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-processed and unspliced viral transcripts.\(^5\)

eIF-5A is a 17 kDa protein present in archaebacteria and eukaryotes which primarily promotes the elongation step of translation.\(^6\)\(^,\)\(^7\)

Activation of eIF-5A involves a unique post-translational modification of a specific lysine residue to the unusual amino acid hypusine (\(^N^4\)-amino-2-hydroxybutylysine). This modification is catalyzed sequentially by two human enzymes, the deoxyhypusine synthase (DHS) and the deoxyhypusine hydroxylase (DOHH).\(^8\)\(^,\)\(^9\)

Targeting the DHS efficiently suppresses the activation of eIF-5A leading to an inhibition of HIV replication without affecting cell proliferation.\(^10\)\(^,\)\(^11\) This has been shown by active compounds like the guanylnucleoside \(\text{CNI-1493}\)\(^(12,\)\(^13\) and analogues of the natural substance, e.g. \(\text{CCL}\).\(^14\)\(^,\)\(^15\) In 2004, the homoeometric 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.\(^16\)

Objective

The in silico designed inhibitor 1 containing an indole core fragment and amino/guanosine moieties showed dose-dependent activity against DHS (IC\(\text{50} = 1.2 \mu M\) and HIV-1 in vitro) without causing cytotoxic effects.\(^17\) 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 the 1,4-substituted Triazole

The triazole was prepared by using click chemistry as a key step to combine the two building blocks. Sox 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%.

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.\(^18\)\(^,\)\(^19\) Both kinds of substituted indoles could be obtained from the same aromatic precursor. The indole formation is a regioselective reaction that was i) either done in situ in 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-alkyltos to steer the sterically less hindered hydroxyl/yl into the 3-position.

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

In vitro DHS Inhibition

The 1,4-substituted triazole 2 showed dose-dependent DHS inhibition with an IC\(\text{50} = 2 \mu M\). This indicates that a smaller aromatic region as well as a hydrogen bond acceptor are preferred for binding to the active site.

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

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 \(\text{B} - \text{I}\) showed low inhibition in the same range.

References


Conclusion

- Nine indole derivatives and one triazole compound were synthesized and screened in vitro.
- The 2.5-substituted indoles 3-7 showed 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.

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