SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL SUBSTITUTEDN^1-[1-BENZYL-3-(3-*TERT*-BUTYLCARBAMOYL-OCTAHYDROISOQUINOLIN-2YL)-2-HYDROXY- PROPYL]-2-[(2-OXO-*2H*-CHROMENE-3-CARBONYL) AMINO] SUCCINAMIDE ANALOGS AS ANTI-VIRAL AND ANTI-HIV AGENTS

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Abstract: A series of novel substituted N^1-[1-benzyl-3-(3-*tert*-butylcarbamoyl-octahydroisoquinolin-2yl)-2-hydroxypropyl]-2-[(2-oxo-*2H*-chromene-3-carbonyl) amino] succinamide analogs has been synthesized and evaluated for their anti-viral activity against HEL, Hela, and Vero cells. These compounds were also screened their anti-HIV activity against HIV-1 (111_B) and HIV-2 (ROD) in MT-4 cells. The compounds (**3d**, **3e**, **3f** and **3h**) which contained halogen or methoxy groups at the 6- or 8-position on the coumarin ring were active against HEL cells. The 6, 8-dibromo and 6-methoxy-8-chloro substituted coumarin analogs (**3d**) and (**3h**) were also active against Hela cells. The 6, 8-dibromo and 6- or 8-methoxy substituted coumarin analogs (**3d**, **3e** and **3h**) exhibited promising activity against Vero Cells. The 6-methyl, 6, 8-dichloro, 6,8-dibromo and 6-chloro substituted coumarin analogs (**3b**, **3d**, **3f** and **3g**) were identified as potent anti-HIV agents against HIV-1 (111_B) and HIV (ROD) in MT-4 cells. A systematic structure-activity relationship (SAR) study to elucidate the essential functional group requirement at the 6- and 8- position of the coumarin ring provides useful on sites for the further optimization of these potential anti-viral and anti-HIV agents.

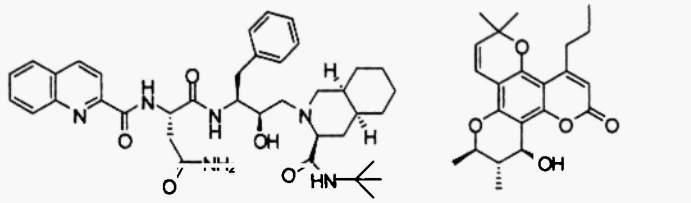
Keywords: Anti-viral activity; anti-HIV activity; N^1-[1-benzyl-3-(3-*tert*-butylcarbamoyl-octahydroisoquinolin-2yl)-2-hydroxypropyl]-2-[(2-oxo-*2H*-chromene-3-carbonyl) amino] succinamide.

1. Introduction

Acquired immunodeficiency syndrome (**AIDS**), a degenerative disease of the immune and central nervous systems, is an enormous world-wide health threat. No cure has been found, and research is aimed at developing chemotherapy against the causative agent, human immunodeficiency virus (**HIV**). Chemotherapy for AIDS has progressed steadily in the past decade.

However, new, effective and less toxic chemotherapeutic agents are still needed. Pseudo peptides containing hydroxyethylamine and related transition state mimics have been found to be potent inhibitors of the aspartic protease of HIV-1 [1]. Inactivation of this enzyme results in premature termination of the post translational processing of the viral

gag and gag-pol polyprotein gene products producing non-infectious virions [2]. Based on the initial pepstatin lead [3], many researchers have reported the synthesis and biological evaluation of HIV-protease inhibitors containing hydroxyethylamine moiety [4]. Saquinavir (**1**) is the first protease inhibitor in this class, and inhibits both HIV-1 & HIV-2 proteases. In this respect, the coumarin moiety is common to many natural and synthetic products associated with anti-HIV and other biological activities [5-9]. For example, the compound (+)-Calanolide A (**2**) is a pyranocoumarin derivative, discovered in extracts from the tropical rainforest tree *Calophyllum lanigerum*, and is a novel inhibitor of the human immunodeficiency virus (**HIV**).



