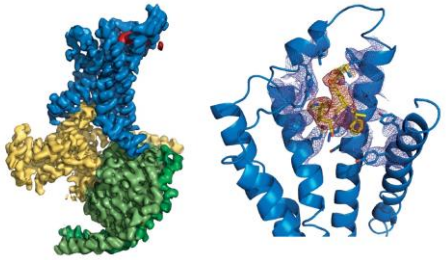
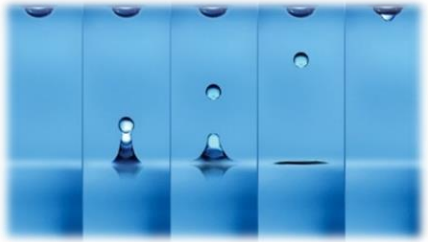
Methods in Drug Development

CH-455



Prof. Christian Heinis

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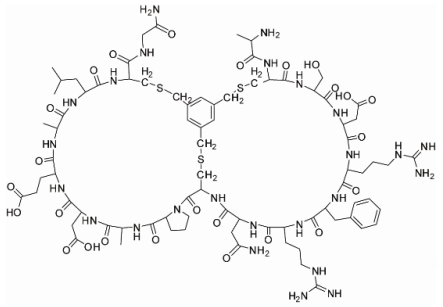
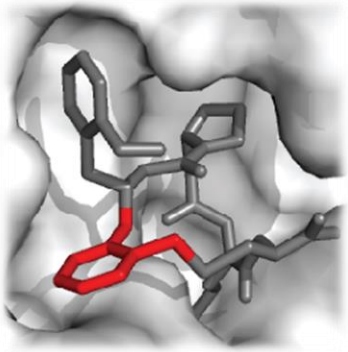
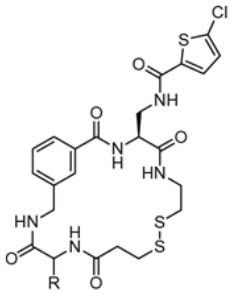
Christian Heinis

- Associate Professor in Bioorganic Chemistry
- Laboratory of Therapeutic Proteins and Peptides (LPPT)
- Research interests
    - New molecule designs (bicyclic peptides, macrocycles)
    - Combinatorial methods (phage display, HTS)
    - Drug discovery and development
    - Translational research
- Contact details
    - BCH 5305, 021 693 93 50, christian.heinis@epfl.ch







Course Organization

Monday, 8:15-9:00 and 9:15-10:00, room: CHB331

	Monday	Tuesday	Wednesday	Thursday	Friday
8:15-9:00					
9:15-10:00					

Course Objectives

Phenotypic screening

Macrocycle drugs

Learning modern methods in drug development

Target identification

Protein degradors

Target validation

Gene editing

Covalent drugs

Single particle cryo-EM

Antibody engineering

DEL libraries

Protein structure prediction

Critically reading and understanding scientific literature

Getting familiar with a new field

Extract key findings

Read & understand complex data

Critically read scientific literature

Example paper

nature chemical biology

ARTICLE

PUBLISHED ONLINE: 1 JUNE 2015 | DOI: 10.1038/NCHEMBIO.1837

SMN2 splice modulators enhance U1–pre-mRNA association and rescue SMA mice

James Palacino[1,3], Susanne E Swalley[1,3]*, Cheng Song[1,3], Atwood K Cheung[1], Lei Shu[1], Xiaolu Zhang[1], Mailin Van Hoosear[1], Youngah Shin[1], Donovan N Chin[1], Caroline Gubser Keller[2], Martin Beibel[2], Nicole A Renaud[1], Thomas M Smith[1], Michael Salcius[1], Xiaoying Shi[1], Marc Hild[1], Rebecca Servais[1], Monish Jain[1], Lin Deng[1], Caroline Bullock[1], Michael McLellan[1], Sven Schuierer[2], Leo Murphy[1], Marcel J J Blommers[2], Cecile Blaustein[1], Frada Berenshteyn[1], Arnaud Lacoste[1], Jason R Thomas[1], Guglielmo Roma[2], Gregory A Michaud[1], Brian S Tseng[1], Jeffery A Porter[1], Vic E Myer[1], John A Tallarico[1], Lawrence G Hamann[1], Daniel Curtis[1], Mark C Fishman[1], William F Dietrich[1], Natalie A Dales[1] & Rajeev Sivasankaran[1]*

