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Polyoxometalates—a new class of potent ecto-nucleoside triphosphate diphosphohydrolase (NTPDase) inhibitors

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Abstract—Polyoxotungstates were identified as potent inhibitors of NTPDases1, 2, and 3. The most potent compound was $K_6H_2[TiW_{11}CoO_{40}]$, exhibiting K_i values of 0.140 μM (NTPDase1), 0.910 μM (NTPDase2), and 0.563 μM (NTPDase3). One of the compounds, $(NH_4)_{18}[NaSb_9W_{21}O_{86}]$, was selective for NTPDases2 and 3 versus NTPDase1. NTPDase inhibition might contribute to the described biological effects of polyoxometalates, including their anti-cancer activity.

Extracellular nucleotides such as ATP, ADP, UTP, and UDP can act on a variety of nucleotide receptors (P2 receptors).[1] The activation of P2 receptors is controlled by ecto-nucleotidases capable of hydrolyzing nucleoside tri- and diphosphates[2] (ecto-nucleoside triphosphate diphosphohydrolases, E-NTPDases). Inhibition of E-NTPDases can result in a potentiation of purinergic signaling, supporting the notion that endogenous ecto-nucleotidases reduce the effective concentration of the released nucleotide.[3–6] Similarly, metabolically stable analogs of ATP are often considerably more effective in causing a biological response than ATP itself. Inhibitors of E-NTPDases could thus represent valuable tools for amplifying the biological effects induced by extracellularly released nucleotides. In addition, inhibition of E-NTPDases is required when studying nucleotide release. Three different E-NTPDases have been known, NTPDase1, 2, and 3.[2] Recently, NTPDase8 has been described as a fourth E-NTPDase.[6,7] NTPDases4, 5, 6, and 7 are intracellular enzymes.

Inhibitors of E-NTPDases should not be dephosphorylated, that is, they should not be substrates of the enzymes. Ideally they would reveal selectivity for individual E-NTPDase isoforms. NTPDase1 hydrolyzes ATP and ADP equally well, while NTPDase2 has a high preference for ATP. NTPDase3 is a functional intermediate, preferably hydrolyzing ATP (ATP/ADP = 5:1, rat enzyme).[2]

Currently, there is a lack of potent and subtype-selective E-NTPDase inhibitors, which are urgently needed as pharmacological tools. Furthermore, such inhibitors may have potential as novel drugs,[2,9] for example, for the treatment of cancer and diseases of the immune system. Inhibition of NTPDases may increase the immune response toward viral or bacterial infections. Local inhalative application may be useful for the treatment of cystic fibrosis and infectious diseases of the lung.

The NTPDase inhibitors known so far are derived from three different chemical classes (i) nucleotides and analogs (e.g., ARL67156, 1), (ii) sulfonated dyes such as Reactive Blue 2 (RB2, 2), and (iii) suramin (3) and derivatives (Fig. 1).[2,9] ARL67156 has been reported to be a moderately potent, but selective, E-NTPDase inhibitor which does not block P2 receptors. However, we recently discovered that ARL67156 only inhibits NTPDases1 and 3, but not NTPDase2 (see Table 1).[10] RB2 and suramin, on the other hand, are potent antagonists at some P2 receptor subtypes and thus can block the effects of nucleotides rather than enhancing them.[11,12]

