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Antiviral activity of UIC-PI, a novel inhibitor of the human immunodeficiency virus type 1 protease

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Abstract

The human immunodeficiency virus type 1 (HIV-1) protease inhibitor UIC-PI (**1**) was developed via structure-based design and incorporated a novel bis-tetrahydrofuran (bis-THF) ligand in the (R)-(hydroxyethyl)sulfonamide based isostere. The EC_{50} and EC_{90} of the compound in acutely-infected H9 cells were <1 and ~ 1 nM, respectively. In chronically infected H9/HIV-1_{IIIB} cells, the EC_{50} and EC_{90} were 20 and 50 nM, respectively. In parallel studies comparing UIC-PI and saquinavir in H9/HIV-1_{IIIB} cells, viral p24 levels in culture supernatants were an order of magnitude lower with UIC-PI than with saquinavir.

Keywords: Human immunodeficiency virus; Protease inhibitor; bis-Tetrahydrofuran; Structure-based design

The etiological agent for acquired immunodeficiency syndrome, considered one of the most formidable challenges in medicine in the 20th century, is the human immunodeficiency virus (HIV) (Barré-Sinoussi et al., 1983; Gallo et al., 1984). Since the discovery that a virally encoded protease is responsible for proteolytic processing of the Gag and Gag–Pol polyproteins to form mature virion proteins, inhibition of this enzyme has become an important target for AIDS chemotherapy (Kramer et al., 1986; Kohl et al., 1988). Indeed, a number of peptidomimetic protease inhibitors in combination with reverse transcriptase inhibitors have been approved by the Food and Drug Administration for the treatment of AIDS (Roberts et al., 1990; Vacca et al., 1994; Kempf et al., 1995; McDonald and Kuritzkes, 1997; Flexner, 1998; Lin, 1997). These treatment regimens have reduced plasma viral burdens, elevated CD4 cell counts and arrested progression of AIDS for many HIV infected individuals (Stein et al., 1996; Schapiro et al., 1996). While the new agents have shown promising indications, poor oral bioavailability, emergence of drug resistance and costly synthesis are still the major limitations.

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As part of our continuing effort on the structure-based design of nonpeptidyl inhibitors, we have developed a number of potent and selective inhibitors incorporating stereochemically defined and conformationally constrained cyclic ether and cyclic sulfone functionalities in the HIV protease substrate binding site (Ghosh et al., 1993b,c, 1994a,b, 1995). The designed ligands effectively replace peptide bonds and mimic the biological mode of action. As shown in Fig. 1, incorporation of a stereochemically defined bis-tetrahydrofuran (bis-THF) ring in the (R)-(hydroxyethyl) sulfonamide based isostere afforded the novel UIC-protease inhibitor (UIC-PI, recently called 'TMC-126') (**1**; Fig. 1) with very potent enzyme inhibitory activity (Ghosh et al., 1998a). Here we examined the effects of UIC-PI (**1**) in acutely- and chronically-infected cell lines, and compared them with those of Ro 31-8959 (saquinavir) (**2**). Our findings have been presented earlier in preliminary form (Pretzer et al., 1996; Ghosh et al., 1999).

