Parasite-specific cyclic nucleotide phosphodiesterase inhibitors to target Neglected Parasitic Diseases (PDE4NPD)

Concept of PDE4NPD

PDE4NPD brings together efforts to tackle kinetoplastid diseases (**human African Trypanosomiasis (sleeping sickness), leishmaniasis, Chagas' disease**) and one major helminth disease (**schistosomiasis**). The consortium will establish a generic **cyclic nucleotide phosphodiesterase (PDE) drug discovery platform** to tackle these (and other) Neglected Parasitic Diseases (NPDs). PDE4NPD will screen PDE-focussed and fragment libraries by employing various **target-centric** biochemical, biophysical and pharmacological PDE studies. The approach builds on insights and technologies that have been developed in the highly successful therapeutic targeting (e.g. Viagra®, Daxas® or Otezla®) of various members of the 11 human PDE families in the human genome. Complementary to these molecular approaches, the consortium will also perform **phenotypic screening** on a large number of parasites, including the malaria parasite *P. falciparum*.

PDE4NPD is establishing a generic PDE drug development platform for tackling a wide variety of parasitic diseases and delivering a range of PDE-based drug clinical candidates

The overall objectives of PDE4NPD:

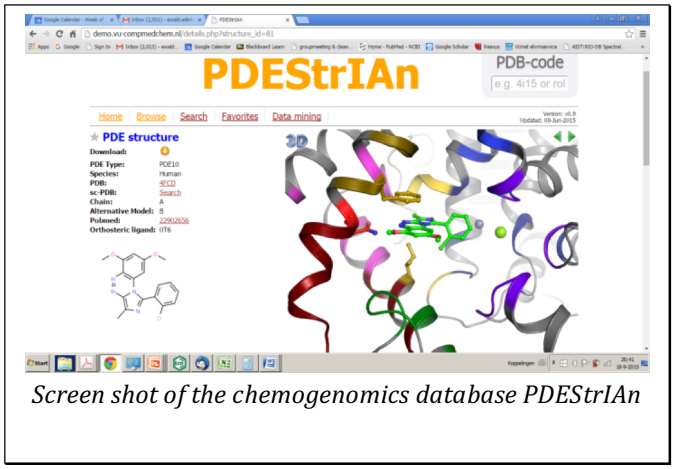
- Establish a generic PDE drug discovery platform that is applicable and available for combating a wide variety of parasitic diseases. This will be achieved by establishing technologies, procedures, understanding and publicly accessible (chemogenomics) databases, that can be used for efficient PDE-based drug discovery for NPDs.
- Demonstrate the effectiveness of the drug discovery platform by delivering several PDE inhibitors as drug candidates.
- Utilize the generic PDE drug discovery platform to tackle unexplored parasite PDE enzymes (e.g. *Schistosoma* spp., *Leishmania* spp., or other relevant *T. brucei* or *T. cruzi* PDE subtypes)
- Accumulate knowledge on targeting PDEs in pathogenic protozoa and helminths in an annotated database *PDEspace* and make this available for the scientific community.
- To successfully disseminate and exploit the generic PDE drug discovery platform results and inhibitors through expansion of the current collaborations, attracting additional funding and stimulating an open-innovation platform.

PDE4NPD progress:

The focus of the PDE4NPD program is on developing anti-parasitic chemotherapy against the kinetoplasts *T. brucei, T. cruzi* and *Leishmania* and the helminth *Schistosoma mansoni* by targeting parasite PDE enzymes.

Establishment of a generic PDE-driven drug discovery platform

PDE4NPD operates as a **public-private drug discovery platform**, which relies on strong interaction between various partners. Next to regular TC's and face-to-face meetings, collaborative work is supported by newly established communication and research tools and relevant databases, *e.g.,* a central website for communication and dissemination, an industry standard, web-based drug discovery database for annotating chemical and screening data (Collaborative Drug Discovery **CDD Vault**) and a proprietary web-based, chemogenomics database **PDEStrIAn**, with information on all published and PDE4NPD PDE X-ray structures. This database has been published and released to the broader scientific community via a web-based portal (www. http://pdestrian.vu-compmedchem.nl), which at the moment (release 28-2-2017) contains the information on 259 different PDE x-ray structures, extracted from the PDB database. The PDE4NPD x-ray structures are only available for PDE4NPD members behind a firewall. This resource is of specific interest to the **PDE4NPD structural biology platform**. PDE4NPD has currently cloned and collected expression plasmids for almost all of the target parasitic PDEs. Moreover, expertise in PDE protein expression and x-ray crystallography has resulted in delivering 48 new crystal structures, since the last report, including one structure with the highest resolution ever for any PDE reported.