1. Saquinavir 2. (+)-Calanolide A

Figure 1. Chemical structures of Saquinavir (**1**) and (+)-Calanolide A (**2**)

The anti-HIV activity of the both Saquinavir (**1**) and (+)-Calanolide A (**2**) (**Figure 1**), prompted us to design and synthesize novel substituted N^1-[1-benzyl-3-(3-*tert*-butylcarbamoyl-octahydroisoquinolin-2yl)-2hydroxypropyl]-2-[(2-oxo-*2H*-chromene-3 carbonyl)amino]succinamide analogs to evaluate their anti-viral and anti-HIV activities.

2. Materials and methods

2.1. General

2-Amino-N^1-[1-benzyl-3-(1-*tert*-butylcarbamoyl-octahydroisoquinolin-2-yl)2-hydroxy-propyl]succinamide (**5**) was prepared by the condensation of *N*-benzyloxy carbonyl-L-asparagine (**4**) with 2-(3-amino-2-hydroxy-4-phenyl-butyl)decahydroisoquinoline-3-carboxylic acid *tert*-butylamide (**3**) using dicyclohexylcarbodiimide (**DCC**) and triethylamine in dry tetrahydrofuran at room temperature for 24 hrs, followed by deprotection with hydrogen and 5%Pd-C. Compound (**5**) was condensed with an appropriate coumarin-3-carboxylic acid (**6a-h**) in the presence of dicyclohexylcarbodiimide (**DCC**) and triethylamine in dry tetrahydrofuran at room temperature for 24 hrs leads to afford the corresponding N^1-[1-benzyl-3-(3-*tert*-butylcarbamoyl-octahydroisoquinolin-2yl)-2-hydroxypropyl]-2-[(2-oxo-*2H*-chromene-3-carbonyl)-amino] succinamide analog (**Figure 2, 7a-h**). All the synthesized compounds were characterized by ^{1}H NMR, and Mass spectral analysis.

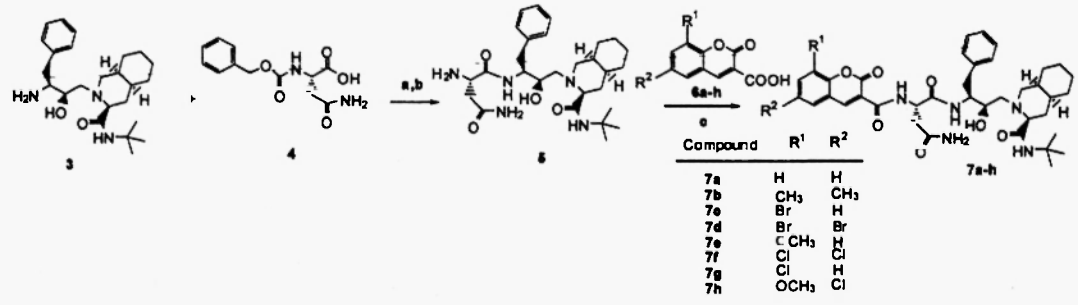


Figure 2. Reagents and Conditions: (a) DCC, THF, TEA, 20-25 ^{0}C, 24hrs, N_2; (b) EtOAc, 5% Pd/C, H_2, 20-25 ^{0}C, 48hrs; (c) DCC, THF, TEA, 20-25 ^{0}C, 24hrs, N_2.

2.2. Spectral data of (7a): ^{1}H NMR (DMSO-d_6): δ 1.26 (s, 9H, $3CH_3$), 1.38-1.44 (m, 11H, $5CH_2$ and CH), 1.64 (m, 1H, CH), 1.66-1.72 (m, 2H, N-CH_2), 2.32 (bs, 1H, OH), 2.62-2.71 (m, 4H, $COCH_2$ and N-CH_2), 3.11 (m,1H, isoquinoline N-CH), 4.05-4.16 (m, 4H, O-CH, N-CH and benzylic-CH_2), 4.84 (m, 1H, N-CH-CO), 5.52 and 5.77 (2bs, 2H, $CONH_2$), 7.10-7.22 (m, 5H, benzylic Ar-H), 7.24 (d, 1H, J= 8.23 Hz, coumarin C_8-H), 7.36-7.52 (m, 3H, coumarin C_5, C_6, C_7-H), 8.2 (bs, 3H, 3NH), 8.89 (s, 1H, coumarin C_4-H); Molecular formula $C_{38}H_{49}N_5O_7$; Mass (HRMS): 687.27 (M+1).