Spinal muscular atrophy (SMA), which results from the loss of expression of the survival of motor neuron-1 (*SMN1*) gene, represents the most common genetic cause of pediatric mortality. A duplicate copy (*SMN2*) is inefficiently spliced, producing a truncated and unstable protein. We describe herein a potent, orally active, small-molecule enhancer of *SMN2* splicing that elevates full-length SMN protein and extends survival in a severe SMA mouse model. We demonstrate that the molecular mechanism of action is via stabilization of the transient double-strand RNA structure formed by the *SMN2* pre-mRNA and U1 small nuclear ribonucleic protein (snRNP) complex. The binding affinity of U1 snRNP to the 5′ splice site is increased in a sequence-

Reading and discussing research papers

At home

- Read publication and if necessary Supplementary Information (SI)
- Make sure that you understand all figures and that you can explain data shown in figures

In lecture

- General introduction to field of paper (by Christian Heinis)
- Introduction to paper (by two students; 2 x 10 minutes)
- Discussion of all data by going through the figures (by all)

Figures

Introduction

nature chemical biology

ARTICLE

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SMA is a debilitating motor neuron disease with an estimated worldwide incidence of 1 in 11,000 live births[1]. SMA is the most common genetic cause of mortality in children, with more than 50% of the affected patients dying before the age of two. The fundamental pathogenesis of SMA results from deficiency in SMN (survival of motor neuron) protein, which causes death of α-motor neurons in the spinal cord. Humans have two SMN genes, *SMN1* and *SMN2*, which produce SMN protein. Full-length SMN (FL-SMN) protein is primarily a product of the *SMN1* gene. Roughly 95% of SMA cases are due to a homozygous deletion of the *SMN1* gene, and the remaining cases are attributed to point mutations in this gene[2–4]. The *SMN2* gene can partially offset the loss of *SMN1*. However, a single nucleotide transition[5,6] in the *SMN2* gene causes altered splicing and exclusion of exon 7, which in turn results in only 10–20% production of full-length SMN mRNA and protein. The remaining mRNA encodes a truncated and unstable protein[7–12]. Phenotypically, there is an inverse relationship between disease severity and *SMN2* copy number in SMA patients, leading to degrees of severity and differences in age of onset ranging from the most common, infantile onset (type I) form to a rare, adult (type IV) form[13].

Splicing is a robustly conserved mechanism of high-precision processing of pre-mRNA into a mature mRNA. Proper assembly of nearly all 213,400 exons in the human genome requires the U1-U2 major spliceosome. The spliceosome acts via a stepwise process to achieve catalytic ligation of the exons and release of an intron 'lariat' structure (reviewed in ref. 14). Although the system must function with precision to ensure proper assembly of transcripts, there is also dynamic regulation of transcript assembly, with estimates that ~90% of all multiexon genes are assembled into more than one splice isoform[15]. This process is regulated through the action of a large collection of splice enhancer and suppressor element cofactors that influence the recruitment of the spliceosome to alternatively spliced exons (reviewed in ref. 16).

Selective enhancement or alteration of exon splicing could be therapeutically beneficial in a broad spectrum of genetic disorders, as aberrant splicing is associated with a large percentage of disease-causing mutations (reviewed in ref. 17). For SMA, efforts to provide therapies have been focused on approaches to enhance expression of the *SMN2* gene or correct aberrant splicing, for instance, by blocking a suppressor element with antisense oligonucleotides[18,19]. Additionally, a recent report described small molecules that enhance exon 7–induced SMN2 levels, both *in vitro* and *in vivo*, albeit without providing information detailing the mechanism of action[20].

In this report, we describe the discovery and advancement of a pyridazine class of orally active, small-molecule enhancers of *SMN* exon 7 inclusion. These splicing modulators elevate FL-SMN protein and in turn extend survival in a severe SMA mouse model. We show that these molecules function via stabilization of the transient double-strand RNA (dsRNA) structure formed between the SMN2 pre-mRNA and U1 snRNP complex, a key component of the spliceosome[14,16,21]. Furthermore, these compounds act by increasing the binding affinity of U1 snRNP to the 5′ splice site (5′ss) in a sequence-selective manner that is discrete from constitutive recognition. Specific mutations of this sequence ablate enhancement of exon inclusion without adversely affecting baseline splicing efficiency. These studies reveal a new mechanism

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demonstrating the feasibility of using small molecules to attain sequence-selective splice modulation, opening the possibility of using similar strategies for other splicing diseases[22–24].

