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Synthesis and Anti-HIV Evaluation of the Novel 2-(*m*-Chlorobenzyl)-4-substituted-7-methyl-1, 1, 3-trioxo-pyrazolo[4, 5-e][1, 2, 4]thiadiazines

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A novel series of 2-(*m*-Chlorobenzyl)-4-substituted-1, 1, 3-trioxo-2*H*, 4*H*-pyrazolo[4, 5-*e*][1, 2, 4] thiadiazines (**7a-k**) were synthesized, and evaluated for their anti-HIV replication in MT-4 cell cultures. Compound (**7a**) showed activity against HIV-1-induced cytopathicity, with an EC_{50} value of 45.6 μM, but none of the compounds exhibited inhibitory activity against HIV-2.

Key words: Synthesis, Pyrazolothiadiazines, HIV-1, NNRTIs, MT-4 cells

INTRODUCTION

Over the past decades, a number of HIV-1 reverse transcriptase (RT) inhibitors have been discovered and introduced to clinical practice. They are divided into two groups: nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). In general, the NNRTIs exhibit higher safety and selectivity than their nucleoside counterparts, leading researchers to study more deeply into these fields (De Clercq *et al.*, 2005). NNRTIs are structurally diverse compounds, with the same inhibition mechanism against HIV-1 replication, a specific allosteric effect arising from the non-competitive binding to a site adjacent to that of the deoxyribonucleoside triphosphate of the enzyme (Joncckheere *et al.*, 2000). However, the therapeutic effectiveness of NNRTIs is limited by the relatively rapid emergence of drug-resistant HIV-1 strains (Das *et al.*, 2005). Therefore, the development of novel, selective, potent and safe NNRTIs remains a high priority in medicinal chemistry research.

In recent studies aimed at the discovery of new NNRTIs, Dr. S. Vega and his colleagues reported that a series of 2, 4-disubstituted-1, 1, 3-trioxo-2*H*, 4*H*-thieno[3, 4-*e*][1, 2, 4] thiadiazines (TTDs), as HIV NNRTIs, were highly effective in the inhibition of the cytopathic effect of HIV-1 and HIV-2 in human T-lymphocyte cells (Arranz *et al.*, 1997; Arranz *et al.*, 1998). Among the lead compounds, the representatives, **QM96521, QM96539**, and **QM96652** (Fig. 1), showed highly potent activity and selectivity. A structure-activity relationship analysis disclosed that double substitutions at the N_2 and N_4 sites of the thieno[3, 4-*e*][1, 2, 4]thiadiazine ring were necessary to maintain the antiviral activity; benzyl or 3-halogen benzyl at the N_2 position and substituents containing π-electrons in the N_4 side chain were also essential for the preservation of the anti-HIV activity.

As a continuation of the study on the structure-activity relationships of TTDs, a new series of 2-(*m*-chlorobenzyl)-4-substituted-1, 1, 3-trioxo-2*H*, 4*H*-pyrazolo[4, 5-*e*][1, 2, 4] thiadiazines (PTDs) were designed and synthesized based on the general principle of bioisosteric replacement in medicinal chemistry. In the PTDs analogues, the active substituents at the N_2 and N_4 positions were preserved unchangeable, and the pyrazole ring was substituted for the thiophene moiety in the TTDs scaffold, due to the known thiophene-pyrazole bioisosterism (Patani and LaVoie, 1996). Herein, a new approach for the synthesis of novel PTDs, as well as the evaluation of their inhibitory effects on HIV replication, is reported.

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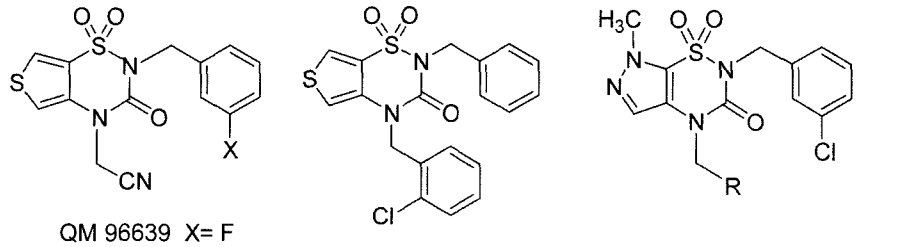