Screen shot of the chemogenomics database PDEStrIAn

In parallel to these research databases and PDE structural biology platform, the partners have assembled a **PDE compound toolbox**, with a *current* selection of 55 structurally diverse human and parasite PDE inhibitors, which has been used in assay development and early hit finding exercises for new parasite PDEs (see WP3). For the parasite PDE enzymes to be studied in the program, the biochemistry partners have established a generic PDE enzyme assay based on LANCE time-resolved FRET technology and a new *T. brucei*-based PDE complementation system, next to an already published yeast PDE complementation system. The LANCE assay has been used for the screening of IOTA's fragment library against 4 different parasitic PDEs (see WP3). Cheminformatic analysis of the screening data of the fragment library, the PDE toolbox, in combination with a detailed analysis of the data in the PDEstrian database, has led to the development of a new **PDE-focussed fragment library** (deliverable D 3.3).

Finally, a generic approach for important **ADMET** evaluation of new compounds had being established within PDE4NPD. Assays that monitoring off-target activity against human PDEs, CYP450s and hERG, next to cytotoxicity against human cells, solubility and metabolic stability measurements had been made all

available within the PDE4NPD program. In order to increase the throughput for metabolic stability measurements PDE4NPD has recently reached out to a major European Pharma company (Boehringer Ingelheim) and they have agreed to screen PDE4NPD compounds for free in their standard ADMET tests.

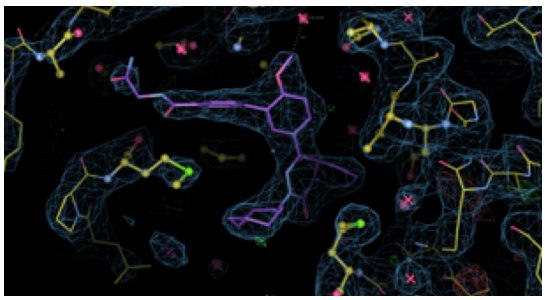
In conclusion, the PDE4NPD partners have provided a clear PDE-focussed drug discovery, that can tackle new parasitic PDEs. In fact, recently the project resources were used by Hemphill & Kunz (University of Bern) to test the validity of GlPDE as drug target to tackle *Giardia Lambia.*

T. brucei PDE4NPD program.

T. brucei expresses 4 different families of PDEs: TbrPDEA, TbrPDEB1 and B2, TbrPDEC and TbrPDED. Seminal work of Prof. Dr. Tom Seebeck (Scientific Advisory Board member) has provided target validation for TbrPDEB1 and TbrPDEB2, whereas TbrPDEC and TbrPDEA have been discarded as drug targets. At present, there is no knowledge concerning the clinical potential of targeting TbrPDED.

At the start of the program, PDE4NPD partners had developed hits against TbrPDEB1. As such, the *T. brucei* research program is the most advanced. Promising compound classes are now undergoing lead optimization that should lead to the selection of a *clinical candidate*. For this, the structure-based chemistry programs have focussed on target potency and selectivity over human PDE4, as well as other drug-like properties. From the start of PDE4NPD, TbrPDEB1 and B2 catalytic domain and full length constructs have been cloned, expressed, purified and are available (in mg quantity) for screening and X-ray crystallography. Currently, 33 X-ray structures for the TbrPDEB1_CD_565-98 construct in complex with different TBrPDEB1 inhibitors, including important PDE4NPD compounds, have been obtained, providing ample options for structure-based drug design. Moreover, recently also TbrPDEB2_CD_600-925 has been crystallised at a resolution of 1.17 angstrom, making it the highest resolution structure known of any PDE.

