Synthesis and *in vitro* Assay of Deoxyhypusine Synthase Inhibitors as Potential Anti-HIV Agents

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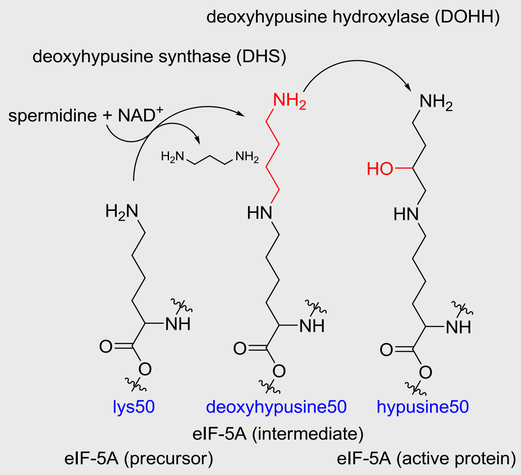
Introduction

HIV chemotherapy mainly focuses on parallel inhibition of several viral enzymes (cART). However, in order to improve cART-related long-term toxicities and decrease the risk of the development of (multi-)drug resistance, it is mandatory to address new potential targets and to identify new anti-retroviral drugs. An avoidance of viral adjustment is possible by inhibition of various host cell-factors that are essential for viral replication. One example is the eukaryotic initiation factor 5A (eIF-5A). This protein acts as a cellular cofactor of the HIV Rev regulatory protein in the process of nucleocytoplasmic transport of incompletely-spliced and unspliced viral transcripts.[1]

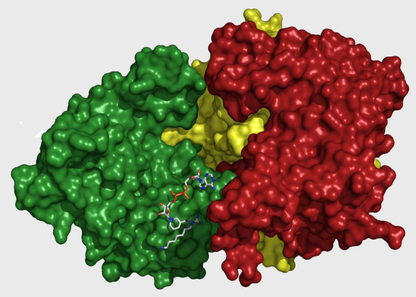


eIF-5A is a 17 kDa protein present in archae and eukaryotes which primarily promotes the elongation step of translation.[1,2]

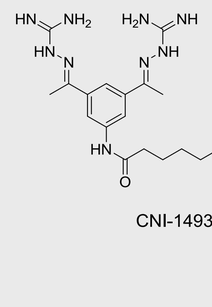
Activation of eIF-5A involves a unique post-translational modification of a specific lysine residue to the unusual amino acid hypusine (N^{ε}-(4-amino-2-hydroxybutyl)lysine). This modification is catalyzed sequentially by two human enzymes, the deoxyhypusine synthase (DHS) and the deoxyhypusine hydroxylase (DOHH).[3]

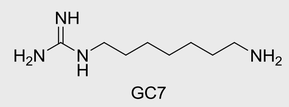
Targeting the DHS efficiently suppresses the activation of eIF-5A leading to an inhibition of HIV replication without affecting cell proliferation.[4] This has been shown by active compounds like the guanylhydrazone CNI-1493[4] and analogues of the natural substrate, e.g. GC7[5].

In 2004, the homotetrameric DHS was co-crystallized with GC7, revealing the active site. It has a size of ca. 17 Å and is mainly encased by negatively charged amino acids.[6]



Crystal structure 1rqd of DHS trimer showing cavity with bound cofactor NAD and inhibitor GC7