Fig. 1. Lead compounds of the TTDs and the newly designed 2, 4-disubstituted-7-methylpyrazolo[4, 5-*e*] [1, 2, 4]thiadiazines (PTDs)

MATERIALS AND METHODS

Chemistry

All melting points were determined on a micromelting point apparatus, and are reported uncorrected. ^{1}H-NMR spectra were obtained on a Brucker Avance-600 (600 MHz) in the indicated solvent. Chemical shifts are expressed in δ units, with TMS as the internal reference. Infrared spectra (IR) were recorded with a Nexus 470FT-IR Spectrometer. Mass spectra were taken on a LC Autosampler Device: Standard G1313A instrument. Flash column chromatography was performed on column packed with silica gel 60 (230-400 mesh). Solvents were of reagent grade and were purified and dried by the standard methods when required. Concentration of the reaction solutions involved the use of a rotary evaporator at reduced pressure.

1-Methyl-5-sulfamoylpyrazole-4-carbohydrazide (2)

A solution mixture of 1-methyl-5-amino-pyrazoloformate **1** (5.1 g, 22 mmol) and hydrazine hydrate (3.8 g, 66 mmol, 3eq), in 25mL ethanol, was refluxed for 10 h. When the reaction was completed, the solvent was evaporated under reduced pressure. The residue was purified by recrystallization from ethanol and white crystals obtained, yield: 2.9 g (61.7%), mp: 166-168°C. IR (KBr, cm^{-1}): 3376-3194 (NH), 3097 (Py-CH), 2960 (CH_3), 1627 (C=O), 1360, 1177 (SO_2); ^{1}H-NMR (DMSO-d_6) δ: 9.76 (s, 1H, CONHNH$_2$), 8.02 (s, 2H, SO_2NH_2), 7.89 (s, 1H, Pyr-H, CH), 4.55 (s, 2H, CONHNH$_2$), 4.05 (s, 3H, CH_3); MS (EI): m/z 220.1(M +1).

1-Methyl-5-sulfamoylpyrazole-4-carboxy azide (3)

To a solution of compound **2** (2.19 g, 10 mmol) in 50 mL 2M hydrochloric acid, a solution of sodium nitrite (98%, 0.85 mg, 1.2 mmol) in 2 mL of water was added dropwise at 10°C. The solution mixture was stirred at this temperature for 2 h, and the precipitate was filtered off, washed thoroughly with cold water and dried under vacuum to yield a white solid (2.3 g, 78.3%). The obtained compounds were pure enough to be used in the following step. IR (KBr, cm^{-1}): 2149, 1216(N_3).

7-Methyl-1, 1, 3-trioxo-2*H*, 4*H*-pyrazolo[4, 5-*e*][1, 2, 4] thiadiazine (5)

A suspended solution of **3** (8.8 g, 40 mmol) in dry toluene (100 mL) was refluxed for 7 h (keep from moisture). The precipitate was filtered and recrystallized from ethanol to give a white solid 6.78 g (84%), mp: 216°C (dec). IR (KBr, cm^{-1}): 3244, 3152 (NH), 3014 (Pyr-CH), 1692 (C=O), 1342, 1141 (SO_2); ^{1}H-NMR (DMSO-d_6) δ: 11.49 (s, 1H, exchanged with deuterium by D_2O addition, NH), 7.40 (s, 1H, Pyr-CH), 3.94 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6) δ: 152.4 (C=O), 125.3 (C-5), 124.3 (C-4a), 123.4 (C-7a), 38.3 (CH_3); MS (EI): m/z 202.2 (M^+). Anal.Calcd for $C_5H_6N_4O_3S$: C, 29.70; H, 2.99; N, 27.71. Found: C, 29.76; H, 3.03; N, 27.66.

2-(*m*-Chlorobenzyl)-7-methyl-1, 1, 3-trioxo-2*H*, 4*H*-pyrazolo[4, 5-*e*][1, 2, 4]thiadiazine (6)