A set of TbrPDEB1 reference inhibitors has been synthesised for assay development purposes, while many novel potential TbrPDEB1 inhibitors have been isolated. Several inhibitors that display preference for TbrPDEB1 over hPDE4 and exhibit submicromolar activity against *T. brucei* whole parasites have been identified. Moreover, for the best TbrPDEB1 compounds direct evidence for targeting of the parasite PDE-specific P-pocket and PDE-mediated anti-parasitic activity has been obtained by cAMP measurements in the parasite. The programme is currently focussing on improving TbrPDEB1/hPDE4 selectivity (> 30 fold), while keeping on target potency (Ki < 100 nM) and obtaining metabolically stable compounds. At this moment the ADMET hurdle is most difficult to tackle.



x-ray structure of one of the PDE4NPD hits bound to the catalytic domain of TbrPDEB1

It has therefore decided that new chemical scaffold for TbrPDEB1 would be advantageous. Screening of the PDE toolbox did not reveal new chemistries and it

was decided that a proposal for the European Lead Factor (IMI) would be prepared. The ELF proposal was accepted in November 2016 and in 2017 a 450.000 high-throughput TbrPDEB1 screening will be performed at ELF using the assays/constructs from the PDE4NPD consortium. As additional backup programs, phenotypic screening as well as a TbrPDED target validation program are integral parts of the *T. brucei* PDE4NPD program. The TbrPDED gene has been cloned and is currently being expressed and evaluated for its PDE functionality.

T. cruzi PDE4NPD program

Like *T. brucei*, *T. cruzi* also expresses 4 different families of PDEs: TcrPDEA, TcrPDEB1 and B2, TcrPDEC and TcrPDED. Based on literature data TcrPDEC was regarded as a valid target for drug discovery at the start of PDE4NPD.[1,2] Initial efforts therefore have focussed on hit-finding in order to start a full Medicinal Chemistry program on TcrPDEC, supported by x-ray crystallography. In parallel, phenotypic screening and a target validation program focussed on the other 3 PDE families were started as part of the *T. cruzi* PDE4NPD program.

So far, PDE4NPD partners have successfully cloned the catalytic domain of TcrPDEC and established TcrPDEC_CD protein purification, x-ray crystallography and enzyme assays. Furthermore, full-length cDNAs of all TcrPDEs have been cloned and along with the catalytic domains of both TcrPDEB1 and B2. The B1 and B2 catalytic domains have also been expressed. For both TcrPDEC and B1, the PDE toolbox and fragment library have been screened, offering a number of interesting tool compounds. Yet, a number of potent TcrPDEC inhibitors hardly affect *T.cruzi* parasites, questioning the validity of TcrPDEC as validated target. To confirm our findings, literature reference TcrPDEC inhibitors were resynthesized and tested in PDE4NPD as being inactive at both TcrPDEC and against *T.cruzi* parasites. As such, TcrPDEC was deprioritized as target.

Early success in phenotypic screening has also led to a productive Medicinal Chemistry *hit exploration* and *optimization* program. Five hits proved to be more active against the intracellular *T. cruzi* form than the reference drug benznidazole. For one selected hit, VUF13527 (NPD-227) more many novel analogues were synthesised to obtain SAR. However, the most promising hits suffer from low microsomal stability and a primary metabolic hotspot was identified. The metabolic issues were tried to be addressed by various analogues, but so far clear improvement is not observed, although a recent N-oxide showed for the first time reasonable metabolic stability. The phenotypic activity of this series of compounds is not related to TcrPDEC or TcrCYP51 activity.

