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Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 1543–1546

A possible improvement for structure-based drug design illustrated by the discovery of a Tat HIV-1 inhibitor

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Received 1 October 2003; revised 5 December 2003; accepted 24 December 2003

Abstract—The HIV-1 Tat protein is a promising target for AIDS therapy, due to its extra-cellular roles against the immune system. From the 2D-NMR structure of Tat, we have designed molecules, called TDS, able to bind to Tat and inhibit HIV-1 replication in vitro. This new family of antivirals is composed of a triphenylene aromatic ring substituted with at least one carbon chain bearing a succinimide group. These ligands are prepared from triphenylene or 2,6,10-trimethylphenylene in 3–6 steps depending on the target molecule.

A decade ago, structure-based drug design or computer-aided drug discovery (CADD) raised the hope that the discovery of new drugs could be accelerated. The reality, however, is far from the expectation since the rate of introduction of new chemical entities to the pharmaceutical market is currently lower than at any time since the Second World War.[1] Focusing on antiviral drugs alone, the current repertoire now includes more than 30 specialized products but only one has been obtained from CADD.[2] The problem is that CADD, based on the use of a single rigid protein structure, faces the difficulty of the dynamic behavior of a receptor, thus accommodating protein flexibility in CADD is a major issue.[3] We have developed a new approach that could contribute to improve CADD by taking into account the dynamic behavior of a receptor and local structural changes induced by mutations. The best way to illustrate the potential of our approach was to choose a highly flexible protein as a target, with possible mutants still having a biological activity. This is the case for the HIV-1 Tat protein.[4]

AIDS is due to the loss of CD4 cells resulting from HIV cytotoxicity but it is also related to the extra-cellular effect of Tat, which is a toxic protein secreted early by HIV infected cells.[4] Tat was first identified as a regulatory protein, essential for the HIV viral cycle by its action of dramatically increasing the HIV gene expression.[5] However, the interest to target Tat for AIDS therapy is due to the discovery of the extra-cellular roles of this protein.[6] Tat acts on different cell lines, such as macrophages and CTL, which are essential for the cellular response of the immune system to eliminate virus-infected cells.[7] The extra-cellular roles of Tat are considered to

Keywords: HIV; Inhibitor; Tat protein.
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doi:10.1016/j.bmcl.2003.12.095

be the major reason for the maintenance of HIV infected cells (or reservoir cells), in spite of the use of antiviral therapy that efficiently blocks the HIV cycle.[7] Consequently, tremendous efforts are currently being made to develop HIV vaccines targeting Tat.[7]

Only two attempts to obtain inhibitors binding on Tat have been reported during the last decade. A benzodiazepine derivative (Ro5-3335 and Ro24-7429) was described as a potential Tat inhibitor,[8] but it turned out that this compound was not binding to Tat but to cycline T, a cellular cofactor essential for Tat.[9] The second study described a tetrahydropyridine derivative able to bind to a polyarginine peptide.[10] This polyarginine peptide binds to TAR but there was no evidence that tetrahydropyridine could bind to the basic region of Tat. However, a number of studies describe molecules that bind to TAR and act as Tat competitors. The most interesting is a compound recently discovered called TR87 that inhibits HIV replication at 5 μM.[11] These molecules can inhibit only the Tat–TAR interaction but have no effect on the other Tat functions such as the immunosupressive effects.

This study describes the synthesis of Tat inhibitors that we call TDS. TDS compounds were designed from docking with a Tat NMR structure.[12] A full Tat protein (86 residues) was chemically synthesized with the six glycine Cα labeled with ^{13}C. This synthetic protein had an activity similar to a regular Tat variant. Homonuclear and heteronuclear ^{1}H–^{13}C 2D Nuclear Magnetic Resonance (NMR) spectra enabled the assignment of the 86 spin systems and the determination of 915 NMR distance constraints.[13] The NMR structure shows that the basic region and the cysteine-rich region are well exposed to the solvent while a part of the N-terminal region and the C-terminal region constitute the core of Tat. The two regions adjacent to the basic region are highly folded and play a hinge-like role to allow the basic region to fit into the Tat nucleotide target TAR. Target regions were selected on Tat to design TDS.[11] The rationale was to design allosteric inhibitors to block the structural changes required for Tat functions.[14]