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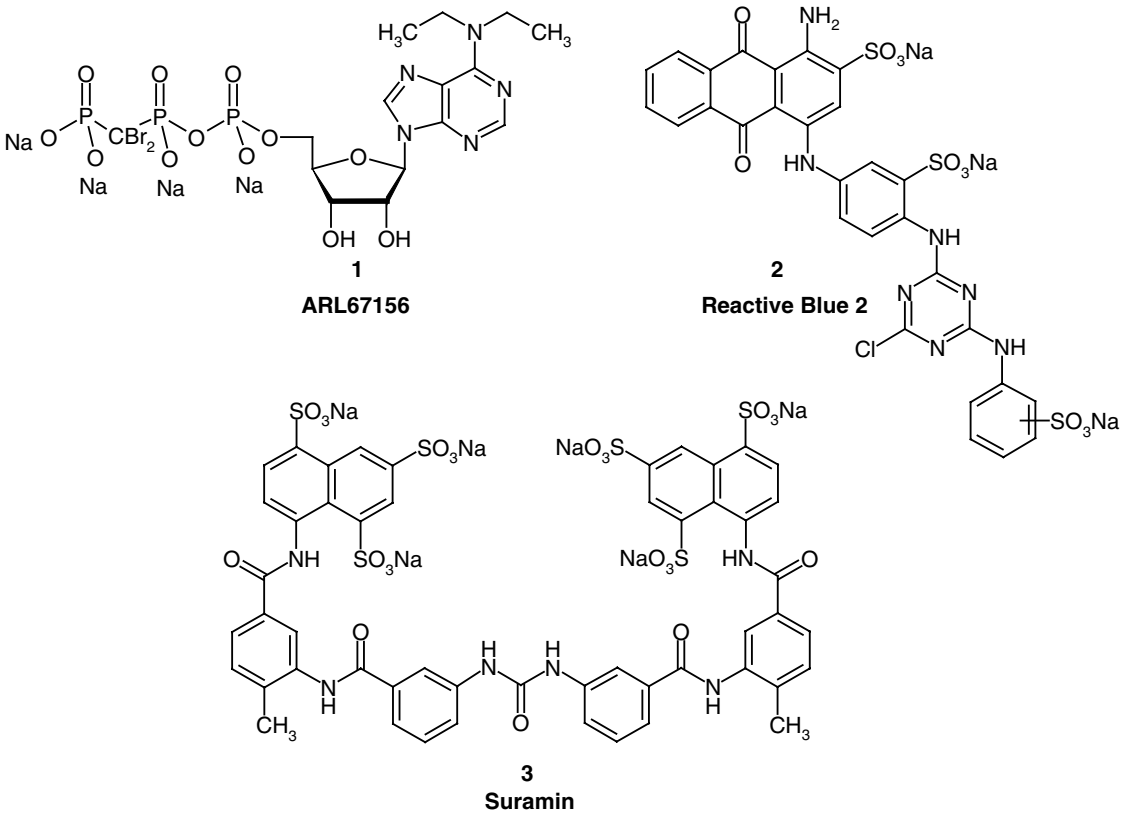


Figure 1. Structures of E-NTPDase inhibitors.

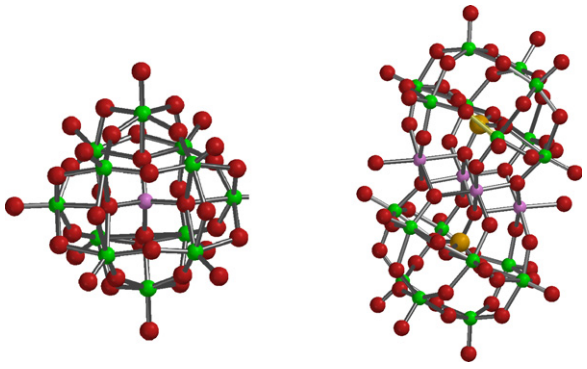
Table 1. Potency of polyoxometalates and standard compounds as inhibitors of rat NTPDase1, 2, and 3 obtained by capillary electrophoresis assays

Compound	Formula or name	Charge at pH 7.4	Stability at pH 7.6[a]	M_r (g/mol) of anions	K_i (μM) ± SEM		
					NTPDase1	NTPDase2	NTPDase3
1[10]	ARL67156	−4	n.d.[b]	747	27.0 ± 0.0	⩾1000[c]	112 ± 0
2[10]	Reactive Blue 2	−3	n.d.	771	20.0 ± 0.0	24.2 ± 0.1	1.10 ± 0.03
3[10]	Suramin	−6	n.d.	901	300 ± 0	65.4 ± 0.0	12.7 ± 0.0
4	$Na_6[H_2W_{12}O_{40}]$	−6	⩾ 14 day	2830	2.58 ± 0.30	28.8 ± 0.2	3.26 ± 0.18
5	$H_3[PW_{12}O_{40}]\cdot H_2O$	−3	ca. 1 h	2859	3.49 ± 0.23	6.17 ± 0.15	8.72 ± 1.81
6	$K_7[Ti_2W_{10}PO_{40}]$	−7	⩾14 day	2606	2.00 ± 0.34	37.4 ± 1.3	4.00 ± 0.26
7	$K_6H_2[TiW_{11}CoO_{40}]\cdot 13H_2O$	−8	ca. 24 h	2770	0.140 ± 0.021	0.910 ± 0.041	0.563 ± 0.113
8	$K_{10}[Co_4(H_2O)_2(PW_9O_{34})_2]\cdot 22H_2O$	−10	⩾14 day	4732	0.480 ± 0.010	1.53 ± 0.20	2.61 ± 0.97
9	$(NH_4)_{18}[NaSb_9W_{21}O_{86}]$	−18	ca. 48 h	6356	>1 mM[d]	3.94 ± 0.78	3.77 ± 0.52

