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Pursuing the Development of New Antiviral Entry Inhibitors

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Introduction

Viral entry is the earliest stage of infection in the viral life cycle, as the virus comes into contact with the host cell and introduces viral material into the cell.[1] Several compounds which block the attachment of HIV gp120 to either the CD4 T cell receptor or the CCR5/CXCR4 co-receptors are currently in clinical development. Most of these compounds have different molecular structures and specific mechanisms of action.[2]

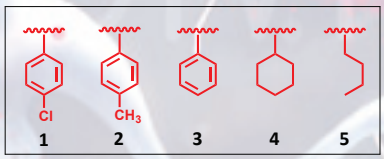
Background and Design

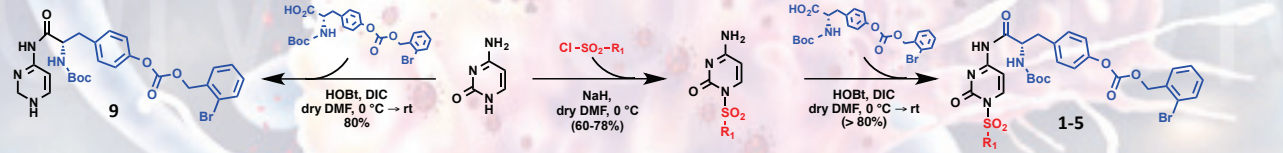
Recently we synthesized a library of potential cytosine-based HIV-1 entry inhibitors. Preliminary results on the ability to inhibit HIV induced cytopathogenic effect in TZM-bl cell culture assay allowed us to identify the cytosine-based compound **1** as interesting CXCR4 inhibitor. Namely, the compound showed a selective inhibitory activity against NL4.3 (X4) viral strain (IC_{50} 20.68 μM versus IC_{50} 112 μM against Bal (R5) viral strain).
To explore and establish the structural parameter associated with the HIV-1 inhibitory activity of this hit compound we performed a series of modifications on its backbone including:

1. Modification of the electronic and lipophilic nature of sulfone group at position *N*-1 of the cytosine ring (compounds **1-5**).

2. Modification of the amino acid residues linked to the exocyclic amine group of cytosine ring (compounds **6-8**).

At the same time we tested the activity of the derivative **9**, lacking the sulfonyl motif and keeping unchanged the protected tyrosine on the *N*-4 position.



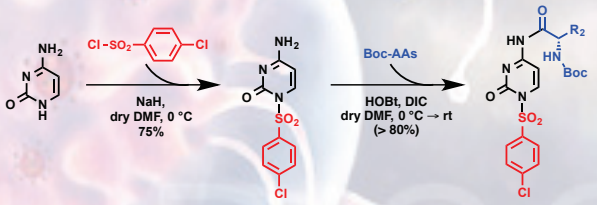


Synthesis

I. To synthesized our compounds the protocol involved the formation of *N*-1 sulfonyl cytosine derivatives through the reaction of cytosine with different sulfonyl chloride under basic conditions (NaH in DMF) at 0 °C. The following coupling with Boc-L-Tyr(2-Br-Z)-OH in anydrous conditions (HOBt, DIC, DMF) provided the compounds **1-5** in high yields (> 80%).

II. For the synthesis of compounds **6-8**, in turn, we choose the of *N*-1-(4-chlorobenzylsulfonyl)cytosine as buliding block and we performed the coupling of the latter with Boc-4-benzoyl-L-Phe-OH (Boc-L-Bpa-OH), or Boc-3-(4-biphenyl)-L-Ala-OH (Boc-L-Bip-OH) or Boc-L-Tyr(Bzl)-OH amino acids residues exploiting the reaction conditions already described (HOBt, DIC, DMF).

III. The strategy was different only for the compound **9**, coming from the easy treatment of cytosine with Boc-L-Tyr(2-Br-Z)-OH (80% yields).



Inhibitory Activity

All these compounds were tested as inhibitors of HIV-1 activity in HeLa (TMZ-bl) cells. In this assay the most potent compounds are **5** and **9**, while compound **2** is also the more citotoxic derivative. Interestingly, these compounds showed a similar micromolar anti HIV-1 activity compared to reference compound **1** against the NL4.3 (X44) viral strain and were also active against BaL (R5) viral strain. Substitution of Tyr moiety, present in all three compounds, for other biphenyl amino acids resulted in a loss of activity of the resulting compounds (**6-8**) against Bal (R5) strain. According to these preliminary results the importance of sulphone groups on the inhibitory activity of these derivatives remains to be defined (**2** and **5** versus **9**). However, these results provide proofs on the major activity of cytosine derivatives with amino acids and give evidence of the importance of the hydrophobic chains on the side chain for the anti HIV activity.

Compound	NL4.3 (X4) EC_{50}[a] (μM)	BaL (R5) EC_{50}[a] (μM)	TZM-bl CC_{50}[b] (μM)
1	20,62	> 112,73	112,73
2	26,46	34,64	82,65
3	32,55	> 101,38	101,38
4	> 20,88	> 20,88	20,88
5	11,56	15,52	105,79
6	28,14	> 90,98	90,98
7	44,03	> 106,40	106,40
8	34,49	> 111,35	111,35
9	36,35	37,17	110,89
AMD3100	1,32	nd	> 1000
Maraviroc	nd	4,07	> 1000

[a]50% effective concentration or compound concentration required to inhibit HIV induced cytopathogenic effect in TZM-bl cell culture
[b]50% cytotoxic concentration in TZM-bl cell culture

1. Walker, B.D; Yu, X.G Nature Rewies Immunology **2013**, *13*, 487-498
2. Grobler, J.A et al. PNAS **2002**, *99*, 6661-6666

In the background: HIV spreads among helper T lymphocytes and macrophages as it compromises the immune system of an infected patient. www.stromastudios.com

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