular distribution of **20/21** within the cellular compartments due to their higher lipophilicity (**20** and **21** cLopP = 1.99 and 2.21,<sup>15</sup> respectively, instead of 1.46 for **VS-II-173**). In addition, compound **20** and **21** showed low cytotoxicity toward non-cancerous NRK cells. This work confirms that pyrazolo[4,3-*a*]phenanthridine is a promising scaffold to further develop AML drug candidates.

### Acknowledgements

The authors (EA, FG, FA, PM) thanks the Région Auvergne-Rhône-Alpes (Pack Ambition International) for financial support. This study was also supported (LH and RA) by the Norwegian Society for Children's Cancer, and the NordForsk NCoE program NordAqua (project No. 82845). MSc Jan-Lukas Førde is thanked for assisting in pilot experiments on AML cells.

### Supplementary data

Material and Methods and <sup>1</sup>H and <sup>13</sup>C NMR spectra of compounds **16–19**, **20–21**.

### References

1. H. Kantarjian, T. Kadia, C. Dinardo, N. Daver, G. Borthakur, E. Jabbour, G. Garcia-Menero, M. Konopleva, F. Ravandi. Acute myeloid leukemia: current progress and future directions. *Blood Cancer J.* 11 (2021) 41. doi: [10.1038/s41408-021-00425-3](https://doi.org/10.1038/s41408-021-00425-3)
2. V. Walhekar, C. Bagul, D. Kumar, A. Muthal, G. Achaiah, R. Kulkarni. Topical advances in PIM kinases and their inhibitors: Medicinal chemistry perspectives. *Biochim. Biophys. Acta Rev. Cancer* 1877 (2022) 188725. doi: [10.1016/j.bbcan.2022.188725](https://doi.org/10.1016/j.bbcan.2022.188725)
3. R. K. Toth, N. A. Warfel. Targeting Pim kinases to overcome therapeutic resistance in cancer. *Mol. Cancer Therap.* 20 (2021) 3–10. doi: [10.1158/1535-7163.MCT-20-0535](https://doi.org/10.1158/1535-7163.MCT-20-0535)
4. V. Suchaud, L. Gavara, F. Giraud, L. Nauton, V. Théry, F. Anizon, P. Moreau. Synthesis of pyrazolo[4,3-*a*]phenanthridines, a new scaffold for Pim kinase inhibition. *Bioorg. Med. Chem.* 22 (2014) 4704–4710. doi: [10.1016/j.bmc.2014.07.011](https://doi.org/10.1016/j.bmc.2014.07.011)
5. R. Bjørnstad, R. Aesoy, Ø. Bruserud, A. K. Brenner, F. Giraud, T. H. Dowling, G. Gausdal, P. Moreau, S. O. Døskeland, F. Anizon, L. Herfindal. A kinase inhibitor with anti-Pim kinase activity is a potent and selective cytotoxic agent toward acute myeloid leukemia. *Mol. Cancer Therap.* 18 (2019) 567–578. doi: [10.1158/1535-7163.MCT-17-1234](https://doi.org/10.1158/1535-7163.MCT-17-1234)

6. G.-D. Zhu, J. Gong, V. B. Gandhi, K. Woods, Y. Luo, X. Liu, R. Guan, V. Klinghofer, E. F. Johnson, V. S. Stoll, M. Mamo, Q. Li, S. H. Rosenberg, V. L. Giranda. Design and synthesis of pyridine–pyrazolopyridine-based inhibitors of protein kinase B/Akt. *Bioorg. Med. Chem.* 15 (2007) 2441–2452. doi: [10.1016/j.bmc.2007.01.010](https://doi.org/10.1016/j.bmc.2007.01.010)
7. U. Bothe, H. Siebeneicher, N. Schmidt, A. Rotgeri, U. Boemer, S. Ring, H. Irlbacher, J. Guenther, H. Steuber, M. Lange, M. Schaefer. Novel carboxamides, method for the production thereof, pharmaceutical preparations comprising them, and use thereof for producing medicaments. PCT Int. Pat. Appl. WO2015091426, 2015.
8. K. Ohrai, M. Adachi, K. Toyama, T. Shimizu, K. Hayashi, M. Minami, Y. Suzuki, M. Sugiyama, M. Ohta, S. Kadono, T. Shiraishi, H. Sato, Y. Watanabe, N. Ishii, M. Sakaitani, M. Hasegawa, Masami. alpha-Amino Acid derivative and pharmaceutical comprising the same active ingredient. Eur. Pat. Appl. EP2045253, 2009.
9. D. J. Slade, N. F. Pelz, W. Bodnar, J. W. Lampe, P. S. Watson. Indazoles: Regioselective Protection and Subsequent Amine Coupling Reactions. *J. Org. Chem.* 74 (2009) 6331–6334. doi: [10.1021/jo9006656](https://doi.org/10.1021/jo9006656)
10. L. Gavara, F. Anizon, P. Moreau. Synthesis of 1,6-dihydropyrrolo[2,3-g]indazoles using Larock indole annulation. *Tetrahedron* 67 (2011) 7330–7335. doi: [10.1016/j.tet.2011.07.029](https://doi.org/10.1016/j.tet.2011.07.029)
11. U. M. Battisti, M. M. Carozzo, G. Cannazza, D. Braghiroli, C. Parenti, L. Brasili, C. Citti, L. Troisi. Efficient synthesis of 5,6-dihydro-8*H*-[1,2,4]thiadiazino[6,5,4-*de*]phenanthridine 4,4-dioxide and 5,6-dihydro-8*H*-[1,2,4]-thiadiazino[6,5,4-*ij*]thieno[2,3-*c*]quinoline 4,4-dioxide. *Tetrahedron Lett.* 53 (2012) 1122–1125. doi: [10.1016/j.tetlet.2011.12.091](https://doi.org/10.1016/j.tetlet.2011.12.091)
12. C. Yeom, Y. Shin, Acetyl Chloride-Mediated Mild and Chemoselective Attachment and Removal of Tetrahydropyranyl (THP) Group. *Bull. Korean Chem. Soc.* 28 (2007), 103–107. doi: [10.5012/BKCS.2007.28.1.103](https://doi.org/10.5012/BKCS.2007.28.1.103)
13. J. W. Beatty, E. A. Lindsey, R. Thomas-Tran, L. Debien, D. Mandal, J. L. Jeffrey, A. T. Tran, J. Fournier, S. D. Jacob, X. Yan, S. L. Drew, E. Ginn, A. Chen, A. T. Pham, S. Zhao, L. Jin, S. W. Young, N. P. Walker, M. R. Leleti, S. Moschutz, N. Strater, J. P. Powers, K. V. Lawson, Kenneth. Discovery of Potent and Selective Non-Nucleotide Small Molecule Inhibitors of CD73. *J. Med. Chem.* 63 (2020) 3935–3955. doi: [10.1021/acs.jmedchem.9b01713](https://doi.org/10.1021/acs.jmedchem.9b01713)

14. J. Bain, L. Plater, M. Elliott, N. Shpiro, J. Hastie, H. McLauchlan, I. Klervernic, S.C. Arthur, D.R. Alessi, P. Cohen. The selectivity of protein kinase inhibitors: a further update. *Biochem. J.* 408 (2007) 297–315. doi: [10.1042/BJ20070797](https://doi.org/10.1042/BJ20070797)
15. T. Sander, J. Freyss, M. von Korff, C. Rufener. DataWarrior: an open-source program for chemistry aware data visualization and analysis. *J. Chem. Inf. Model.* 55 (2015) 460–473. doi: [10.1021/ci500588j](https://doi.org/10.1021/ci500588j)