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.

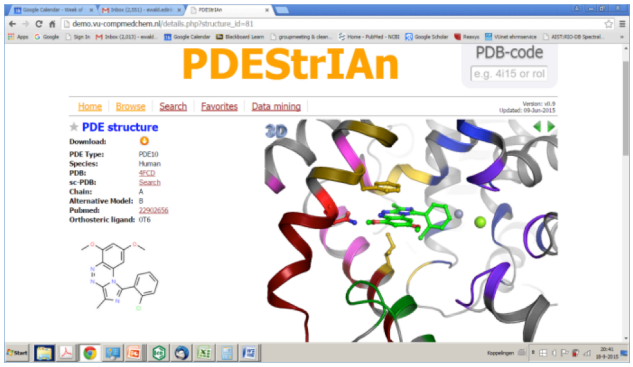
PDE4NPD progress

The current 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 meeting, the 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 is about to be released to the broader scientific community via web-based access. Next to these research databases, the partners have assembled a **PDE compound toolbox**, with a *current* selection of 49 structurally diverse human and parasite PDE inhibitors, which are used for assay development and early hit finding exercises for any new parasite PDE. The toolbox compounds are also valuable assets for phenotypic screening against various parasites. For the parasite PDE enzymes to be studied in the program, the biochemistry partners have also established a generic PDE enzyme assay based on the LANCE time-resolved FRET technology and a new *T.brucei*-based PDE complementation system.

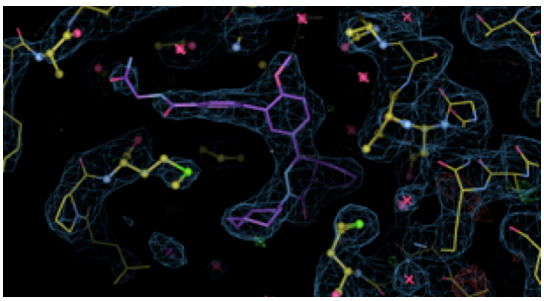


Screen shot of the chemogenomics database PDEstrian

Finally, a generic approach for important ADMET evaluation of new compounds is established within PDE4NPD. Importantly, assays that allow for monitoring off-target activity against human PDEs, CYP450s and hERG, next to cytotoxicity against human cells, solubility and metabolic stability measurements are now all available within the PDE4NPD program.

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 on the clinical potential of targeting TbrPDED. At the start of the program, PDE4NPD partners had already developed several hits series against TbrPDEB1 and TbrPDEB2. As such, the *T. brucei* research line is the most advanced. These 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 focus on target potency, selectivity over human PDE4 as well as other drug-like properties.


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

Since the start of PDE4NPD, TbrPDEB1 and B2 catalytic domain and full length constructs have been cloned, expressed, purified and are available (mg

quantity) for screening and X-ray crystallography. Currently, 11 X-ray structures for the TbrPDEB1_CD_565-98 construct in complex with different TBrPDEB1 inhibitors, including two lead compounds have been obtained. A set of TbrPDEB1 reference inhibitors has been synthesised for assay development purposes, whereas more than 50 novel potential TbrPDEB1inhibitors have been isolated. For the first time, inhibitors that display preference for TbrPDEB1 over hPDE4 and exhibit sub-micromolar activity against *T. brucei* whole parasites have been identified. Moreover, for the TbrPDEB1 lead compounds direct evidence for PDE-mediated anti-parasite activity was obtained by cAMP measurements in the parasite.

As backup program, phenotypic screening as well as a TbrPDED target validation program are integral part of the *T.brucei* PDE4NPD program. The TbrPDED gene has been cloned and is currently being expressed and evaluated for its PDE functionality. Phenotypic screening against *T. brucei brucei* has resulted in the identification of 16 hits (pIC_{50} range = 5.4-6.5), which are available for back-up programs.

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 is regarded as a valid target for drug discovery. Efforts so far 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 are 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 and enzyme assays. The purified protein can also be crystalized, but so far the x-ray diffraction is not yet optimal. Furthermore, the full length TcrPDEC cDNA has been cloned, next to the catalytic domains of both TcrPDEB1 and B2.

The established TcrPDEC_CD enzyme assay has been used to start a fragment-based drug discovery approach next to the screening of the PDE compound toolbox. Screening of the PDE toolbox and the fragment library has resulted in the identification of a variety of interesting hits, which are currently being triaged for hit prioritization and medicinal chemistry exploration.

Moreover, 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 (NDP-227) more than 50 novel analogues were synthesized to obtain SAR. The most promising hits suffer from low microsomal stability and a primary metabolic hotspot was identified. The metabolic issues are being addressed and at present, more than 15 additional analogues were synthesized. These new derivatives are now available for biological evaluation.

Leishmania PDE4NPD program

Also *Leishmania* expresses 4 different families of PDEs: LmPDEA, LmPDEB1 and B2, LmPDEC and LmPDED. Based on unpublished work from the lab of Prof Seebeck showing that LmPDE knock-out is inconclusive and the success of our own internal data on LmjPDEB1 inhibitors, the consortium decided early in the Project to pursue a chemical biology approach using the potent LmjPDE inhibitors discovered by the PDE4NPD consortium, before embarking on more resource-intensive gene

cloning and medicinal chemistry efforts. The available chemical tools will be used to obtain proof of concept of PDE inhibitors as promising anti-Leishmanial agents.
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. Phenotypic screening against L. infantum has resulted in the identification of 27 hits (pIC_{50} range = 5.0-5.9). Besides being active against intracellular amastigotes (in mouse macrophages), 17 hits also showed activity against extracellular promastigotes.