The results are means ± SEM of three separate experiments each run in duplicate.
[a] At least 50% of cluster structure was intact after time indicated.
[b] n.d., not determined.
[c] 50% inhibition at 1 mM.
[d] 15% inhibition at 1 mM.

Polyoxometalates are anionic complexes that are relatively stable, some even highly stable in aqueous solutions at biological pH values.[13]

They contain transition metal ions, such as tungsten, molybdenum, vanadium, etc., which are bridged by oxygen atoms. In addition to applications in catalysis, separations, analysis, and as electron-dense imaging agents, some of these complexes have been shown to exhibit biological activity in vitro as well as in vivo ranging from anti-cancer, antibiotic, and antiviral to antidiabetic effects.[13–15] Due to their negative charges they bear resemblance to nucleotides. A set of six polyoxotungstates (**4**–**9**) having different charge, size, and shape were selected and investigated for their potency to inhibit ecto-nucleotidases: $[H_2W_{12}O_{40}]^{6-}$ (**4**), $[PW_{12}O_{40}]^{3-}$ (**5**), $[Ti_2W_{10}PO_{40}]^{7-}$ (**6**), $[TiW_{11}CoO_{40}]^{6-}$ (**7**), $[Co_4(H_2O)_2(PW_9O_{34})_2]^{10-}$ (**8**), and $[NaSb_9W_{21}O_{86}]^{18-}$ (**9**). The metatungstate **4** shows a highly symmetric cluster structure consisting of corner- and edge-sharing WO_6 octahedrons,[16] **5**–**7** are Keggin type polyanions,[17–19] **8** forms trivacant Keggin-derived sandwiches,[20] and **9** is an inorganic cryptate with sodium encapsulated in the central cavity.[21] The x-ray structures of **7** and **8** are



$[TiW_{11}CoO_{40}]^{8-}$ $[Co_4(H_2O)_2(PW_9O_{34})_2]^{10-}$

7 8

Figure 2. Structures of E-NTPDase inhibitors **7** (typical Keggin type structure) and **8** (trivacant Keggin-derived sandwich).

shown in Figure 2. Polyoxotungstates **4** and **5** were purchased (Fluka) and used without further purification. Compounds **6**,[22] **7**,[19,23] **8**,[20] and **9**[24] were prepared and purified according to published procedures. Elemental analysis (ICP-MS: ELAN 9000, Perkin-Elmer for Co, P, Sb, Ti, W; AAS: AAS 4100, Perkin-Elmer for K, Na) and IR spectra confirmed the composition of the polyoxotungstates. All polyoxotungstates investigated show typical charge transfer bands between 240 and 260 nm (bridging oxygen in W–O–W). After degradation of the cluster structure this band disappears. Thus, this peak was chosen to study the hydrolytic stability of the compounds using UV-vis spectroscopy. Experiments were performed with aqueous solutions of compounds (10 μM) at pH 7.6 (HCl–borate buffer). The results are summarized in Table 1.