To a solution of compound **5** (1.0 g, 5 mmol, 1eq) in dry DMF (20 mL), sodium hydride (60% dispersion in mineral oil, 0.2 g, 5 mmol, 1eq) was added in portions, under inert atmosphere (N_2), with the temperature maintained below 10°C. After 15 minutes of stirring, 3-chlorobenzyl chloride (0.85 g, 5 mmol, 1eq) was added dropwise to the mixture, which was stirred at room temperature for 20 min and at 50°C for a further 20 h (checked by TLC). After evaporation of the solvent under reduced pressure, the crude product was separated by flash column chromatography (ethyl acetate/cyclohexane 1:3), and further purified by recrystallization to give a white solid 0.71 g (43.4%), mp: 187-188°C. IR (KBr, cm^{-1}): 3223 (NH), 1678 (C=O), 1334, 1181 (SO_2); ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 11.39(s, 1H, exchanged with deuterium by D_2O addition, NH), 7.45 (s, 1H, PyH), 7.31-7.40 (m, 4H, PhH), 4.97 (s, 2H, NCH_2), 4.03 (s, 3H, CH_3); MS (EI): m/z 327.3 (M+1).

General Procedure for the Preparation of 2-(*m*-Chlorobenzyl)-4-Substituted-7-methyl-1, 1, 3-trioxo-2*H*, 4*H*-pyrazolo[4, 5-*e*][1, 2, 4]thiadiazine Derivatives (7a-k)

To a solution of compound **6** (1.6 g, 5 mmol, 1eq) in dry DMF (20 mL), sodium hydride (60% dispersion in mineral oil, 0.2 g, 5 mmol, 2eq) was added in portions, under an inert atmosphere (N_2), with the temperature maintained

below 10°C. After 30 min of stirring, the alkyl halide (RX, 5 mmol, 1eq) was added dropwise to the mixture. The mixture solution was stirred at room temperature for 20 min and at 30-60°C for a further 12-20 h (checked by TLC). After evaporation of the solvent under reduced pressure, the crude product was purified by recrystallization from ethanol.

2-(*m*-Chlorobenzyl)-4-cyanolmethyl-7-methyl-1, 1, 3-trioxo-2*H*, 4*H*-pyrazolo[4, 5-*e*][1, 2, 4]thiadiazine (7a)
A mixture of compound **6** and chloroacetonitrile was heated at 40-50°C for 20 h, and then purified by recrystallization from ethanol to give compound **7a** as a brown solid (55%); mp: 124-126°C. IR (KBr, cm^{-1}): 1700(C=O), 1345, 1190 (SO_2); ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 8.01 (s, 1H, PyH), 7.45 (s, 1H, PhH), 7.34-7.39 (m, 3H, PhH), 5.09 (s, 2H, NCH_2), 5.04 (s, 2H, NCH_2), 4.09 (s, 3H, CH_3). MS (EI): m/z 366.3(M+1).

2-(*m*-Chlorobenzyl)-4-benzyl-7-methyl-1, 1, 3-trioxo-2*H*, 4*H*-pyrazolo[4, 5-*e*][1, 2, 4]thiadiazine (7b)
A mixture of compound **6** and benzyl bromide was heated at 30-40°C for 12 h, and then purified by recrystallization from ethanol to give compound **7b** as a white solid (66%); mp: 140-142°C. IR (KBr, cm^{-1}): 1669 (C=O), 1330, 1193 (SO_2); ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 7.82 (s, 1H, PyH), 7.25-7.41 (m, 9H, PhH), 5.11 (s, 2H, NCH_2), 5.06 (s, 2H, NCH_2), 4.06 (s, 3H, CH_3); MS (EI): m/z 417.5 (M+1).

2-(*m*-Chlorobenzyl)-4-(*o*-bromobenzyl)-7-methyl-1, 1, 3-trioxo-2*H*, 4*H*-pyrazolo[4, 5-*e*][1, 2, 4]thiadiazine (7c)
A mixture of compound **6** and 2-bromobenzyl bromide was heated at 30-40°C for 12 h, and then purified by recrystallization from ethanol to give compound **7c** as a white solid (67%); mp: 124-126°C. IR (KBr, cm^{-1}): 1695 (C=O); 1330, 1192 (SO_2); ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 7.75 (s, 1H, PyH), 7.67 (dd, 1H, *J*=7.86 Hz, *J*=1.01 Hz, PhH), 6.95 (d, 1H, *J*=7.48 Hz, PhH), 7.24-7.40 (m, 6H, PhH), 5.11 (s, 2H, NCH_2), 5.04 (s, 2H, NCH_2), 4.09 (s, 3H, CH_3); MS: m/z 495.2 (M^+).