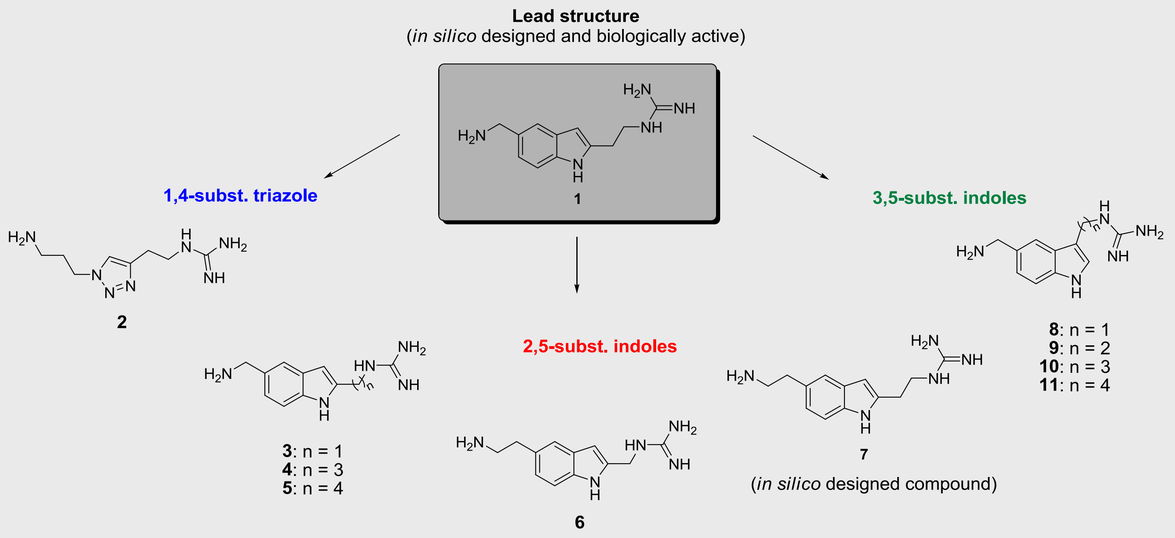




Objective

The *in silico* designed inhibitor **1** containing an indole core fragment and amino/guanidino moieties showed dose-dependent activity against DHS (IC_{50} ≈ 12 µM) and HIV-1 *in vitro* without causing cytotoxic effects.[7] This hit compound has been employed as a lead structure for further optimization of binding affinity and development of new potential drugs.

Here, we present the synthesis and biological evaluation of a second substance identified in the initial virtual screening and several new compounds with modifications regarding the substitution pattern, alkyl chain lengths and the aromatic scaffold.



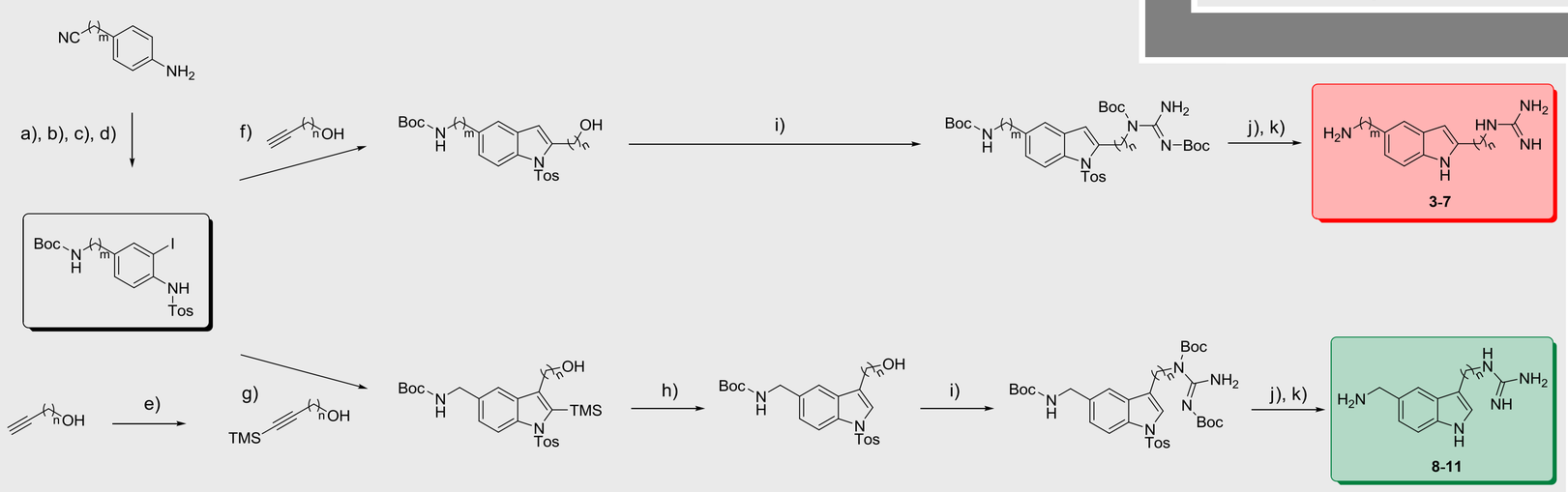
Synthesis of 2,5- and 3,5-substituted Indoles

The indole compounds were prepared according to the optimized synthesis protocol of the lead structure as well as modified literature known procedures.[7,8] Both kinds of substituted indoles could be obtained from the same aromatic precursor. The indole formation is a regioselective reaction that was

f) either done by *in situ* C-C cross coupling and cyclisation in order to get 2-substituted indoles in yields of 91-98%

g) or by direct cyclisation using TMS-alkynols to steer the sterically less hindered hydroxyalkyl into the 3-position.