RESULTS

Identification of small-molecule *SMN2* splicing modulators

To identify small-molecule modulators of *SMN2* exon 7 inclusion, we used an NSC34 motor neuron cell line expressing an *SMN2* minigene reporter for high-throughput screening (HTS). The designed pair of *SMN2*-matched reporter constructs indicated either exon 7 inclusion (full-length reporter) or exon 7 exclusion (Δ7 reporter) based on in-frame luciferase expression (**Fig. 1a**). A screen of the Novartis compound library (~1.4×10^6 compounds) resulted in a hit rate of <1% (**Supplementary Results**, **Supplementary Table 1**). We selected hits on the basis of complementary changes in forward and reverse reporter activity, relative to control, that fit the anticipated profile for *SMN2* splicing modulators (**Fig. 1b**). Molecules that scored positively in the HTS and elicited dose responsiveness were assessed via qPCR to confirm the desired splicing activity and by ELISA to confirm increased SMN protein levels in SMNΔ7 mouse myoblasts, indicating efficacy on the endogenous gene.

One of the scaffolds identified from the HTS efforts contained a pyridazine core group as the core functionality (**Fig. 1c**). Compound optimization aimed at maximizing cellular potency guided by the SMN ELISA assay in both mouse myoblasts and SMA patient fibroblasts led to the identification of compounds NVS-SM1 (1) and NVS-SM2 (2) as well as inactive analogs NVS-SM3 (3) and NVS-SM4 (4) (**Fig. 1d** and **Supplementary Fig. 1a**,**b**). We observed favorable pharmacokinetics profiles of the two active compounds in rodents. Both NVS-SM1 and NVS-SM2 showed high plasma exposure, good bioavailability and, notably, good distribution to the brain, a primary target tissue (**Supplementary Fig. 1c**).

In vivo efficacy of SMN splicing modulators

As NVS-SM1 and NVS-SM2 were suitable *in vivo* tools, we sought to explore whether the cellular potency we observed translated to *in vivo* activity. For this purpose, we used the C/+ SMA mouse model[25]. After oral administration, both compounds produced dose-dependent elevations of *SMN2*-FL transcript and SMN protein in brain and spinal cord (**Fig. 2a**,**b**), thus establishing translatability and a pharmacokinetic-pharmacodynamic relationship. Although both compounds showed dose-responsive activity in the C/+ model (**Fig. 2a**,**b**), NVS-SM1 exhibited efficacy at lower doses and exposures, and thus this analog was studied further in additional cellular and animal models. NVS-SM1 showed robust activity across disease-relevant induced pluripotent stem cell (iPSc)-derived neurons (**Supplementary Fig. 2a**,**b**). We also demonstrated the desired transcript response in SMA-type III PBMCs after NVS-SM1 treatment, suggesting that this readout could serve as a peripheral pharmacodynamic marker in the clinic (**Supplementary Fig. 2c**).

In the cellular setting, we observed that SMN protein levels remained elevated after compound treatment, which is in agreement with published findings[26] and suggests that SMN protein has a long half-life. We sought to confirm this finding in the C/+ *in vivo* model, as this could affect the design of longer-term efficacy studies. In fact, a single, 30 mg per kg body weight oral dose of NVS-SM1 resulted in significant ($P < 0.05$) and durable SMN protein elevation in brain for up to 160 h (**Fig. 2c**).

To evaluate the efficacy of NVS-SM1, we used the SMNΔ7 mouse model, which displays a severe phenotype. In the specific colony used for this study, death typically occurs before postnatal day 15 (ref. 27). We were pleased to observe that, in addition to a dose-dependent elevation of SMN protein in the brain (**Fig. 2d**), oral administration of NVS-SM1 improved body weight (**Fig. 2e**) and extended lifespan, with 50% of the animals in the 1 mg per kg body weight group and 62% of animals in the 3 mg per kg body weight group showing increased survival (**Fig. 2f**).