Enzyme inhibition assays were performed using a recently developed capillary electrophoresis-based online method.[10] Membrane preparations of Chinese hamster ovary cells expressing recombinant rat NTPDase1, 2, or 3 were used[10] and microassays were performed within the capillary followed by electrophoretic separation of substrate and products, and subsequent quantitative determination by UV detection.[25] For the determination of IC_{50} and K_i values of polyoxometalates (Table 1) as NTPDase inhibitors, 6–8 different concentrations of inhibitor spanning about three orders of magnitude were used, while a fixed substrate concentration of 320 μM ATP was employed for all three NTPDases. Under the applied conditions less than 10% of substrate was converted by the enzymes.

All of the polyoxometalates tested were potent inhibitors of NTPDases (Table 1). However, significant differences were found in activities and selectivities for certain isoenzymes. The simple metatungstate **4** was a potent NTPDase1 and 3 inhibitor (K_i 2.58 and 3.26 μM), and showed about 10-fold lower inhibitory activity for NTPDase2. Compound **6** gave very similar results (see Fig. 3). Compounds **5**, **7**, and **8** were non-selective inhibitors of all three NTPDases (**5**: K_i 3.49–8.72 μM, **7**: K_i 0.140–0.910 μM (see Fig. 3), and **8**: K_i 0.480–2.61 μM). Compound **5** exhibits limited stability (see Table 1) but the assay conditions (fresh preparation of test solution, fast assay within a few minutes) were such that degradation should be negligible. In contrast to all other investigated polyoxometalates, compound **9** did not inhibit NTPDase1, but was a potent inhibitor of NTPDase2 and 3.

The inhibitory potency of the polyoxometalates appeared to correlate neither with the size or shape of the complexes nor with the number of negative charges (Table 1).

In comparison with the standard NTPDase inhibitors ARL67156 (**1**), Reactive Blue 2 (**2**), and suramin (**3**) the investigated polyoxotungstates were generally more potent. The most potent NTPDase inhibitor of the present series, compound **7**, was >140-fold more potent at NTPDase1, and >26-fold more potent at NTPDase2 than the standard compounds. Polyoxometalate **9** will allow for the first time to selectively inhibit NTPDases2 and 3 without affecting the highly expressed NTPDase1.

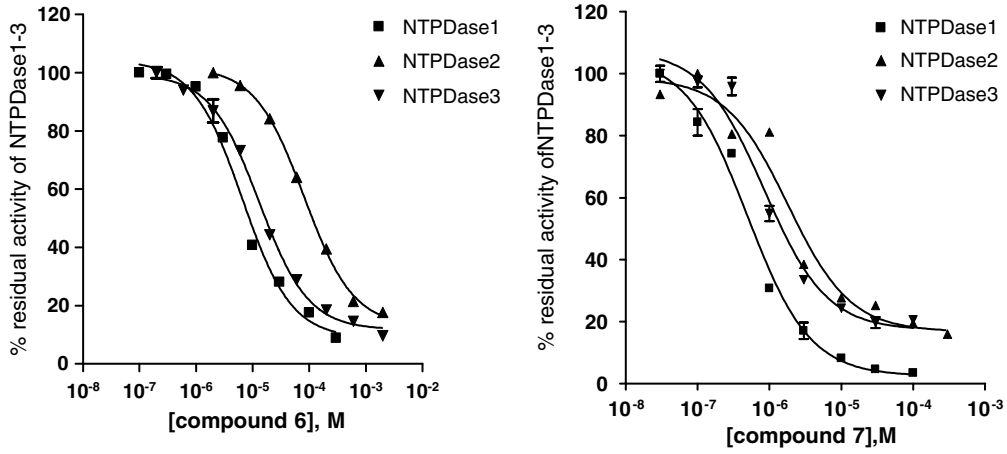


Figure 3. Concentration-inhibition curves for compounds **6** and **7**.