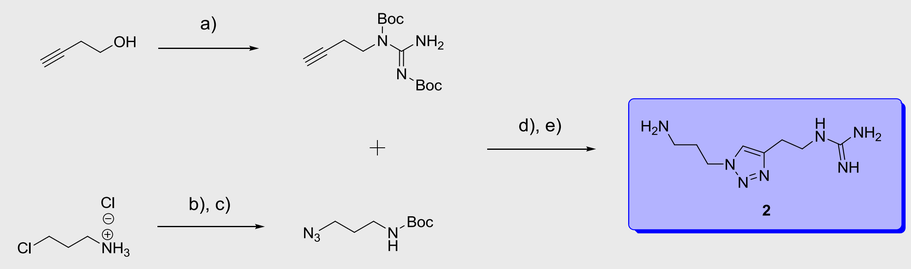
After TMS cleavage again the same reaction protocols were used for all derivatives, that is introduction of the guanidino moiety using Mitsunobu-conditions and removal of the protecting groups as the final steps.



Reagents and conditions: a) ICl, $CaCO_3$, CH_3OH, rt, 15 h, m = 1: 79%; **b)** (i) BH_3·THF, THF, reflux, 4 h, (ii) 2 M HCl, reflux, 1 h; **c)** Boc_2O, NEt_3, DMAP, CH_2Cl_2, 40 °C, 6 h, 85-87% (over 2 steps); **d)** TosCl, pyridine, CH_2Cl_2, rt, 41 h, 88-95%; **e)** (i) *n*-BuLi, THF, TMSCl, –78 °C → rt, 20 h, (ii) 5 M H_2SO_4, 0 °C → rt, 4 h, 97-98%; **f)** CuI, $Pd(PPh_3)_2Cl_2$, NEt_3, DMF, 85 °C, 17 h, 91-98%; **g)** LiCl, DABCO, $Pd(OAc)_2$, DMF, 100 °C, 16-24 h, 23-80% (TMS was cleaved for n = 1); **h)** TBAF, THF, 0 °C → rt, 1 h, 84-92%; **i)** *N*,*N*'-Di-Boc-guanidine, PPh_3, DIAD, THF, reflux, 3 h, 57-96%; **j)** 5 M NaOH, CH_3OH, rt, 40 h, 50-94%; **k)** (i) 2 M HCl/CH_3CN 1:1, rt, 22 h, (ii) RP chromatography, (iii) sephadex LH-20, 9-75%.

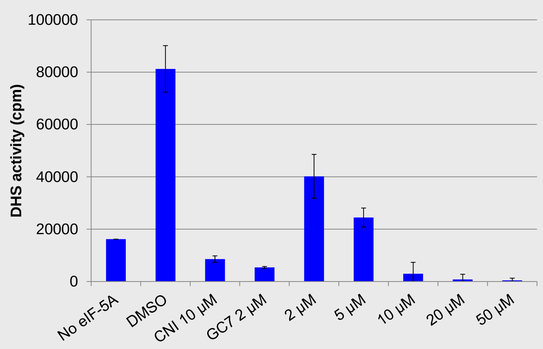
Synthesis of the 1,4-substituted Triazole

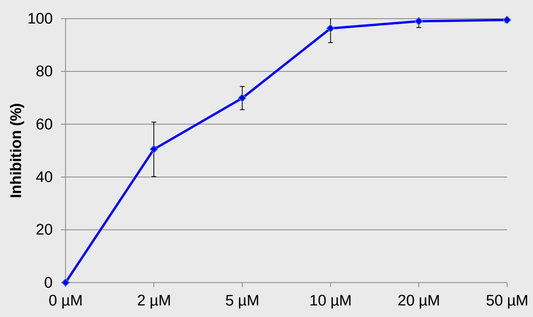
The triazole was prepared by using click chemistry[9] as a key step to combine the two building blocks. Boc protecting groups were applied for the guanidino function as well as for the amino group. The target compound was obtained in an overall yield of 20%.