The synthesis of the protease inhibitor UIC-PI (**1**) is depicted in Fig. 2. H9 cells were obtained from Dr Robert Gallo through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH, cultured in RPMI-1640 medium (Irvine Scientific, Santa Ana, CA) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (RPMI/10), and split 1:6 every 3–4 days. Chronically infected H9/HIV-1_{IIIB} cells were kindly provided by T. El-Beik and J. McGrath (San Francisco General Hospital), and were cultured as described above for H9 cells. HIV-1_{IIIB} was obtained from culture supernatants of acutely infected SupT-1 cells (p24 = 1450 ng/ml; $TCID_{50}$ on Sup-T1 cells = 1×10^4 per ml). H9 cells were infected with HIV-1 at 10 ng viral p24/10^6 cells (MOI = 6.9×10^{-5}) for 2 h, then washed three times in medium and plated at a density of 5×10^5 per ml in RPMI/10 with or without treatments. Every 3–4 days 75–80% of the medium was replaced with fresh medium with or without treatments. H9/HIV-1_{IIIB} cells were washed and plated at 1×10^5 cells per well with or without treatments, then cultured the same as uninfected H9 cells, with replacement of treatments every 3–4 days. Infection was monitored by ELISA determination of p24 antigen in culture supernatants (Konopka et al., 1990; Pretzer et al., 1997). The protease inhibitors **1** and **2** were dissolved and serially diluted in DMSO. The final dilution into the medium was such that the DMSO concentration in all the inhibitor-treated wells and a DMSO control was 0.2%. For 50% tissue culture infectious dose ($TCID_{50}$) determinations, supernatants were collected from experimental wells on the 10th day after the beginning of treatment and stored at −80 °C. Prior to testing they were thawed and clarified by centrifugation and filtration through a 0.45 μm filter. $TCID_{50}$ was determined according to Johnson and Byington (Johnson and Byington, 1990). Supernatants to be tested were serially diluted (1:4) in medium from column to column of 96-well plates, and H9 cells were added to a final dilution of 2×10^4 per well. Cells were split 1:2 every few days as needed. Plates were observed for the presence of syncytia and scored when the development of syncytia in new wells ceased.

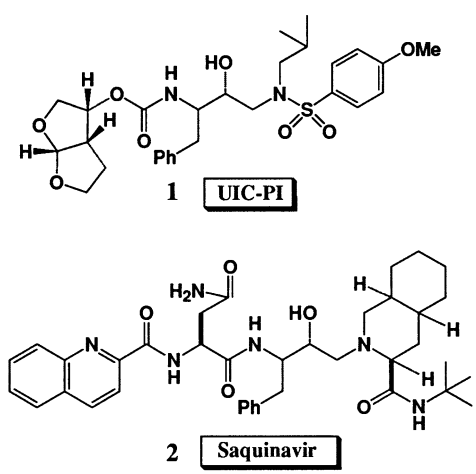

Fig. 1. Molecular structures of UIC-PI and Saquinavir.

Continuous treatment of chronically infected H9/HIV-1_{IIIB} cells with UIC-PI resulted in a dose-responsive inhibition of viral p24 production between 10 and 50 nM (Fig. 3A). The EC_{50} was determined to be 20 nM on day 7 and 30 nM on day 10. In two separate experiments, 50 nM UIC-PI reduced p24 levels by approximately 90% on days 7 through 11. $TCID_{50}$ determinations on

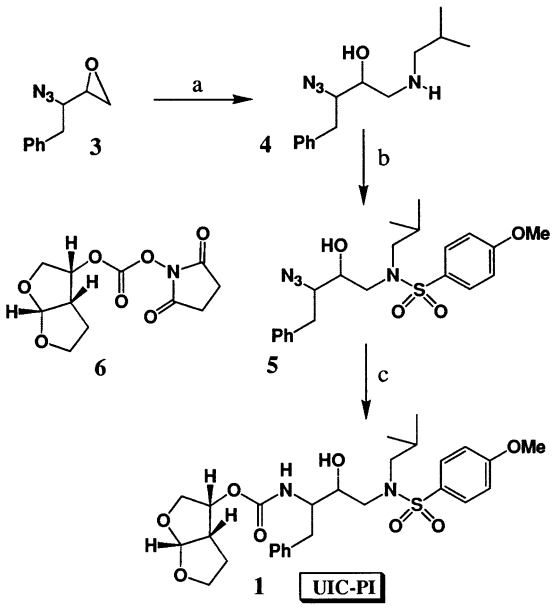

Fig. 2.

supernatants obtained from cultures 10 days post-treatment showed a dose-dependent decrease (Fig. 3B). Although, 1 nM UIC-PI did not cause any reduction in p24 levels, it reduced the $TCID_{50}$ by 50%. This observation suggests that although, viral particles were being released at 1 nM inhibitor at a level similar to controls, the infectivity of the virions were diminished, possibly due to the release of immature virions. UIC-PI was not cytotoxic to H9 cells in the concentration range studied (1–100 nM), as determined by a modified Alamar blue assay (Konopka et al., 1996).