2-(*m*-Chlorobenzyl)-4-(*p*-bromobenzyl)-7-methyl-1, 1, 3-trioxo-2*H*, 4*H*-pyrazolo[4, 5-*e*][1, 2, 4]thiadiazine (7d)
A mixture of compound **6** and 4-bromobenzyl bromide was heated at 30-40°C for 12 h, and then purified by recrystallization from ethanol to give compound **7d** as a white solid (68%); mp: 84-86°C. IR (KBr, cm^{-1}): 1668 (C=O), 1339, 1194 (SO_2); ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 7.86 (s, 1H, PyH), 7.53 (d, 2H, *J*=7.85 Hz, PhH), 7.32 (d, 1H, *J*=7.17 Hz, PhH), 7.23 (d, 2H, *J*=8.20 Hz, PhH), 7.36-7.40 (m, 3H, PhH), 5.07 (s, 2H, NCH_2), 5.05 (s, 2H, NCH_2), 4.06 (s, 3H, CH_3); MS (EI): m/z 495.3(M^+).

2-(*m*-Chlorobenzyl)-4-(*o*-fluorobenzyl)-7-methyl-1, 1, 3-trioxo-2*H*, 4*H*-pyrazolo[4, 5-*e*][1, 2, 4]thiadiazine (7e)
A mixture of compound **6** and 2-fluorobenzyl chloride was heated at 40-50°C for 20 h, and then purified by recrystallization from ethanol to give compound **7e** as a white solid (64%); mp: 100-102°C. IR (KBr, cm^{-1}): 1679 (C=O), 1328, 1190 (SO_2); ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 7.80 (s, 1H, PyH), 7.14-7.40 (m, 8H, PhH), 5.17 (s, 2H, NCH_2), 5.04 (s, 2H, N-CH_2), 4.07(s, 3H, CH_3). MS(EI): m/z 435.5 (M+1).

2-(*m*-Chlorobenzyl)-4-(*p*-fluorobenzyl)-7-methyl-1, 1, 3-trioxo-2*H*, 4*H*-pyrazolo[4, 5-*e*][1, 2, 4]thiadiazine (7f)
A mixture of compound **6** and 4-fluorobenzyl chloride was heated at 40-50°C for 20 h, and then purified by recrystallization from ethanol to give compound **7f** as a white solid (63%); mp: 122-124°C. IR (KBr, cm^{-1}): 1669 (C=O), 1332, 1194 (SO_2); ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 7.87 (s, 1H, PyH), 7.14-7.41 (m, 8H, PhH), 5.09 (s, 2H, NCH_2), 5.05 (s, 2H, NCH_2), 4.05 (s, 3H, CH_3); MS (EI): m/z 435.5 (M+1).

2-(*m*-Chlorobenzyl)-4-(o-chlorobenzyl)-7-methyl-1, 1, 3-trioxo-2*H*, 4*H*-pyrazolo[4, 5-*e*][1, 2, 4]thiadiazine (7g)
A mixture of compound **6** and 2-chlorobenzyl chloride was heated at 40-50°C for 20 h, and then purified by recrystallization from ethanol to give compound **7g** as a white solid (60%); mp: 116-118°C. IR (KBr, cm^{-1}): 1695 (C=O), 1330, 1192 (SO_2); ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 7.15 (s, 1H, PyH), 7.49 (s, 1H, PhH), 7.41 (dd, 1H, *J*=7.97 Hz, *J*=1.03 Hz, PhH), 6.96 (dd, 1H, *J*=7.69 Hz, *J*=0.88 Hz, PhH), 7.19-7.39 (m, 5H, PhH), 5.20 (s, 2H, NCH_2), 5.09 (s, 2H, NCH_2), 4.15 (s, 3H, CH_3); MS (EI): m/z 451.4(M^+).

2, 4-Di(*m*-chlorobenzyl)-7-methyl-1, 1, 3-trioxo-2*H*, 4*H*-pyrazolo[4, 5-*e*][1, 2, 4]thiadiazine (7h)
A mixture of compound **6** and 3-chlorobenzyl chloride was heated at 40-50°C for 20 h, and then purified by recrystallization from ethanol to give compound **7h** as a white solid (80%); mp: 136-138°C. IR (KBr, cm^{-1}): 1668 (C=O); 1340, 1195 (SO_2); ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 7.84 (s, 1H, PyH), 7.33-7.41 (m, 8H, PhH), 5.11 (s, 2H, NCH_2), 5.06 (s, 2H, NCH_2), 4.06 (s, 3H, CH_3); MS (EI): m/z 451.4 (M+).