Next, we evaluated whether NVS-SM1 elicited sustained efficacy by comparing two additional cohorts; the first cohort continued with 1 mg per kg body weight daily dosing of NVS-SM1 for an additional two weeks until day 49, whereas the second cohort received only

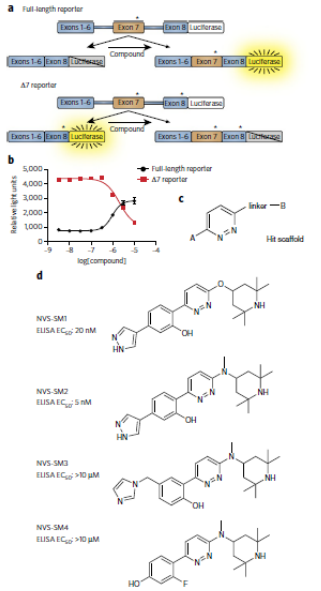
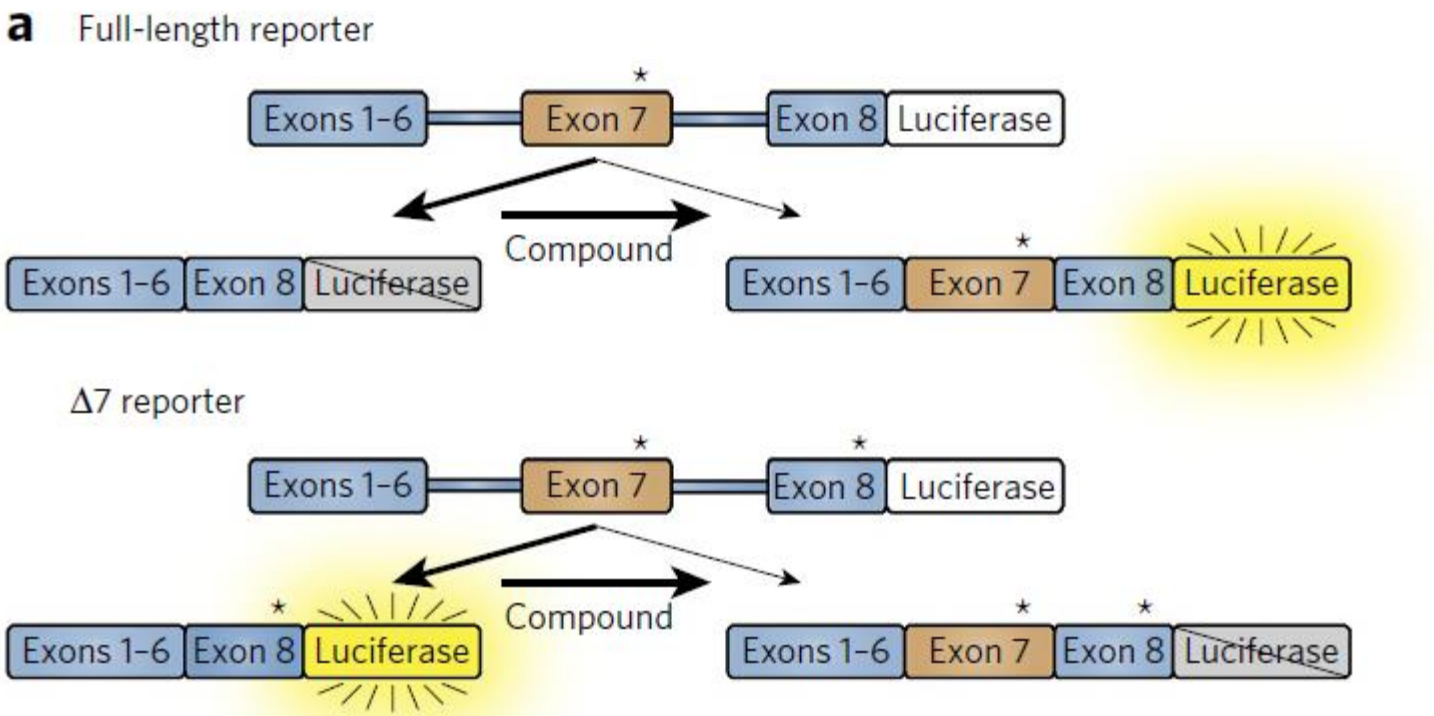


Figure 1 | Screening paradigm and molecules. (**a**) Schematic representation of SMN minigene constructs. Full-length construct (top) expresses luciferase in frame when exon 7 is included in transcript. Δ7 construct (bottom) expresses luciferase in frame when exon 7 is skipped in transcript. Asterisks indicate insertion of C nucleotides to adjust reading frame for constructs and to ensure that luciferase is in frame for the appropriate splice variant. (**b**) Data from example hit from the high-throughput screen showing elevation of full-length reporter luciferase signal (black) and a concomitant reduction in Δ7 reporter luciferase signal (red). Data represent mean ± s.e.m. ($n = 2$). (**c**) Hit scaffold structure for pyridazine molecules. (**d**) Chemical structures of NVS-SM1, NVS-SM2, NVS-SM3 and NVS-SM4 with SMN ELISA half-maximum effective concentration (EC_{50}) values.