Virus production by acutely infected H9 cells was more susceptible to the inhibitory effect of UIC-PI than that by chronically infected cells; UIC-PI had an EC_{50} of 0.7 nM and an EC_{90} of ~ 1 nM. The more pronounced effect of UIC-PI on HIV-1 production by acutely infected cells compared with chronically infected cells may be due to the lower level of virus production in the former.

The antiviral effects of UIC-PI were compared with that of Ro 31-8959 (saquinavir) synthesized in our laboratory (Fig. 4). In chronically infected H9/HIV-1_{IIIB} cells, 10 nM UIC-PI was as effective

Fig. 2. Synthesis of UIC-PI reagents and conditions: (a) $Me_2CHCH_2NH_2$, 2-propanol, 84 °C, 12 h; (b) *p*-MeO-$C_6H_4SO_2Cl$, aq. $NaHCO_3$, CH_2C_{12}; (c) **6**, Et_3N, H_2, 10%Pd-C, THF. Synthesis of UIC-PI. Reaction of azido epoxide **3** with isobutylamine in 2-propanol at 84 °C for 12 h provided the amine derivative **4**. Reaction of amine **4** with *p*-methoxybenzene-sulfonyl chloride in methylene chloride in the presence of aqueous $NaHCO_3$ provided the corresponding sulfonamide derivative **5** (Ghosh et al., 1998b; Kim et al., 1995). Catalytic hydrogenation of **5** over 10% Pd–C in ethyl acetate furnished the corresponding amine which was reacted with the mixed carbonate **6** in methylene chloride in the presence of triethylamine at 23 °C for 12 h to provide the inhibitor **1** (Ghosh et al., 1996). Saquinavir (**2**) was prepared as described previously (Ghosh et al., 1993a). To a stirred solution of **3** (546 mg, 2.88 mmol) in isopropanol (7 ml) was added isobutylamine (426 mg, 5.76 mmol), and the resulting mixture was heated at 84 °C for 12 h. After this period, the reaction mixture was evaporated under reduced pressure and the crude product was dried under vacuum. The residue was chromatographed over silica gel (50% ethyl acetate/hexane) to furnish azido alcohol **4** (670 mg). To a stirred solution of **4** in CH_2Cl_2 (7 ml) were added *p*-methoxybenzenesulfonyl chloride (593 mg, 2.88 mmol) followed by 10% aqueous $NaHCO_3$ solution (7 ml). The resulting mixture was stirred at 23 °C for 12 h. The layers were separated and the organic layer was dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure afforded a residue which was chromatographed over silica gel (25% ethyl acetate/hexane) to obtain azido sulfonamide **5** (1.1 g, 89%) as an oil. To a stirred solution of [3*R*, 3*aS*, 6*aR*]-3-hydroxyhexahydrofuro[2,3-*b*]furan (650 mg, 5 mmol; prepared according to the procedure of Ghosh and Chen (1995) in dry CH_3CN (50 ml) at 23 °C were added disuccinimidyl carbonate (1.92 g, 7.5 mmol) and triethylamine (2.5 ml). The resulting mixture was stirred at 23 °C for 12 h. The reaction was quenched with saturated aqueous $NaHCO_3$ (20 ml) and the mixture was concentrated under reduced pressure. The residue was extracted with CH_2Cl_2 (2×50 ml) and the combined organic layers were washed with brine (10 ml) and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure gave a residue which was chromatographed over silica gel (50% ethyl acetate/hexane) to furnish (3*R*, 3*aS*, 6*aR*) 3-hydroxyhexahydrofuro[2,3-*b*]furanyl-succinimidyl carbonate **6** (840 mg) as a white solid (m.p. 129–131 °C). To a stirred solution of azide **5** (1 g, 2.31 mmol), succinimidyl carbonate **6** (626 mg, 2.31 mmol) and triethylamine (0.5 ml) in tetrahydrofuran (15 ml) at 23 °C was added 10% palladium on carbon catalyst (200 mg). The resulting mixture was hydrogenated under a balloon filled with hydrogen for 12 h. The catalyst was removed by filtration over celite and the filtrate was concentrated under reduced pressure to give a residue. The above residue was purified by silica gel chromatography (75% ethyl acetate/hexane) to furnish the inhibitor **1** (934 mg, 72%) as a white solid (m.p. 65–67 °C). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.71 (d, 2H, $J = 8.5$ Hz), 7.29–7.20 (m, 5H), 6.98 (d, 2H, $J = 7.0$ Hz), 5.65 (d, 1H, $J = 5.2$ Hz), 5.01 (m, 2H), 3.95–3.82 (m, 7H), 3.69 (m, 2H), 3.0–2.7 (m, 6H), 1.85 (m, 1H), 1.64–1.45 (m, 3H), 0.92 (d, 3H, $J = 6.5$Hz), 0.89 (d, 3 H, $J = 6.6$ Hz); ^{13}NMR ($CDCl_3$, 100 MHz): δ 163.01, 155.37, 137.54, 129.57, 129.39, 129.27, 128.45, 126.48, 114.29, 109.20, 73.31, 72.75, 70.7, 69.52, 58.76, 55.57, 55.03, 53.64, 45.26, 35.54, 27.19, 25.72, 20.06, 19.79.