TDS1 turned out to be our first lead compound (Fig. 1). This new family of antivirals is composed of a triphenylene ring with functional groups, such as a succinimide, connected to the triphenylene by an aliphatic chain (Fig. 1). Fluorescence experiments showed that TDS compounds bind in a hydrophobic pocket in Tat and that the succinimide is essential for this binding.[12] Virological tests showed that 30 μM of TDS1 is necessary to inhibit the HIV-1 cytotoxicity with MT4 cells and cytotoxicity in human cells appears at 100 μM.[12] However, TDS1 can inhibit the Tat TAR interaction (Fig. 2A). The small TDS molecule prevents binding of the protein to its 59-bp TAR RNA target, obtained by in vitro transcription.[15] It is interesting to note that the Tat TAR interaction is not inhibited by TDS1 if the complex has already formed (Fig. 2B). To observe the inhibition of the Tat–TAR complex, Tat must be pre-incubated with TDS1 for 30 min before adding TAR. Structural changes are required for Tat to bind to TAR.[16] This experiment suggests that TDS1 inhibits Tat function by blocking Tat structural changes. Moreover, it shows that the TDS1 binding site is preserved in two different Tat variants, one from Europe (Tat Bru) and one from Africa (Tat Mal). These two variants have a similar folding but mutations induce local structural variations.[17] The TDS binding site is not affected by these structural variations.[12]

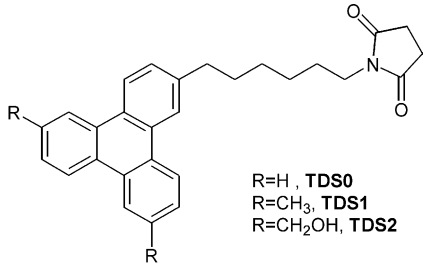


Figure 1. TDS compounds.

Synthesis of the **TDS0** ligand was accomplished in a four-step sequence from the commercially available triphenylene **1** as described in Scheme 1. Triphenylene **1** was treated with glutaric acid monomethyl ester chloride **2**[18] in the presence of an excess of anhydrous aluminum

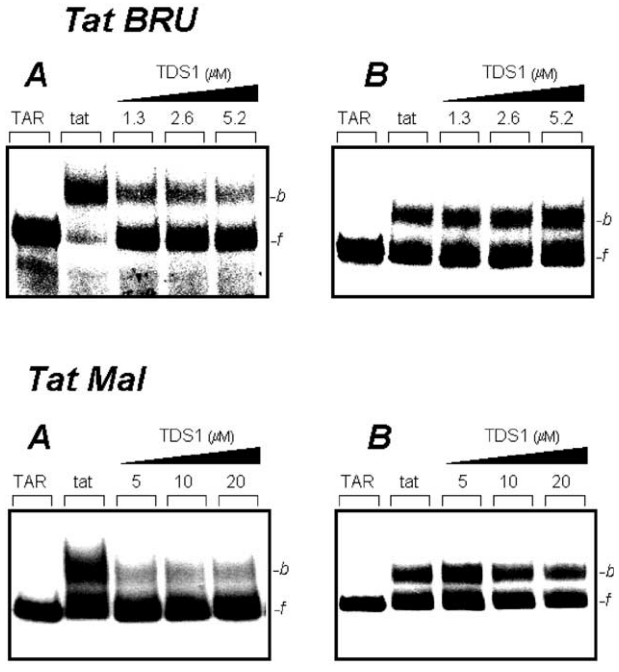
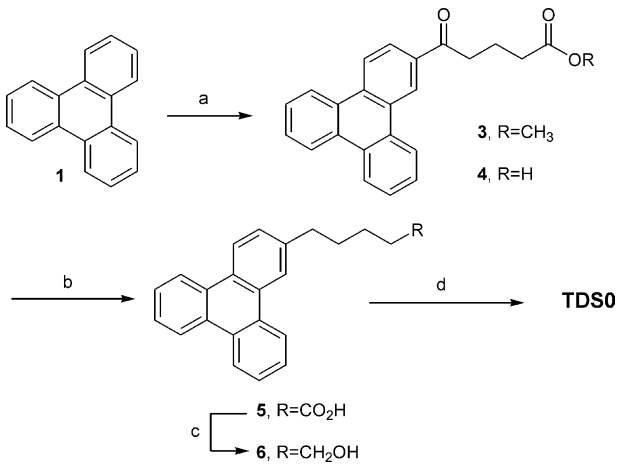