Figures


a
Full-length reporter
Exons 1-6
Exon 7
Exon 8
Luciferase
Compound
Exons 1-6
Exon 8
Luciferase
Exons 1-6
Exon 7
Exon 8
Luciferase
Δ7 reporter
Exons 1-6
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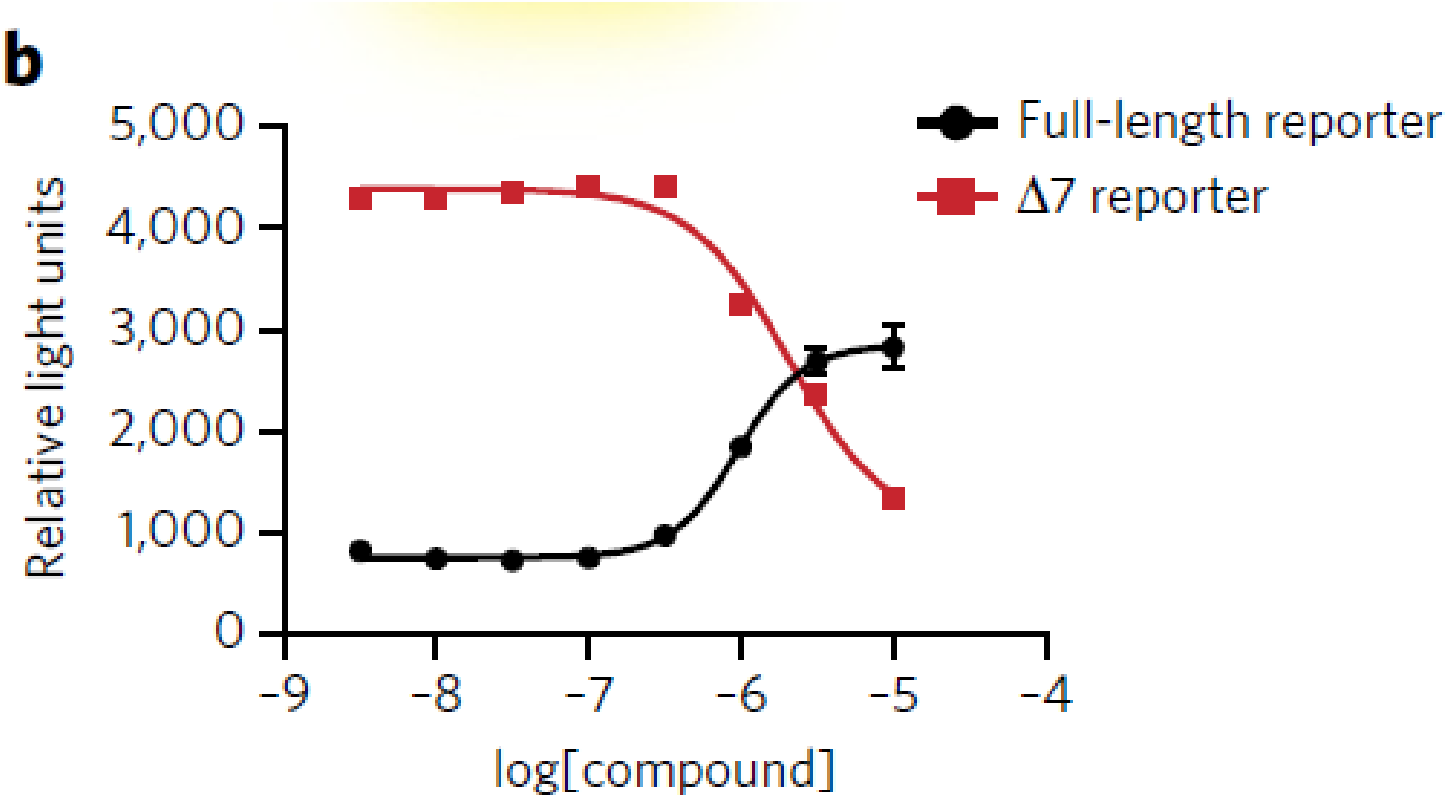
Exon 8
Luciferase