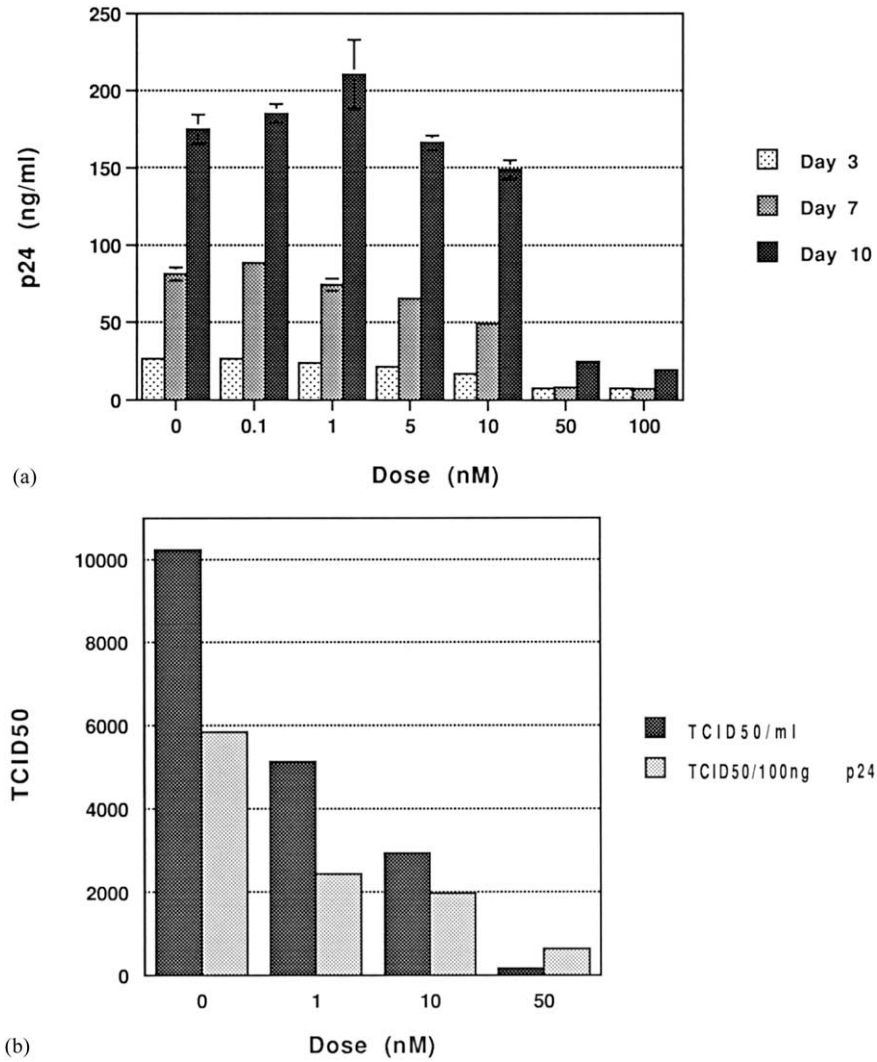

Fig. 3. Inhibition of HIV-1 production in chronically infected H9/HIV-1_{IIIB} cells by continuous treatment with UIC-PI. (A) Viral p24 levels measured on days 3, 7 and 10 after the initiation of treatment. The vertical lines indicate the standard deviation. (B) Tissues culture infectious dose, 50% end-point, ($TCID_{50}$) of day 10 supernatants of H9/HIV-1_{IIIB} cells treated with UIC-PI.