2-(*m*-Chlorobenzyl)-4-(*p*-chlorobenzyl)-7-methyl-1, 1, 3-trioxo-2*H*, 4*H*-pyrazolo[4, 5-*e*][1, 2, 4]thiadiazine (7i)
A mixture of compound **6** and 4-chlorobenzyl chloride was heated at 40-50°C for 20 h, and then purified by recrystallization from ethanol to give compound **7i** as a white solid (61%); mp: 102-104°C. IR (KBr, cm^{-1}): 1668 (C=O), 1339, 1194 (SO_2). ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 7.87(s, 1H, PyH), 7.28-7.41 (m, 8H, PhH), 5.10 (s, 2H,

NCH_2), 5.05 (s, 2H, NCH_2), 4.06 (s, 3H, CH_3); MS (EI): m/z 451.3 (M^+).

2-(*m*-Chlorobenzyl)-4-(2, 4-dichlorobenzyl)-7-methyl-1, 1, 3-trioxo-2*H*, 4*H*-pyrazolo[4, 5-e][1, 2, 4]thiadiazine(7j) A mixture of compound **6** and 2,4-dichlorobenzyl chloride was heated at 45-55°C for 20 h, and then purified by recrystallization from ethanol to give compound **7j** as a white solid (63%); mp:100-102°C. IR (KBr, cm^{-1}): 1694 (C=O), 1333, 1191 (SO_2); ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 7.78(s, 1H, PyH), 7.68 (d, 1H, *J*=2.13Hz, PhH), 7.09 (d, 1H, *J*=8.39 Hz, PhH), 7.32-7.40 (m, 5H, PhH), 5.13 (s, 2H, NCH_2), 5.03 (s, 2H, NCH_2), 4.08 (s, 3H, CH_3); MS (EI): m/z 485.3 (M+).

2-(*m*-Chlorobenzyl)-4-(*o*-cyanobenzyl)-7-methyl-1, 1, 3-trioxo-2*H*, 4*H*-pyrazolo[4, 5-e][1, 2, 4]thiadiazine(7k) A mixture of compound **6** and 2-cyanobenzyl chloride was heated at 50-60°C for 20 h, and then purified by recrystallization from ethanol to give compound **7k** as a white solid (55%); mp: 120-122°C. IR (KBr, cm^{-1}): 2222 (CN), 1700 (C=O); 1330, 1198(SO_2); ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 7.39(s, 1H, PyH), 7.89 (d, 1H, *J*=7.69 Hz, PhH), 7.19 (d, 1H, *J*=7.89Hz, PhH), 7.31-7.84 (m, 6H, PhH), 5.29 (s, 2H, NCH_2), 5.03 (s, 2H, NCH_2), 4.09 (s, 3H, CH_3); MS (EI): m/z 442.4 (M+1).

In vitro anti-HIV activity

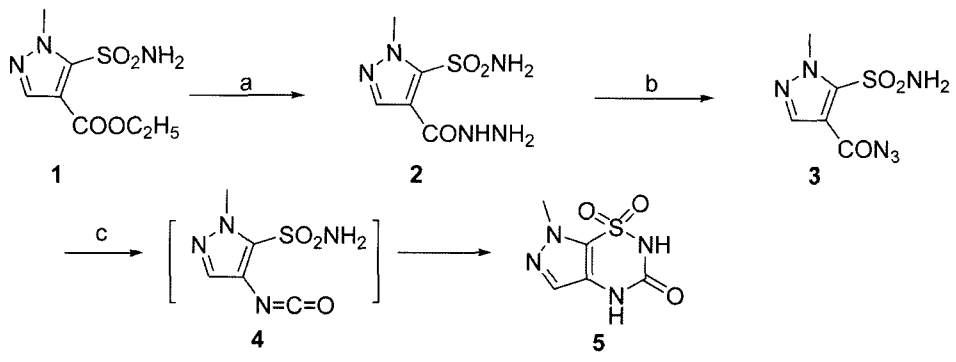
The activities of the compounds **6** and **7a-k** against HIV-1 (IIIB strain) and HIV-2 (strain ROD) multiplication in acutely infected cells were based on the inhibition of the virus-induced cytopathogenicity in MT-4 cells. Briefly, 50 μL of culture medium, containing 1×104 cells, was added to each well of flat-bottomed microtitre trays containing 50 uL of culture medium, with or without various concentrations of the compounds under test, and then 20 μL of a HIV suspension, containing 100 CCID50 (50% cell culture infective dose), added. After 5 days of incubation at 37°C, the number of viable cells was determined by the MTT method. The cytotoxicity of the compounds was evaluated in parallel with their antiviral activity, based on the viability of mock-infected cells, as monitored by the MTT method (Pauwels *et al.*, 1988; Witvrouw *et al.*, 1998). The precursors, **QM96539**, **QM96639**, and **QM96652,** were used as references, with the values of EC_{50} and CC_{50} for comparative purposes, with Nevirapine used as the reference drug.