Reagents and conditions: a) *N*,*N*'-Di-Boc-guanidine, PPh_3, DIAD, THF, reflux, 3 h, 92%; **b)** NaN_3, H_2O, 80 °C, 22 h, 86%; **c)** Boc_2O, NEt_3, CH_3OH, rt, 24 h, 88%; **d)** $CuSO_4$, Na ascorbate, THF/H_2O 1:1, rt, 20 h, 79%; **e)** (i) 2 M HCl/CH_3CN 1:1, rt, 22 h, (ii) RP chromatography, (iii) sephadex LH-20, 37%.

In vitro DHS Inhibition

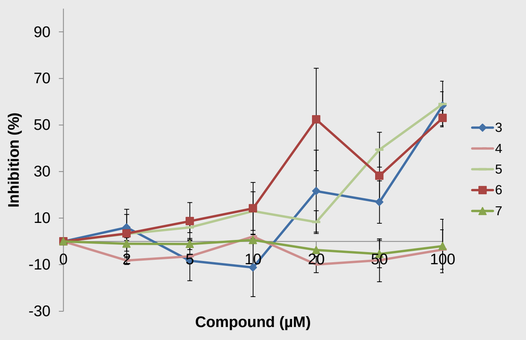




Inhibition of DHS by the **1,4-subst. triazole** (reference: CNI-1493, GC7)

The 1,4-substituted triazole **2** showed dose-dependent DHS inhibition with an IC_{50} ≈ 2 µM. This indicates that a smaller aromatic region as well as a hydrogen bond acceptor are preferred for binding to the center of the active site.

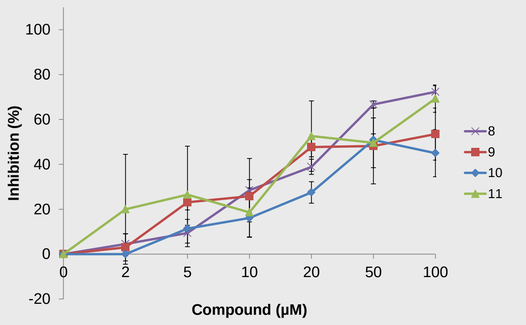
Regarding the 2,5-substituted indoles, compounds **4** and **7** were inactive against DHS, compounds **3**, **5** and **6** showed only similar weak inhibition. As a consequence, the result taken from the virtual screening could not be confirmed.



Inhibition of DHS by the **2,5-subst. indoles**

Moreover, no proper correlation between functional group distance and binding affinity could be observed.

The alkyl chain length at the 3-position seems to have no significant impact on DHS activity since all compounds **8-11** showed low inhibition in the same range.



Inhibition of DHS by the **3,5-subst. indoles**

Conclusion

- Nine indole derivatives and one triazole compound were synthesized and screened *in vitro.*
- The 2,5-substituted indoles **3-7** did not show improved binding affinity as compared to the lead structure **1**.
- The 3,5-substituted indoles **8-11** showed low DHS inhibition regardless of the alkyl chain length.
- **The 1,4-substituted triazole 2 efficiently inhibited the DHS at 2 µM**.

References

[1] Bevec, D.; Jaksche, H.; Oft, M.; Woehl, T.; Himmelspach, M.; Pacher, A.; Schebesta, M.; Koettnitz, K.; Dobrovnik, M. *Science* **1996**, *271*, 1858-1860. **[2]** Saini, P.; Eyler, D. E.; Green, R.; Dever, T. E. *Nature* **2009**, *459*, 118-121. **[3]** Park, M. H. *J. Biochem.* **2006**, *139*, 161-169. **[4]** Hauber, I.; Bevec, D.; Heukeshoven, J.; Krätzer, F.; Horn, F.; Choidas, A.; Harrer, T.; Hauber, J. *J. Clin. Invest.* **2005**, *115*, 76-85. **[5]** Jakus, J.; Wolff, E. C.; Park, M. H.; Folk, E. J. *J. Biol. Chem.* **1993**, *268*, 13151-13159. **[6]** Umland, T. C.; Wolff, E. C.; Park, M. H.; Davies, D. R. *J. Biol. Chem.* **2004**, *279*, 28697-28705. **[7]** Schröder, M.; Kolodzik, A.; Pfaff, K.; Priyadarshini, P.; Krepstakies, M.; Hauber, J.; Rarey, M.; Meier, C. *ChemMedChem* **2014**, *9*, 940-952. **[8]** Larock, R. C.; Yum, E. K.; Refvik, M. D. *J. Org. Chem.* **1998**, *63*, 7652-7662. **[9]** Liang, L.; Astruc, D. *Coord. Chem. Rev.* **2011**, *255*, 2933-2945.

Acknowledgement

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