as 50 nM Ro 31-8959. Viral p24 levels in culture supernatants were an order of magnitude lower with UIC-PI than with saquinavir. While 50 nM UIC-PI almost completely inhibited HIV-1 production over the course of the experiment, 50 nM Ro 31-8959 allowed considerable virus release into the culture medium. In acutely infected H9 cells, both inhibitors were similarly effective in inhibiting completely virus production at 10 and 50 nM (data not shown). At 1 nM, however, essentially no viral p24 was observed with UIC-PI, while Ro 31-8959 permitted considerable virus production (p24 levels about 50% of untreated controls). The inhibitory effect of UIC-PI is probably further amplified by the reduction in the spread of infection in the culture.

The inhibitor UIC-PI evolved from structure-based design strategies. It contains a stereochemically defined bis-THF ligand as the P2-ligand and an (R)-(hydroxyethyl)sulfonamide isostere containing p-methoxybenzenesulfonamide functionality as the P2′-ligand. It displayed an enzyme

Ki = 1.1 ± 0.4 nM ($n = 4$), which is comparable to that of saquinavir (Ki = 1.4 ± 0.2 nM, $n = 3$). Encouraged by this enzyme inhibitory activity, we investigated the effect of UIC-PI in chronically infected H9/HIV-1_{IIIB} cells and acutely infected H9 cells. The data in Fig. 4 indicate that UIC-PI has an EC_{50} value of approximately 20 nM in chronically infected cells. In parallel experiments, saquinavir displayed an EC_{50} value of about 50 nM. For comparison, Perno et al. (1998) have reported that in chronically infected H9/LAI cells, for which the EC_{50} of saquinavir was 48 ± 1 nM (in close agreement with our results with H9/HIV-1_{IIIB} cells), the EC_{50} values for ritonavir and KNI-272 were 945 ± 435 and 442 ± 8 nM, respectively. In CEM-SS cells chronically infected with HIV-1_{IIIB}, the EC_{50} for the protease inhibitor AG1343 (nelfinavir) was 39 nM (Patick et al., 1996). The EC_{50} of amprenavir (VX-478) was 410 ± 80 nM for chronically infected H9IIIB cells (St.Clair et al., 1996). Thus, UIP-PI appears to be considerably more effective against HIV-1 than ritonavir and amprenavir in chronically infected cells.

In acutely infected H9 cells, although both inhibitors were equally effective at higher concentrations, UIC-PI was highly inhibitory even at 1 nM, while saquinavir was essentially ineffective at this concentration. In acutely infected MT-2 cells, the EC_{50} values for saquinavir, KNI-272 and ritonavir were reported to be 21 ± 1, 59 ± 3 and 63 ± 2 nM, respectively (Perno et al., 1998). The first study on ritonavir (ABT-538) reported EC_{50} values of between 22 and 130 nM for various HIV-1 strains (Kempf et al., 1995). For acute infection of various cells with a number of HIV-1 strains, the EC_{50}s for AG1343 were between 10 and 60 nM (Patick et al., 1996). In acutely infected peripheral blood lymphocytes, the mean