The pharmacological effects expected from NTPDase inhibitors[8] and those described for polyoxymetalates,[13–15] including some of the compounds investigated in this study, are amazingly similar. It is tempting to speculate that some of the effects of polyoxometalates observed in in vitro and in vivo studies might be due to ecto-nucleotidase inhibition. In particular the anticancer activity of polyoxometalates, including **5** and **7**, might be explained by an enhanced activation of P2 receptors due to an increase in the extracellular nucleotide concentrations. Likewise, protection against viral or bacterial infection by polyoxymetalates may be enhanced by NTPDase inhibition, in addition to further mechanisms, such as prevention of virus entry into the cells or direct inhibition of DNA polymerases (e.g., HIV reverse transcriptase) or HIV protease. Furthermore, antidiabetic activities of polyoxometalates have been described. These may also be due to NTPDase inhibition, since activation of P2Y receptors on pancreatic islet cells by nucleotides will lead to an increase in insulin release.[26]

In order to investigate whether polyoxometalates are selective for NTPDases versus P2 receptors we exemplarily investigated their affinity for the $P2Y_{12}$ receptor subtype, a G protein-coupled receptor expressed on platelets that is activated by ADP and blocked by ATP.[27] At a test concentration of 10 μM, compounds **4**–**7** and **9** inhibited binding of the $P2Y_{12}$ antagonist radioligand [^{3}H]PSB-0413[28] to the receptors only moderately (20–40%) indicating that their K_i values are above 10 μM. Only compound **8** showing a unique structure (Fig. 2) was more potent (K_i 1.30 ± 0.16 μM). Thus, except for **8**, all compounds investigated, including the most potent derivative **7**, can be described as selective at least versus the $P2Y_{12}$ receptor subtype. Further studies at the other P2Y and the P2X receptor subtypes are in progress. In future experiments the nature of inhibition, for example, competitive versus non-competitive, is to be elucidated.

In conclusion, we identified polyoxometalates as a novel class of E-NTPDase inhibitors, which belong to the most potent E-NTPDase inhibitors described to date. Inhibition of E-NTPDases may explain or contribute to some of the observed in vitro and in vivo effects of polyoxometalates, including anti-cancer activity, protection against viral and bacterial infections, and antidiabetic activity.