2.3. Spectral data of (7b): ^{1}H NMR (DMSO-d_6): δ 1.28 (s, 9H, $3CH_3$), 1.36-1.43 (m, 11H, $5CH_2$ and CH), 1.65 (m, 1H, CH), 1.68-1.75 (m, 2H, N-CH_2), 2.30 (s, 6H, $2CH_3$), 2.13 (s, 1H, OH), 2.64-2.75 (m, 4H, $COCH_2$ and N-CH_2), 3.15 (m,1H, isoquinoline N-CH), 4.12-4.18 (m, 4H, O-CH, N-CH and benzylic-CH_2), 4.86 (m, 1H, N-CH-CO), 5.56 and 5.80 (2bs, 2H, $CONH_2$), 7.12-7.45 (m, 6H, Ar-H), 7.28 (d, 1H, J= 8.23 Hz, coumarin C_7-H), 8.15 (bs, 3H, 3NH), 8.90 (s, 1H, coumarin C_4-H); Molecular formula $C_{40}H_{53}N_5O_7$; Mass (HRMS): 715.37 (M+1).

2.4. Spectral data of(7c): ^{1}H NMR (DMSO-d_6): δ 1.46 (s, 9H, $3CH_3$), 1.47-1.54 (m, 11H, $5CH_2$ and CH), 1.74 (m, 1H, CH), 1.83-1.86 (m, 2H, CH_2), 2.42 (m, 2H, N-CH_2), 2.65 (m, 2H, $COCH_2$), 3.13 (m,1H, isoquinoline N-CH), 4.10-4.23 (m, 4H, O-CH, N-CH and benzylic-CH_2), 4.78 (bs, 1H, OH), 4.88 (m, 1H, N-CH-CO), 6.85 and 6.94 (2bs, 2H, $CONH_2$), 7.31-7.42 (m, 5H, benzylic Ar-H), 7.45-7.56 (m, 3H, coumarin C_5, C_6, C_7-H), 8.05 (bs, 3H, 3NH), 8.76 (s, 1H, coumarin C_4-H); Molecular formula $C_{38}H_{48}BrN_5O_7$; Mass (HRMS): 765.17 (M+1).

2.5. Spectral data of (7d): ^{1}H NMR (DMSO-d_6): δ 1.46 (s, 9H, $3CH_3$), 1.49-1.55 (m, 11H, $5CH_2$ and CH), 1.76 (m, 1H, CH), 1.82-1.87(m, 2H, CH_2), 2.41 (m, 2H, N-CH_2), 2.63 (m, 2H,$COCH_2$), 3.10 (m,1H, isoquinoline N-CH), 4.11-4.25 (m, 4H, O-CH, N-CH and benzylic-CH_2), 4.86 (bs, 1H, OH), 4.92 (m, 1H, N-CH-CO), 6.84 and 6.97 (2bs, 2H, $CONH_2$), 7.33-7.45 (m, 5H, benzylic Ar-H), 7.83 (s, 1H, coumarin C_7-H), 8.10 (s, 1H, coumarin C_5-H), 8.02 (bs, 3H, 3NH), 8.78 (s, 1H, coumarin C_4-H); Molecular formula $C_{38}H_{47}Br_2N_5O_7$; Mass (HRMS): 845.18 (M+1).

2.6. Spectral data of (7e): ^{1}H NMR (DMSO-d_6): δ 1.47 (s, 9H, $3CH_3$), 1.48-1.54 (m, 11H, $5CH_2$ and CH), 1.76(m, 1H,CH), 1.80-1.86 (m, 2H, CH_2), 2.40 (m, 2H, N-CH_2), 2.66 (m, 2H, $COCH_2$), 3.13 (m,1H, isoquinoline N-CH), 3.78 (s, 3H, OCH_3), 3.82-4.25 (m, 4H, O-CH, N-CH and benzylic-CH_2), 4.81 (bs, 1H, OH), 4.95 (m, 1H, N-CH-CO), 6.85 and 7.12 (2bs, 2H, $CONH_2$), 7.35-7.47 (m, 5H, benzylic Ar-H), 7.33-7.56 (m, 3H, coumarin C_5-H, C_6-H, C_7-H), 8.12 (bs, 3H, 3NH), 8.77 (s, 1H, coumarin C_4-H); Molecular formula $C_{39}H_{51}N_5O_8$; Mass (HRMS): 717.35 (M+1).