Leishmania PDE4NPD program

Leishmania also expresses 4 different families of PDEs: LmPDEA, LmPDEB1 and B2, LmPDEC and LmPDED. Initially, this program was given less priority in view of available resources and the initial advice by SAB members on the importance of PDEs in *Leishmania*. Yet, in view of the continuous high medical need and the platform function of PDE4NPD, since the Hamburg meeting PDE4NPD has followed up SAB advice to also pay attention to *Leishmania.* Expression profiling, gene cloning and PDE expression and purification are all progressing, allowing

[1] Kunz *et al.* FEBS J. **2005**;272(24):6412-22

[2] King-Keller *et al.* Antimicrob Agents Chemother. 2010 Sep;54(9):3738-45

e.g. PDE toolbox and fragment screening against LmPDEB1, next to initial x-ray pilots.
At the same time, all PDE inhibitors being developed in the program are also being screened phenotypically against Leishmania, which has led to the identification of interesting hit series. In one of these hit series, imidazole compounds of CSIC partner, in vivo proof of concept has been obtained for the first time, despite suboptimal pharmacokinetics. A NPD molecule that increases parasitic cAMP levels also shows up to 60% improvement in an in vivo *Leishmania* model. Currently, partner UA has planned a new in vivo experiment, in which exposure to the NPD compounds will be higher (ip administration instead of p.o).

S. mansoni PDE4NPD program

No knowledge exists about the expression and/or role of SmPDEs, other than a published genomic sequence showing ten potential PDE genes. The *S. mansoni* research program was thus the least advanced at the start. The PDE4NPD challenge is primarily at the level of *target validation* via both gene cloning and phenotypic screening of a set of PDE4NPD inhibitors. So far, the PDE4NPD partners have confirmed the presence of at least 10 PDE genes using genomic DNA from a clinically relevant strain of *S. mansoni* from partner TBRI. PDE4NPD has now cloned 8 full length PDE sequences using cDNA generated from adult worms and obtained a full expression profile of all 10 PDEs in various developmental stages. It appears that expression levels of various PDEs varies greatly during various life cycles and is generally higher in male than in females.
The cloned PDEs are currently lined up for functional characterization using standard PDE expression and purification and using the *T.brucei* and yeast PDE complementation systems. A highly expressed PDE SmPDE4a, has already been validated as functional PDE enzyme, has initially been crystallized at 5 angstrom, has been used for fragment screening of the IOTA fragment library and has been characterized pharmacologically with the PDE toolbox. The PDE toolbox screening revealed the the drug Roflumilast (NDP-006) as potent inhibitor, offering the option of drug repurposing.
Phenotypic screening of 184 compounds (incl. the PDE toolbox) against *S. mansoni* whole worms has resulted in 35 compounds showing worm killing, including Roflumilast. Interestingly, most compounds have stronger effects on males than females and are also much more active against the production of worm eggs than actual worm killing, which happens often at relatively high concentrations. These remarkable findings will be further studied in the next period. Moreover, an in vivo experiment is planned with Roflumilast.

Expected PDE4NPD results and impact

In line with the priorities of the WHO and other global initiatives, the major impact of PDE4NPD will be to contribute to the generic development of new drugs for neglected diseases. PDE4NPD expects to provide TbrPDEB1 inhibitors as clinical candidates for *T.brucei*, whereas for *T.cruzi* advanced lead molecules are expected, either based on the present phenotypic hits or on validated TcrPDEs. For *Leishmania* and *S.mansoni* PDE4NPD will result in the identification and target validation of parasite PDEs, although also for *Leishmania* the currently identified phenotypic hits

might provide advanced leads for drug development. Finally, PDE4NPD expects to tackle at least one additional parasitic disease by collaborative efforts with public and/or private parties outside the current PDE4NPD consortium.