Figure 2. Inhibition of Tat–TAR binding with TDS1. In the panels A, the two Tat variants were incubated with TDS1 for 30 min prior to adding the TAR RNA. In the panels B, the two Tat variants were incubated with TAR RNA prior to adding TDS1. The letters b and f indicate respectively a free Tat RNA and a Tat–TAR complex. The 59 nucleotide TAR RNA containing the essential UUU pyrimidine bulge was prepared by in vitro T3 RNA polymerase transcription. Binding reaction mixtures (20 μL) contained 0.2 nmol of radiolabeled TAR RNA, 0–100 ng of Tat in TK buffer (50 mM Tris pH 7.4, 20 mM KCl, 0.1% Triton X-100). Complexes were separated from unbound RNA by electrophoresis in non-denaturing 8% polyacrylamide gels containing 0.1% Triton X-100. The gels were pre-run for 30 min before loading the sample (25 μL). The electrophoresis was continued for 90 min at about 200 V. The relative amounts of free (f) and/or bound (b) RNA were determined by phosphor imaging.

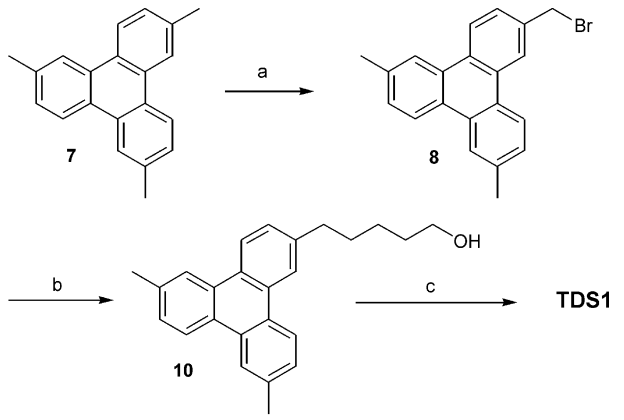


Scheme 1. Reagents and conditions: (a) **2**, $AlCl_3$, CH_2Cl_2, rt, overnight, 65%; (b) Zn dust, $HgCl_2$ (cat.), HCl concd, Toluene/H_2O, reflux, 24 h, 70%; (c) LAH, Et_2O, 0 °C to rt, 3 h, 95%; (d) *N*-succinimide, PPh_3, DEAD, THF, −30 °C to rt, overnight, 95%.

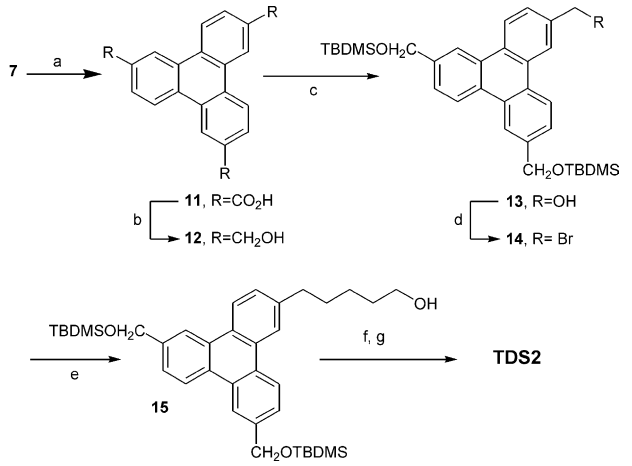
trichloride in dichloromethane to provide the ketoester **3** with 65% yield. In our previous approach, we used glutaric anhydride instead of **2**, but the corresponding ketoacid **4** was isolated with only 25% yield. The ketoester **3** was submitted to the Clemmensen reduction, under typical acidic conditions with zinc dust and catalytic amounts of mercuric chloride, leading to reduction of the ketone with simultaneous hydrolysis of the methyl ester to afford the acid **5** with 70% yield. The acid **5** was reduced with an excess of lithium aluminum hydride (LAH) to give the primary alcohol **6**, which was converted to the first target molecule **TDS0** with 41% yield under Mitsunobu conditions[19] using succinimide in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine (Scheme 1).