2.7. Spectral data of (7f): ^{1}H NMR (DMSO-d_6): δ 1.48 (s, 9H, $3CH_3$), 1.51-1.56 (m, 11H, $5CH_2$ and CH),1.75 (m, 1H, CH), 1.80-1.88 (m, 2H, CH_2), 2.46 (m, 2H, N-CH_2), 2.63 (m, 2H, $COCH_2$), 3.14 (m,1H, isoquinoline N-CH), 3.80-4.23 (m, 4H, O-CH, N-CH and benzylic-CH_2), 4.84 (bs, 1H, OH), 4.92 (m, 1H, N-CH-CO), 6.82 and 7.14 (2bs, 2H, $CONH_2$), 7.35-7.48 (m, 5H, benzylic Ar-H), 7.53 (s, 1H, coumarin C_7-H), 7.98 (s, 1H, coumarin C_5-H), 8.10 (bs, 3H, 3NH), 8.76 (s, 1H, coumarin C_4-H); Molecular formula $C_{38}H_{47}Cl_2N_5O_7$; Mass (HRMS): 755.39 (M+1).

2.8. Spectral data of (7g): ^{1}H NMR (DMSO-d_6): δ 1.47 (s, 9H, $3CH_3$), 1.48-1.56 (m, 11H, $5CH_2$ and CH), 1.77 (m, 1H, CH), 1.81-1.84 (m, 2H, CH_2), 2.43 (m, 2H, N-CH_2), 2.67 (m, 2H, $COCH_2$), 3.15 (m,1H, isoquinoline N-CH), 4.14-4.26 (m, 4H, O-CH, N-CH and benzylic-CH_2), 4.81 (bs, 1H, OH), 4.84 (m, 1H, N-CH-CO), 6.83 and 6.91 (2bs, 2H,

$CONH_2$), 7.30-7.44 (m, 5H, benzylic Ar-H), 7.43-7.58 (m, 3H, coumarin C_5, C_6, C_7-H), 8.12 (bs, 3H, 3NH), 8.79 (s, 1H, coumarin C_4-H); Molecular formula $C_{38}H_{48}ClN_5O_7$; Mass (HRMS): 721.36 (M+1).

2.9. Spectral data of (7h): ^{1}H NMR (DMSO-d_6): δ 1.46 (s, 9H, $3CH_3$), 1.47-1.54 (m, 11H, $5CH_2$ and CH), 1.75 (m, 1H, CH), 1.86-1.88 (m, 2H, CH_2), 2.45 (m, 2H, N-CH_2), 2.68 (m, 2H, $COCH_2$), 3.23 (m,1H, isoquinoline N-CH), 3.78 (s, 3H, OCH_3), 3.85-4.34 (m, 4H, O-CH, N-CH and benzylic-CH_2), 4.83 (bs, 1H, OH), 4.96 (m, 1H, N-CH-CO), 6.87 and 7.22 (2bs, 2H, $CONH_2$), 7.34-7.49 (m, 5H, benzylic Ar-H), 7.57 (s, 1H, coumarin C_7-H), 7.72 (s, 1H, coumarin C_5-H), 8.10 (bs, 3H, 3NH), 8.78 (s, 1H, coumarin C_4-H); Molecular formula $C_{39}H_{50}ClN_5O_8$; Mass (HRMS): 751.37 (M+1).