For more information: www.PDE4NPD.eu or PDE4NPD@gmail.com

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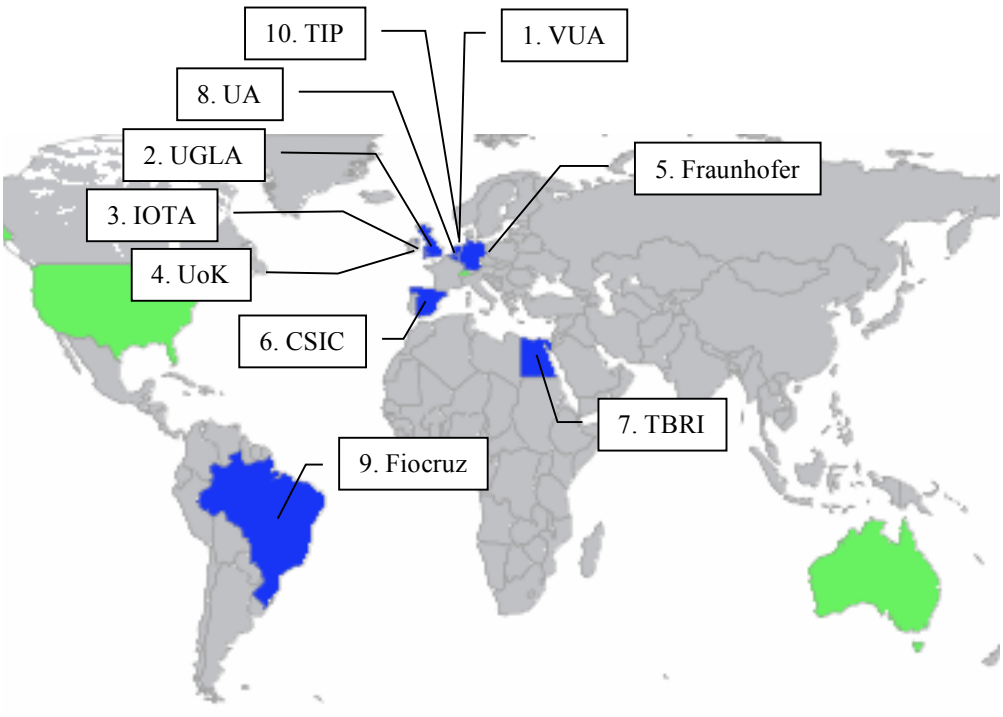
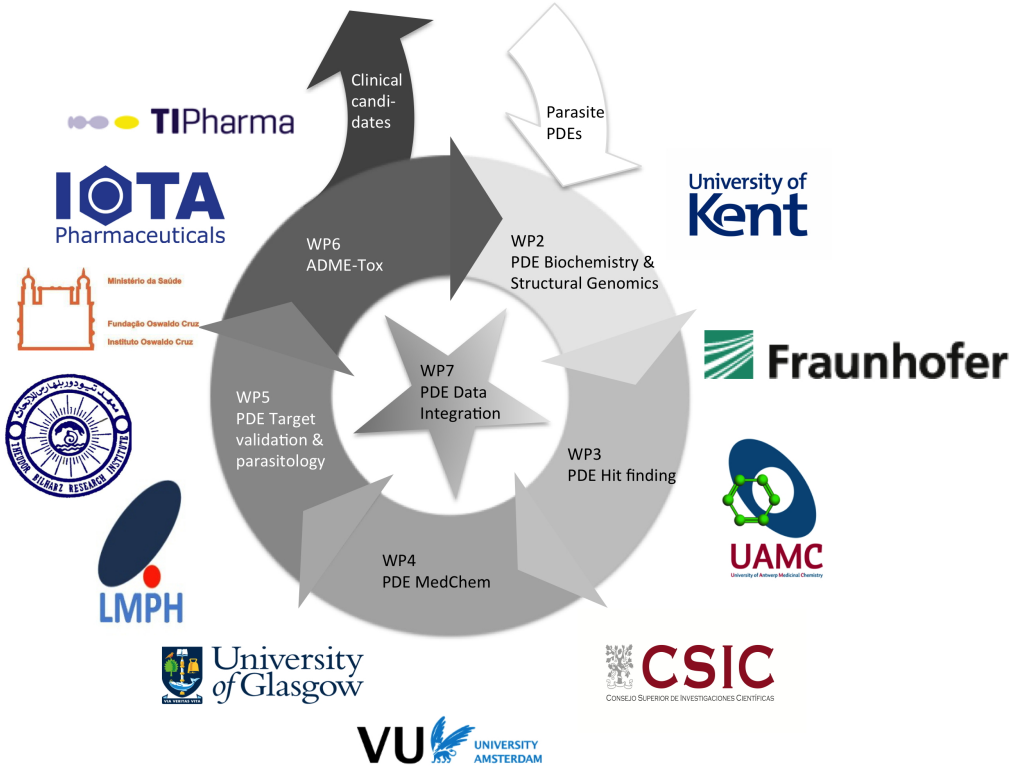


PDE4NPD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Parasite PDEs
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