TDS1 and **TDS2** were obtained from 2,6,10-trimethyltriphenylene[20] **7** as described in Schemes 2 and 3. Treatment of 2,6,10-trimethyltriphenylene **7** with a slight excess of *N*-bromosuccinimide (NBS) and 10% benzoylperoxide (BPO) as radical initiators in diluted CCl_4 (0.02 M) under reflux led to the corresponding monobromomethyl derivative **8**, which was isolated with 40% yield after purification, with 20% recovery of the starting material **7**. Elaboration of the chain was accomplished using the coupling reaction of the monobromomethyl **8** with an excess (6 equiv) of Grignard reagent **9**,[21] in the presence of a catalytic amount of $LiCuBr_2$, to furnish the desired alcohol **10** with 50% yield. Finally, **TDS1** was synthesized from alcohol **10** using the Mitsunobu procedure previously described for **TDS0** (Scheme 2).

It was anticipated that the last target ligand **TDS2** could be prepared from the triol **12**, which would arise from the reduction of the corresponding triacid **11**. Following a known procedure, 2,6,10-trimethyltriphenylene **7** was oxidized with aqueous $Na_2Cr_2O_7$ at 250 °C in an autoclave,[22] to afford the triacid **11** with 88% yield. After careful purification,[23] the triacid **11** was reduced with an excess of LAH in refluxing THF to the corresponding triol **12** in moderate yield. To complete the preparation



Scheme 2. Reagents and conditions: (a) BPO, AIBN (cat.), CCl_4, reflux, 2–3 h, 40%; (b) **9** MgCl-$(CH_2)_4$-OMgBr, $CuLiBr_2$, THF, −78 °C to rt, overnight; (c) *N*-succinimide, PPh_3, DEAD, THF, rt, overnight, 50%.



Scheme 3. Reagents and conditions: (a) $Na_2Cr_2O_7$, H_2O, autoclave, 250 °C, 18 h, 88%; (b) LAH, THF, reflux, 12 h, 85%; (c) 2 equiv TBDMS–Cl, imidazole, DMF, rt, overnight, 46%; (d) NBS, Me_2S, CH_2Cl_2, −20 °C to 0 °C, 3 h, 89%; (e) **9**, $CuLiBr_2$, THF, −10 °C, 50%; (f) *N*-succinimide, PPh_3, DEAD, THF, −10 °C to rt, 48 h, 80%.

of **TDS2**, triol **12** was treated with 2 equiv of *tert*-butyldimethylsilyl chloride (TBDMSCl) in the presence of imidazole in DMF. Careful purification of the crude mixture by chromatography on a silica gel column resulted in the isolation of the desired disilyl ether **13** with 25% yield, as well as the corresponding monosilyl ether (25%) and trisilyl ether (25%). These were recycled after silyl ether cleavage with tetra-*n*-butylammonium fluoride (TBAF). The hydroxyl group of **13** was cleanly converted to the corresponding bromide derivative **14** with a good yield using NBS and dimethylsulfide. Using the coupling reaction previously described for **TDS1**, alcohol **15** was isolated in 55% yield from the bromide **14**. **TDS2** was finally obtained from **15**, after treatment under Mitsunobu conditions with succinimide and removal of both TBDMS protecting groups under standard TBAF cleavage conditions (Scheme 3).

The application of this strategy to prepare analogues with various chain lengths or containing aromatic systems is currently being explored to improve the biological activity of these ligands.

In conclusion, the great hope that drug design or CADD could replace random tests in drug discovery has not yet been fulfilled due to the flexibility of proteins. This study suggests that the discovery of ligands could be improved by the design of chemical functional groups (or probes) not rigidified to a central structure (or scaffold). The rationale is that different possible orientations of a probe could compensate for the structural variations of proteins that are due to flexibility or mutations. We hope that this approach will contribute to improving CADD by taking into account the dynamic behavior of a receptor and local structural changes induced by mutations. Tat was certainly the best way to illustrate the potential of our approach, due to its high flexibility.

Acknowledgements

The authors are deeply thankful to the American Scientific Committee, represented by Drs. David Ho, Michael Saag and John Bartlett, for having selected the TDS project for the GlaxoSmithKline (GSK) Drug Discovery and Development Award. E.L.P. and J.L. thank ANVAR (Agence Nationale de la Valorisation de la Recherche), ANRS (Agence Nationale de la Recherche sur le SIDA) and SIDACTION for their financial support. In addition, the authors are deeply indebted to the CNRS (Centre National de la Recherche Scientifique) for its constant support of the TDS project. We also would like to thank Dr. Harald Bock (UPR-CNRS 864, Pessac, France) for experimental details of the compound **7** procedure.

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