3. Results and discussion

3.1. *Anti-viral activity*

All the synthesized compounds (**7a-h**) were evaluated their anti-viral activity against HEL, Hela cells, and Vero cells. Cytotoxicity was verified in mock-infected HEL, Hela cells and Vero cells. The anti-viral activity assays were based on inhibition of virus-induced cytopathicity in the above cultures, and the activity of the compounds was compared with standard anti-viral drugs such as Brivudin and Ribavirin. Briefly, the confluent cell culture in 96-well micro liter plates were inoculated with 100 CC1D50 of virus, ICC1D50 being the virus dose required to in feet 50% of the cells in cultures. After a 1 hr virus adsorption period, residual virus was removed, and the cells were incubated in the presence of varying concentration 400μg/ml, 100μg/ml, 80 μg/ml and 16μg/ml, of the test compounds. Viral cytopathicity was recorded as soon completion in the control virus occurred in treated cell cultures. SAR analysis of the substituent at the 6- and 8- position of coumarin ring revealed some interesting trends. The 6, 8-dibromo coumarin (**7d**), 6, 8-dichloro coumarin (**7f**), 6-methoxy coumarin (**7e**) and 6-methoxy-8-chloro coumarin (**7h**) analogs showed good anti-viral activity against HEL cells. The 6, 8-dibromo coumarin (**7d**) and 6-methoxy-8-chloro coumarin (**7h**) analogs were active against Hela cells. In the case of Vero cells, the 6, 8-dibromo coumarin (**7d**), 6-methoxy coumarin (**7e**) and 6-methoxy-8-chloro coumarin (**7h**) analogs showed good activity, but the 6, 8-dichloro coumarin (**7f**) exhibited very poor activity against Vero cells. The results are shown in **(Tables 1-3)**.

Table 1: Cytotoxic and anti-viral activity of substituted N^1-[1-benzyl-3-(3-*tert*-butylcarbamoyl-octahydroisoquinolin-2yl)-2-hydroxypropyl]-2-[(2-oxo-*2H*-chromene-3-carbonyl)amino] succinamide derivatives against HEL cell cultures.

Compound	Min. Cytotoxic Conc. (μg/ml)	Minimum inhibitory Conc. (μg/ml)		
		[a]A	[a]B	[a]C
7[a]	80	>16	>16	>16
7b	80	>16	>16	>16
7c	80	16	>16	>16
7d	400	>80	>80	>80
7e	400	>80	>80	>80
7f	100	>80	>80	>80
7g	80	>16	>16	>16
7h	100	>80	>16	>80
Brivudin	>400	>400	-	-
Ribavirin	>400	9.6	-	>80

[a]A: Vesicular Stomatitis virus, B: Coxysackie virus B4, C: Respiratory Syncytial virus.

Table 2: Cytotoxic and anti-viral activity of substituted N^1-[1-benzyl-3-(3-*tert*-butylcarbamoyl-octahydroisoquinolin-2yl)-2-hydroxypropyl]-2-[(2-oxo-*2H*-chromene-3-carbonyl) amino] succinamide derivatives against Hela cell cultures.

Compound	Min.Cytotoxic Conc. (μg/ml)	Minimum inhibitory Conc. (μg/ml)				
		[a]A	[a]B	[a]C	[a]D	[a]E
7[a]	≥80	>16	>16	>16	>16	>16
7b	≥80	>16	>16	>16	>16	>16
7c	80	>16	>16	>16	>16	>16
7d	≥16	>16	>16	>16	>80	>80
7e	≥16	>3.2	>3.2	>3.2	>3.2	>3.2
7f	≥16	>16	>16	>16	>16	>16
7g	≥16	>16	>16	>16	>16	>16
7h	≥80	>3.2	>16	>16	>16	>80
Brivudin	≥400	0.015	>80	3.2	>400	>400
Ribavirin	≥400	>400	240	80	80	>400

[a]A: Herpes simplex virus-1 (**KOS**), B: Herpes simplex virus-2 (**G**), C: Vaccinia virus, D: Vesicular stomatitis virus, E: Herpes simplex virus-1 (**TK-KOS ACV**).

Table 3: Cytotoxic and anti-viral activity of substituted N^1-[1-benzyl-3-(3-*tert*-butylcarbamoyl-octahydroisoquino lin-2yl)-2-hydroxypropyl]-2-[(2-oxo-*2H*-chromene-3-carbonyl)amino]succinamide derivatives against Vero cell cultures.