References and notes

1. Burnstock, G. *Curr. Top. Med. Chem.* **2004**, *4*, 793.
2. Zimmermann, H. *Naunyn Schmiedeberg's Arch. Pharmacol.* **2000**, *362*, 299.
3. Zimmermann, H. *Nat. Med.* **1999**, *5*, 987.
4. Crack, B. E.; Pollard, C. E.; Beukers, M. W.; Roberts, S. M.; Hunt, S. F.; Ingall, A. H.; McKechnie, K. C.; IJzerman, A. P.; Leff, P. *Br. J. Pharmacol.* **1995**, *114*, 475.
5. Bültmann, R.; Driessen, B.; Goncalves, J.; Starke, K. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1995**, *351*, 555.
6. Sesti, C.; Koyama, M.; Broekman, M. J.; Marcus, A. J.; Levi, R. *J. Pharmacol. Exp. Ther.* **2003**, *306*, 238.
7. Bigonnesse, F.; Levesque, S. A.; Kukulski, F.; Lecka, J.; Robson, S. C.; Fernandes, M. J.; Sevigny, J. *Biochemistry* **2004**, *43*, 5511.
8. Knowles, A. F.; Li, C. *Biochemistry* **2006**, *45*, 7323.
9. (a) Gendron, F. P.; Benrezzak, O.; Krugh, B. W.; Kong, Q.; Weisman, G. A.; Beaudoin, A. R. *Curr. Drug Targets* **2002**, *3*, 229; (b) Gendron, F. P.; Halbfinger, E.; Fischer, B.; Beaudoin, A. R. *Adv. Exp. Med. Biol* **2000**, *486*, 119.
10. Iqbal, J.; Vollmayer, P.; Braun, N.; Zimmermann, H.; Müller, C. E. *Purinergic Signal.* **2005**, *1*, 349.
11. (a) Müller, C. E. *Curr. Pharm. Des.* **2002**, *8*, 2353; (b) Brunschweiger, A.; Müller, C. E. *Curr. Med. Chem.* **2006**, *12*, 325.
12. Kennedy, C. *Arch. Int. Pharmacodyn. Ther.* **1990**, *303*, 30.
13. *Polyoxometalate Chemistry: From Topology via Self-Assembly to Applications*; Pope, M. T., Müller, A., Eds.; Kluwer: Dordrecht-Boston-London, 2001.
14. Rhule, J. T.; Hill, C. L.; Judd, D. A.; Schinazi, R. F. *Chem. Rev.* **1998**, *98*, 327.
15. Hasenknopf, B. *Front. Biosci.* **2005**, *10*, 275.
16. Pope, M. T.; Varga, G. M. *Chem. Commun.* **1966**, 653.
17. Gabriel, J. C. P.; Nagarajan, R.; Natarajan, S.; Cheetham, A. K.; Rao, C. N. R. *J. Solid State Chem.* **1997**, *129*, 257.
18. Meißner, T.; Bergmann, R.; Oswald, J.; Rode, K.; Stephan, H.; Richter, W.; Zänker, H.; Kraus, W.; Emmerling, F.; Reck, G. *Transit. Met. Chem.* **2006**, *31*, 603.
19. Kraus, W.; Stephan, H.; Röllich, A.; Matějka, Z.; Reck, G. *Acta Crystrallogr.* **2005**, *E61*, i35.
20. Evans, H. T.; Tourné, C. M.; Tourné, G. F.; Weakly, T. J. R. *J. Chem. Soc., Dalton Trans.* **1986**, 2699.
21. Fischer, J.; Ricard, L.; Weiss, R. *J. Am. Chem. Soc.* **1976**, *98*, 3050.
22. Domaine, P. J.; Knoth, W. H. *Inorg. Chem.* **1983**, *22*, 818.
23. Chen, Y.; Liu, J. *Synth. React. Inorg. Met.-Org. Chem.* **1997**, *27*, 239.
24. Jasmin, C.; Chermann, J.-C.; Hervé, G.; Tezé, A.; Souchay, P.; Boy-Lousteau, C.; Raybaud, N.; Sinoussi, F.; Raynaud, M. *J. Nat. Cancer Inst.* **1974**, *53*, 469.
25. All experiments were carried out using a P/ACE MDQ capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) equipped with a UV detection system coupled with a diode-array detector (DAD). The capillary temperature was kept constant at 37 °C. The temperature of the sample storing unit was adjusted to 10 °C. The electrophoretic separations were carried out using an eCAP polyacrylamide-coated fused-silica capillary [30 cm (20 cm effective length) × 50 μm internal diameter (ID) × 360 μm outside diameter (OD), obtained from CS-Chromatographie (Langerwehe, Germany)]. The separation was performed using an applied current of −60 μA and a data acquisition rate of 8 Hz. Analytes were detected using direct UV absorbance at 210 nm. The capillary was conditioned by rinsing with water for 2 min and subsequently with buffer (phosphate 50 mM, pH 6.5) for 1 min. Sample injections were made at the cathodic side of the capillary. The CE running buffer consisted of 50 mM K_2HPO_4, pH 6.5. The reaction buffer contained 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, and 10 mM Hepes, pH 7.4. The automation cycle consisted of: (i) washing with water for 2 min (40 psi; 1 psi = 6894.76 Pa), (ii) equilibration with the CE running buffer for 1 min (40 psi), (iii) injection of a plug of reaction solution containing 320 μM ATP (substrate) in reaction buffer and various concentrations of inhibitor stock solutions in reaction buffer, (iv) injection of a plug of suitably diluted enzyme, (v) injection of another plug of reaction solution as in (iii), (vi) and finally injection of a plug of water. The plugs were then allowed to react, while

the capillary ends were dipped into water, for a predetermined waiting period of 5 min. Then, a current of −60 μA was applied. Each electropherogram was recorded over 7 min.

26. Hillaire-Buys, D.; Chapal, J.; Bertrand, G.; Petit, P.; Loubatieres-Mariani, M. M. *Fundam. Clin. Pharmacol.* **1994**, *8*, 117.
27. Membranes were prepared from human outdated platelets from the blood bank. Radioligand binding assays were performed using 5 nM [^{3}H]PSB-0413[28] and 100 μg of protein in Tris–HCl buffer 50 mM, pH 7.4. Competition by test compounds was determined. The mixture was incubated for 1 h at rt followed by filtration through GF/B filters. Nonspecific binding was determined with 1 mM ADP. At least three independent experiments were performed each in triplicate.
28. El-Tayeb, A.; Griessmeier, K.; Müller, C. E. *Bioorg. Med. Chem. Lett.* **2005**, 5450.