RESULTS AND DISCUSSION

Chemistry

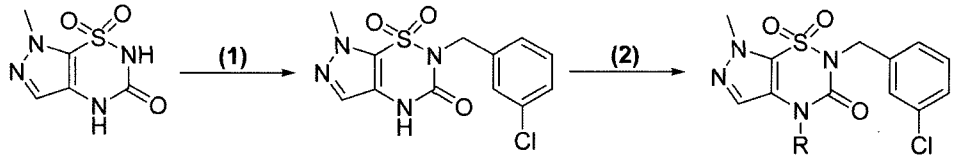
The nucleus ring of 7-methyl pyrazolo[4, 5-e][1, 2, 4] thiadiazine-1, 1, 3(2H, 4H)-trioxide **5** was prepared in parallel to the thiophene and the regioisomeric 6-methyl pyrazole series (Arranz *et al.*, 1997) (Scheme 1), which was started from the hydrazinolysis of ethyl 1-methyl-5-sulfamoyl-pyrazole-4-carboxylate **1**, a commercially available product, with hydrazine hydrate by refluxing in ethanol, forming the hydrazide **2** in excellent yield. Carboxy azide **3** was obtained by the reaction of compound **2** with sodium nitrite in diluted hydrochloric acid at a temperature of 10°C, and was pure enough as the white solid, without further purification, for use in the next step of the ring closure reaction. Subsequently, by refluxing compound **3** in anhydrous toluene, a classical Curtius rearrangement was carried out, through the intermediacy of isocyanate **4,** to afford the new regioisomer **5** in good yield.

2-(m-Chlorobenzyl)-7-methyl-1, 1, 3-trioxo-2H, 4H-pyrazolo[4, 5-e][1, 2, 4]thiadiazine (**6**) was prepared, starting from the nuclear ring **5**, with one equimolar of sodium hydride in DMF solvent at 10°C, followed by the addition of one equimolar of 3-chlorobenzyl chloride at 50°C for 20 h, with the mono N_2-substituted product **6** prepared in good yields (Martinez *et al.*, 2000a, 2000b; Arranz *et al.*, 1999, 2000) (Scheme 2). The regioselectivity of alkylation at the N_2 position of nuclear ring **5** is mainly due to the more acidic hydrogen at N_2 position and easier deprotonation than that at the N_4 position under the con-



Reagents: (a) $N_2H_4 \cdot H_2O$/EtOH (b) 2N HCl , $NaNO_2$, H_2O (c) △/toluene

Scheme 1. Synthesis of the nucleus ring of 7-methylpyrazolo[4, 5-e] [1, 2, 4]thiadiazine



Reagents: (1) NaH/3-chlorobenzyl chloride (1:1) (2) NaH/RX (1:1)

Scheme 2. Synthesis of compounds **6** and **7a-k**

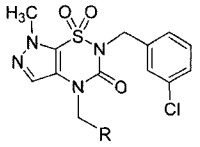
dition used, which was due to the strong electric withdraw effect of the sulfonyl group. The N_2-alkylated structure was further confirmed by means of a NOE experiment and the sequences of HMBC for long-distance proton/carbon correlation. The proton of N_2-CH_2 was shown to be correlated exclusively with the quaternary carbon C-3, which is different from the proton of N_4-CH_2, which would correlate with both C-3 and C-4a.

The 2-(*m*-Chlorobenzyl)-4-substituted-7-methyl-1, 1, 3-trioxo-2*H*, 4*H*-pyrazolo[4, 5-*e*][1, 2, 4]thiadiazine derivatives (**7a-k**) were easily prepared by alkylation at the N_4 position of compound **6**, using different alkyl halides under the same conditions as used for the alkylation of compound **5** (Scheme 2). Compounds **7a-k** are listed in Table I, and were structurally characterized by their ^{1}H-NMR, IR and MS spectra.

