Supporting Information

Development of a selective and highly sensitive fluorescence assay for nucleoside triphosphate diphosphohydrolase1 (NTPDase1, CD39)

Sang-Yong Lee,a,* Xihuan Luo,a Vigneshwaran Namasivayam,a Jennifer Geiss,a Salahuddin Mirza,a Julie Pelletier,b Holger Stephan,c Jean Sévigny,b,d and Christa E. Müller a

a PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany

b Centre de Recherche du CHU de Québec – Université Laval, Québec City, QC, Canada

c Institute of Radiopharmaceutical Cancer Research, Helmholtz-Zentrum Dresden - Rossendorf, Bautzner Landstrasse 400, 01328 Dresden, Germany

d Département de Microbiologie-Infectiologie et d’Immunologie, Faculté de Médecine, Université Laval, Quebec City, QC, Canada

* Correspondence: Dr. Sang-Yong Lee, PharmaCenter Bonn, Pharmaceutical Sciences Bonn (PSB), Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany.
E-mail: s6saleee@uni-bonn.de, Fax: +49-228-73-2567

Table of contents

Fig. S1. CE chromatograms with different incubation times for the hydrolysis of PSB-170621A to its enzyme product PSB-170621B p.2
Fig. S2. LC-MS chromatogram of PSB-170621B p.2
Fig. S3. Calibration curves for the forward and reverse operation p.3
Fig. S4. Structural formulae of the investigated enzyme inhibitors p.3
Table S1. Kinetic data of PSB-170621A at different ectonucleotidases p.4
Fig. S1. CE chromatograms with different incubation times for the hydrolysis of PSB-170621A to its enzyme product PSB-170621B. The separation conditions for the reverse operation were: 50 mM phosphate buffer (pH 6.5), effective capillary length of 10 cm x 50 μm (id), electrokinetic injection (6 kV, 30 s), separation at 15 kV and detection at 488 nm (excitation) and 520 nm (emission). The samples were diluted 100-fold with reaction buffer before CE measurements.
**Fig. S2.** LC-MS chromatogram of PSB-170621B. (A) Only one peak with a retention time of 5.8 min was observed in the UV/VIS chromatogram, (B) Two M/z values were obtained for the single peak in the UV/VIS chromatogram (M/z 805,2219 with z = 1 and M/z 403,1153 with z = 2).

**Fig. S3.** Calibration curves for the forward operation (A) and the reverse operation (B).

**Fig. S4.** Structural formulae of the investigated inhibitors.
Table S1. Kinetic data of PSB-170621A at different ectonucleotidases

<table>
<thead>
<tr>
<th></th>
<th>NTPDases</th>
<th>NPPs</th>
<th>APs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NTPDase1</td>
<td>NTPDase2</td>
<td>NTPDase3</td>
</tr>
<tr>
<td>$K_m$ (µM)</td>
<td>19.6</td>
<td>55.5</td>
<td>13.3</td>
</tr>
<tr>
<td>$k_{cat}$ (10^{-3} \text{ x s}^{-1})</td>
<td>119</td>
<td>4.94</td>
<td>22.5</td>
</tr>
<tr>
<td>$k_{cat}/K_m$ (M$^{-1}$ x s$^{-1}$)</td>
<td>6070</td>
<td>89.0</td>
<td>1690</td>
</tr>
</tbody>
</table>