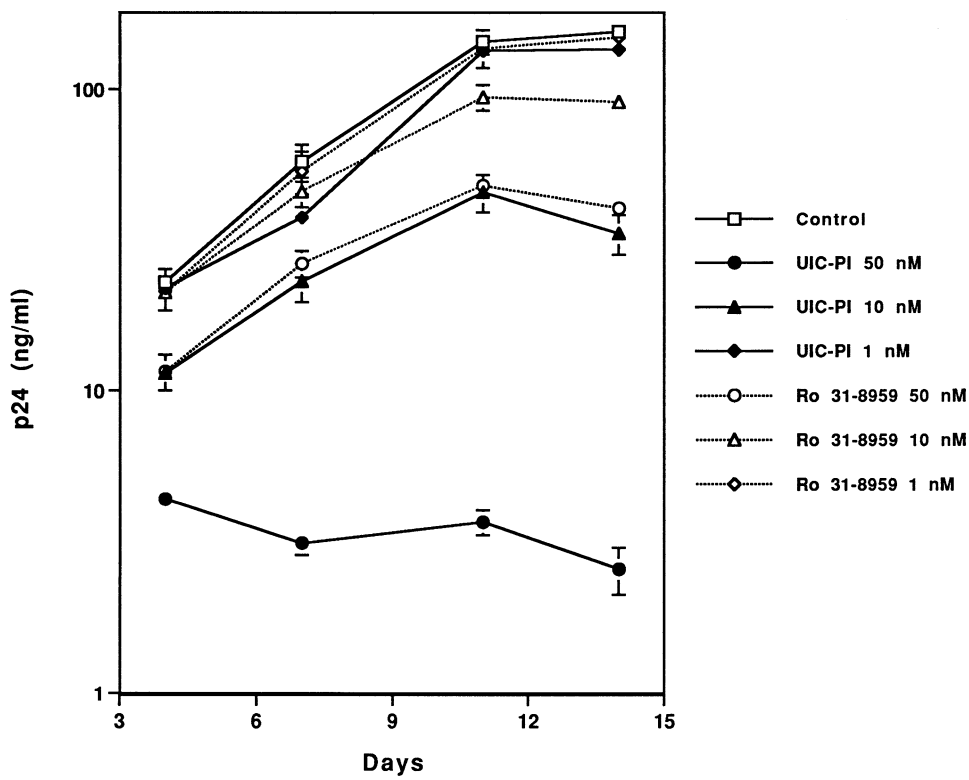

Fig. 4. Comparison of the effects of UIC-PI and Ro 31-8959 (Saquinavir) in chronically infected H9/HIV-1_{IIIB} cells. Cells were treated continuously with the indicated doses, and viral p24 levels in culture supernatants were measured on days 4, 7, 11 and 14 following the initiation of treatment. The vertical lines indicate the standard deviation.

EC_{50} for amprenavir against AZT-sensitive isolates was 12 nM, while that against AZT-resistant isolates was 19 nM (St.Clair et al., 1996). These data indicate that UIC-PI may be more effective against HIV-1 than KNI-272, ritonavir, AG1343 and amprenavir.

The origin of the antiviral properties of UIC-PI may be related to specific ligand-binding site interactions in the active site (Ghosh et al., 1998a). Based upon an energy-minimized model of UIC-PI in the HIV-1 protease active site, it appeared that both oxygen atoms of the bis-THF ligand are within hydrogen bonding distance to ASP 29 and Asp 30 residues. In addition, the 4-methoxyl oxygen on the benzenesulfonamide group is within hydrogen-bonding distance to ASP 29′ and Asp 30′ NH, and this may account for the strong antiviral activity of UIC-PI. Thus, incorporation of a stereochemically defined bis-THF ligand in the (R)-(hydroxyethyl)sulfonamide isostere leads to a novel inhibitor with potent antiviral properties.

These antiviral properties of UIC-PI may positively affect the drug-resistance profile of the inhibitor. Thus, it will be important to examine the activity of UIC-PI against strains of HIV-1 that are resistant to currently available protease inhibitors (De Clercq, 1995; Flexner, 1998; Boden and Markowitz, 1998), as well as, whether it is a substrate to the MDR1 multidrug transporter (Pgp) (Lee and Gottesman, 1998; Kim et al., 1998). Other aspects of UIC-PI that need further exploration are its antiviral activity in the presence of potentially inhibitory serum components such as α_1-acid glycoprotein (Kageyama et al., 1994; Zhang et al., 1999) or albumin (Koeplinger and Zhao, 1996), as well as its oral bioavailability and pharmacokinetics (Lin, 1997). In the future, it may be possible to optimize the structure of UIC-PI to enhance its antiviral and pharmacokinetic properties. It will also be of interest to ascertain whether liposome-mediated delivery of UIC-PI to HIV-1-infected macrophages will enhance its antiviral effect, as we have observed previously in the case of a first-generation protease inhibitor (Pretzer et al., 1997).

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