In vitro anti-HIV activity

The activity and cytotoxicity results of the newly designed and synthesized PTD derivatives, **6** and **7a-k**, for the inhibition of HIV-induced cytopathogenicity are listed in Table I. As expected from the chemical structure, compound **7a** (N_2-*m*-chlorobenzyl, N_4-CH_2CN) showed active against HIV-1-induced cytopathicity, with an EC_{50} value of

Table I. Anti-HIV activities, cytotoxicities and selectivity indexes of compounds **6** and **7a-k**



No.	CH_2R	EC_{50} (μM)[a]		CC_{50}[b] (μM)	SI[c]	
		HIV-1 III_B	HIV-2 ROD		HIV-1 III_B	HIV-2 ROD
6	H	>223.9	>232.2	231.6	<1	<1
7a	**CH_2CN**	**45.6**	**>175.6**	**201.3**	**4**	**<1**
7b	Benzyl	>300.1	>300.1	>300.1	×1[e]	×1
7c	2-Br-benzyl	>252.3	>252.3	>252.3	×1	×1
7d	4-Br-benzyl	>252.3	>252.3	>252.3	×1	×1
7e	2-F-benzyl	>278.5	>257.8	257.8	×1	×1
7f	4-F-benzyl	>208.9	>216.1	208.9	×1	×1
7g	2-Cl-benzyl	>277.2	>277.2	>277.2	×1	×1
7h	3-Cl-benzyl	>277.2	>277.2	>277.2	×1	×1
7i	4-Cl-benzyl	>277.2	>246.1	246.1	×1	×1
7j	2,4-Cl_2-benzyl	>257.5	>257.5	>257.5	×1	×1
7k	2-CN-benzyl	>283.1	>233.3	233.3	×1	×1
QM96539[d]		0.09		>340	>3778	
QM96639[d]		0.05		93.6	1872	
QM96652[d]		0.10		>119.0	>1190	
Nevirapine		0.03		683	22,767	

[a]EC_{50}: dose of compound required to achieve 50% protection of MT-4 cells from HIV-1 induced cytotoxicity, as determined by the MTT method. [b]CC_{50}: dosage required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method. [c]SI: selected index (CC_{50}/ EC_{50}). [d]The synthesis and antiviral properties of these compounds have previously been described. [e]The SI values: ×1 stand for 1or<1. All data represent the mean values of at least two separate experiments.

45.6 μM, CC_{50} value of 201.3 μM and SI=4. However, the potency of compound **7a** was much lower than the corresponding lead compounds, **QM96539** and **QM96639**, which bear N_2-*m*-halogenated benzyl and N_4-cyanomethyl substituents in the TTDs. In order to further investigate the clinical value of the active compound, the anti-HIV-1 activity against two NNRTI-resistant strains, S0561945 (RT K103N) and S0772126 (RT Y181C) was evaluated, but it was found to have completely lost its activity against these resistant strains.

Of the compound tested, only **7a** was found to exhibit inhibitory activity against HIV-1 replication, although other compounds, such as **7e,** were structurally constructed with active substituents at the N_2 and N_4 positions, similar to the TTDs lead compound **QM96652**. The lack of the activity of PTD derivatives is probably due to the strong hydrophilicity or the spatial restriction of the 1-methyl pyrazole ring moiety in the PTDs compared to the thiophene counterpart in the TTDs, since PTD molecules do not permit the "butterfly-like" conformation assumed to exist in the other HIV-1 NNRTIs (Schaefer *et al.*, 1993), which reduces their interaction with the binding site of the HIV-1 enzyme. In addition, all the synthesized compounds were screened for activity against HIV-2 (strain ROD), but none was found.

In summary, a series of PTD derivatives have been designed and evaluated for their anti-HIV-1 (IIIB strain) and anti-HIV-2 (strain ROD) activities in MT-4 cell cultures. Only compound **7a** showed active against HIV-1-induced cytopathicity, but none of the compounds exhibited inhibitory activity against HIV-2.