S. mansoni PDE4NPD program

No knowledge exists on the expression and or role of SmPDE's, other than a published genomic sequence showing ten potential PDE genes. The *S.mansoni* research program is thus the least advanced and the challenge is primarily at the level of *target validation* via both gene cloning and phenotypic screening of a set of PDE4NPD inhibitors. In the last period, the PDE4NPD partners have confirmed the presence of at least 7 PDE genes using genomic DNA from a clinically relevant strain of *S. mansoni* and have already cloned 4 full length PDE sequences using cDNA generated from adult worms. These PDEs currently await functional characterization, but based on the obtained structural information all 4 putative proteins show all characteristics of the PDE protein family.
Phenotypic screening of 103 compounds (incl. the PDE toolbox) against *S. mansoni* whole worms has resulted in 7 compounds showing worm killing but at relatively high concentrations. Interestingly, only male worms were killed by the identified hits. These remarkable findings will be further studied in the coming months. .

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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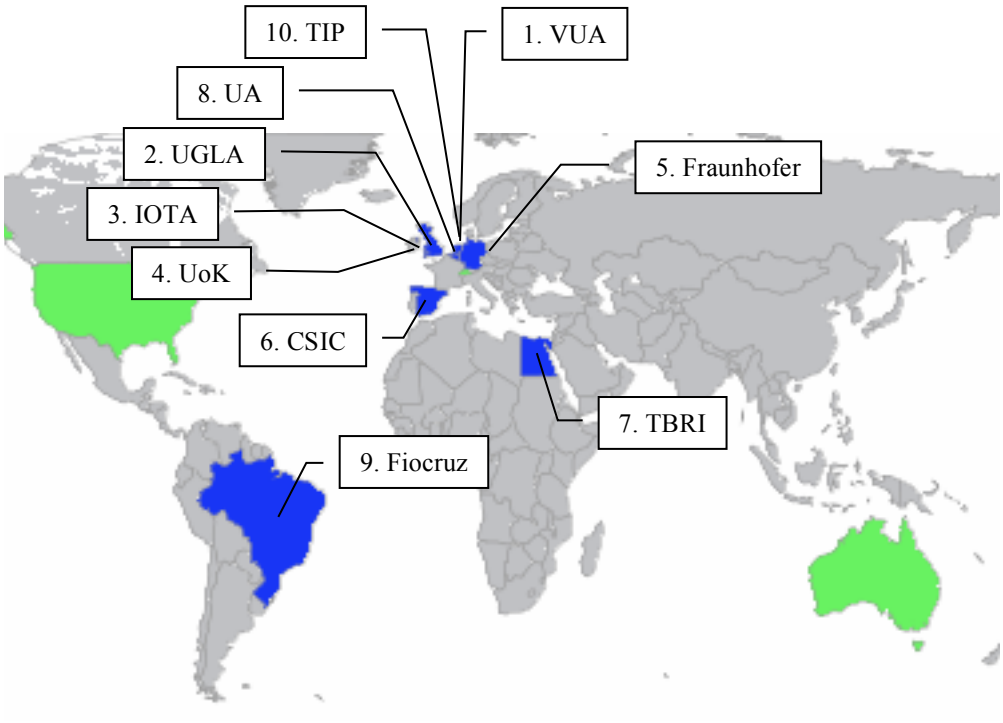
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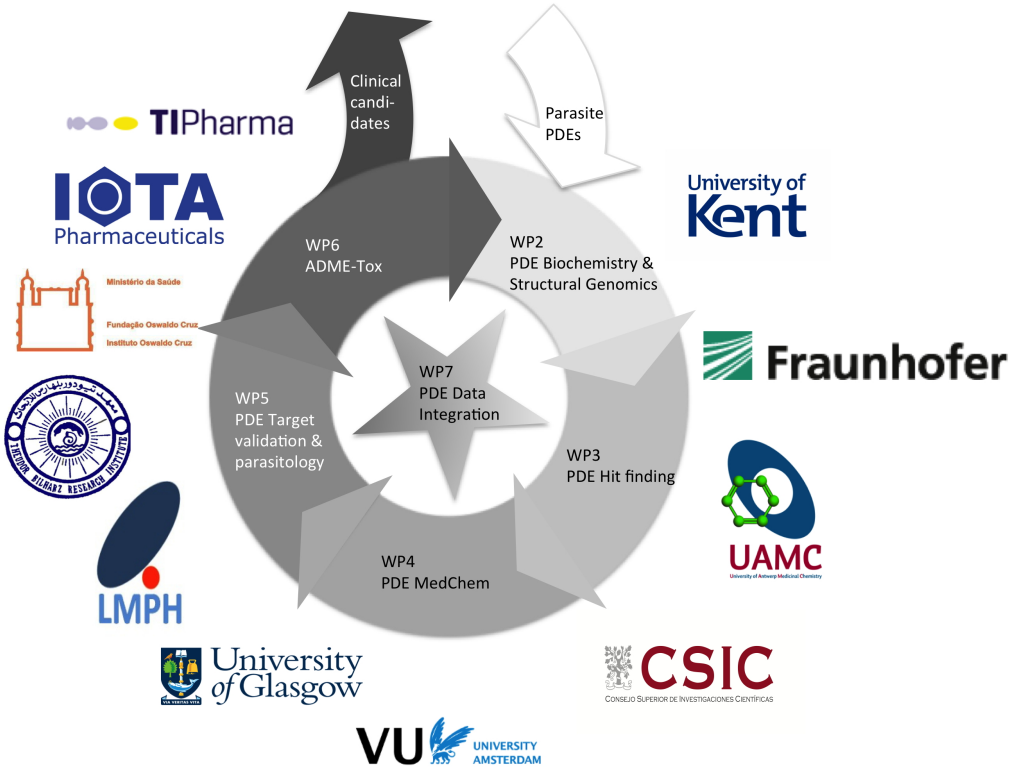
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