Figures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5,000
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-6
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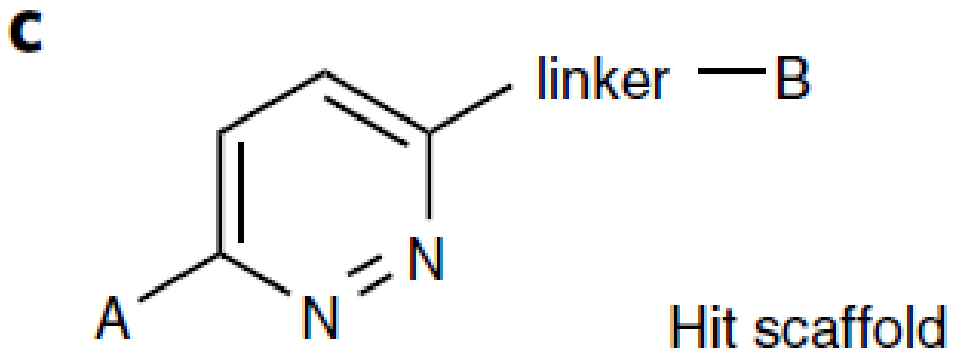
log[compound]
Full-length reporter
Δ7 reporter

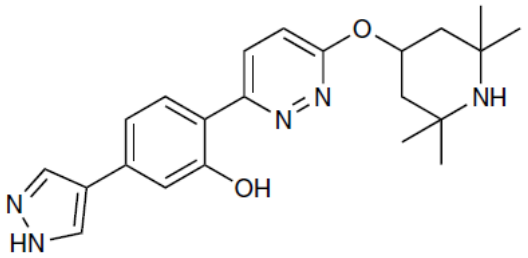
Figures



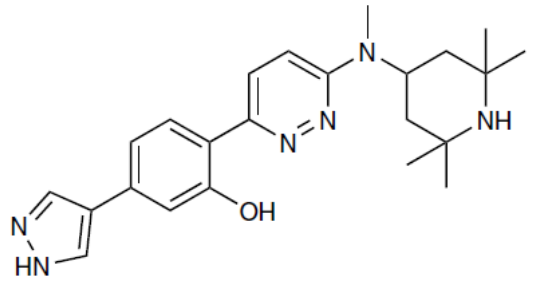
Figures

d

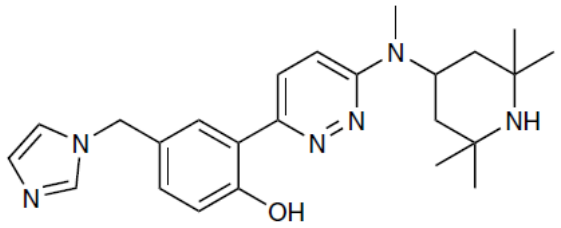
NVS-SM1
ELISA EC_{50}: 20 nM



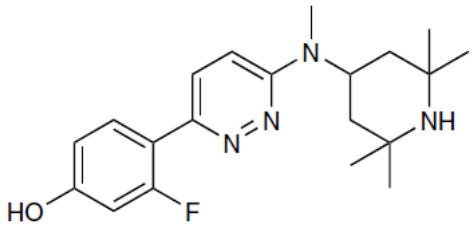
NVS-SM2
ELISA EC_{50}: 5 nM



NVS-SM3
ELISA EC_{50}: >10 μM



NVS-SM4
ELISA EC_{50}: >10 μM



Course Material

http://moodle.epfl.ch

CH-455

- Papers (PDF)
- Supplementary information (SI, as PDF)
- Figures (PPT presentations)
- General introduction to fields (PPT presentations)

Assessment

- Written exam at the end of the course (80%)
- Oral presentations during the course (20%)

Exam

- Written exam (2 hrs)
- Questions about the 12 papers

  (I will show figures and ask mostly questions of understanding)
- It is important to read and fully understand the research papers
- No need to memorize information