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REFERENCES

Arranz, M. E., Vega, S., and Díaz, J. A., Synthesis of Hetero [1,2,4]thiadiazine 1,1-Dioxides. *Heterocycles*, 45, 1767-1774 (1997).

Arranz M. E., Diaz, J. A., Ingate, S. T., Witvrouw, M., Pannecouque, C., Balzarini, J., De Clercq, E., and Vega, S., Novel 1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine derivatives as non-nucleoside reverse transcriptase inhibitors that inhibit human immunodeficiency virus type 1 replication. *J. Med. Chem.*, 41, 4109-4117 (1998).

Arranz, M. E., Díaz, J. A., Ingate, S. T., Witvrouw, M., Pannecouque, C., Balzarini, J., De Clerq, E., and Vega, S., Synthesis and anti-HIV activity of 1,1,3-trioxo-2*H*,4*H*-thieno [3,4-*e*] [1,2,4]thiadiazines (TTDs): a new family of HIV-1 specific non-nucleoside reverse transcriptase inhibitors. *Bioorg. Med. Chem.*, 7, 2811-2822 (1999).

Arranz, M. E., Díaz, J. A., Vega, S., Campos-Toimil, M., Orallo, F., Cardelus, I., Llenas, J., and Fernandez, A. G., Synthesis and pharmacological evaluation of 2, 3-dihydro-3-oxo-4*H*-thieno [3, 4-*e*][1, 2, 4]thiadiazine 1, 1-dioxidesas voltage-dependent calcium channel blockers. *Eur. J. Med. Chem.*, 35, 751-759 (2000).

Das, K., Lewi, P. J., Hughes, S. H., and Arnold, E., Crystallography and the design of anti-AIDS drugs: conformational flexibility and positional adaptability are important in the design of non-nucleoside HIV-1 reverse transcriptase inhibitors. *Prog. Biophys. Mol. Biol.*, 88, 209-231 (2005).

De Clercq, E., Recent highlights in the development of new antiviral drugs. *Curr Opin Microbiol.*, 8, 552-560 (2005).

Joncckheere, H., Anne, J., and De Clercq, E., The HIV-1 reverse transcription (RT) process as target for RT inhibitors. *Med. Res. Rev.*, 20, 129-154 (2000).

Martinez, A., Gil, C., Abasolo, M. I., Castro, A., Bruno, A. M., Perez, C., Prieto, C., and Otero, J., Benzothiadiazine dioxide dibenzyl derivatives as potent human cytomegalovirus inhibitors: Synthesis and comparative molecular field analysis. *J. Med. Chem.*, 43, 3218-3225 (2000a).

Martinez, A., Gil, C., Perez, C., Castro, A., Prieto, C., Otero, J., Andrei, G., Snoeck, R., Balzarini, J., and De Clercq E., Nucleoside human cytomegalovirus inhibitors: Synthesis and antiviral evaluation of (chlorophenylmethyl)benzothiadiazine dioxide derivatives. *J. Med. Chem.*, 43, 3267-3273 (2000b).

Patani, G. P. and LaVoie, E. J., Bioisosterism: A rational approach in drug design. *Chem. Rev.*, 96, 3147-3176 (1996).

Pauwels, R., Balzarini, J., Baba, M., Snoeck, R., Schols, D., Herdewijn, P., Desmyter, J., and De Clercq, E., Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. *J. Virol. Methods.*, 20,309-321 (1988).

Schaefer, W., Gunar Friebe, W., Leinert, H., Mertens, A., Poll, T., Vonder Saal, W., Zilch, H., Nuber, B., and L. Ziegler, M., Non-nucleoside inhibitors of HIV-1 reverse transcriptase: molecular modeling and x-ray structure investigations. *J. Med. Chem.*, 36, 726-732 (1993).

Witvrouw, M., Arranz, M. E., Pannecouque, C., Declerq, R., Jonckheere, H., Schmit, J. C., Vandamme, A. M., Dia, J. A., Ingate, S. E., Desmyter, J., Esnouf, E., Meervelt, L. V., Vega, S., Bazarni, J., and De Clercq, E., 1,1,3-Trioxo-2H,4H-thieno [e-3,4][1,2,4]thiadiazines (TTDs) derivatives: a new class of nonnucleoside human immunodeficiency virus type 1 (HIV-1) reverse transcriptase inhibitors with anti-HIV-1 activity. *Antimicrob. Agents. Chemother.*, 42, 618 -623 (1998).