Compound	Min.Cytotoxic Conc. (μg/ml)	Minimum inhibitory Conc. (μg/ml)				
		[a]A	[a]B	[a]C	[a]D	[a]E
7[a]	80	>16	>16	>16	>16	>16
7b	80	>16	>16	>16	>16	>16
7c	80	>16	>16	>16	>16	>16
7d	>80	>80	>80	>80	>80	>80
7e	>80	>80	>80	>80	>80	>80
7f	>16	>10	>10	>10	>10	>10
7g	>16	>16	>16	>16	>16	>16
7h	≥80	>80	>80	>80	>80	>80
Brivudin	≥400	>400	>400	>400	>40	>400
Ribavirin	≥400	48	48	>400	>400	16

3.2. *Anti-HIV activity*

All the synthesized compounds (**7a-h**) were screened their anti-HIV activity against the replication of HIV-1 (111_B) and HIV (ROD) in MT-4 cells [10]. These cells were grown and maintained in RPM 1640 medium supplemented with 10% heat in activated fetal calf serum (**FCS**), 2mm glutamine, 0.10% sodium bicarbonate and 20μg/ml Gentamicin (culture medium). HIV-1 (HTLV-111 BLA1) and HIV-2 (LAV-2 ROD) were used in all experiments. The virus strains were propagated in MT-4 cells. Titers of virus stock were determined in MT-4 cells and the virus stock was stored at -70 ^{0}C until used. The inhibitory effects of the compounds on HIV-1 and HIV-2 replication were monitored by inhibition of virus-induced cytopathic effect in MT-4 cells and were estimated by the MTT method. Briefly, 50μl of HIV-1 and HIV-2 (100-300 $CCID_5$) were added to flat-bottomed micro liter trays with 50ml of medium containing various concentrations of the test compounds. MT-4 cells were added at a final concentration of $6x10^5$cells/ml. After 5 days of incubation at 37 ^{0}C, the number of viable cells were determined by the 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (**MTT**) method. Azidothymidine (**AZT**) was used as the standard drug. The 6-chloro coumarin analog (**7g**) showed greater activity against both HIV-1 and HIV-2, whereas the 6-bromo coumarin analog (**7c**) exhibited very poor activity against HIV-1 and HIV-2. Both the 6, 8-dibromo coumarin (**7d**) and 6,8-dichloro coumarin (**7f**)analogs were active against HIV-1 and HIV-2. The 6-methyl substituted coumarin derivative (**7b**) showed substantial effect on the both HIV-1 and HIV-2. Moreover, the 6-methoxy substituted coumarin derivatives (**7e**, and **7h**) did not show any anti-HIV activity. The results were shown in (**Table-4**).

Table 4: Anti-HIV activity of substituted N^1-[1-benzyl-3-(3-*tert*-butylcarbamoyl-octahydroisoquinolin-2yl)-2 hydroxypropyl] -2-[(2-oxo-2*H*-chromene-3-carbonyl)amino]succinamide derivatives against HIV-1 (111_B) and HIV-2 (ROD).

Compound	Strain	EC_{50} (μg /ml)	CC_{50} (μg /ml)
7a	HIV-1 (111_B)	>10	>10
	HIV-2 (ROD)	>10	>10
7b	HIV-1 (111_B)	>12	>26
	HIV-2 (ROD)	>12	>26
7c	HIV-1 (111_B)	>09	>09
	HIV-2 (ROD)	>09	>09
7d	HIV-1 (111_B)	>24	>24
	HIV-2 (ROD)	>24	>24
7e	HIV-1 (111_B)	>14	>14
	HIV-2 (ROD)	>14	>14
7f	HIV-I ($I11_B$)	>14	>26
	HIV-2 (ROD)	>14	>26
7g	HIV-1 (111_B)	>20	>32
	HIV-2 (ROD)	>20	>32
7h	HIV-1 ($I11_B$)	>10	>10
	HIV-2 (ROD)	>10	>10
AZT	HIV-1 (111_B)	0.0012	65.90
	HIV-2 (ROD)	0.0012	65.90

In summary, we have described the synthesis and structure-active relationships of a series of N^1-[1-benzyl-3-(3-*tert*-butylcarbamoyl-octahydroisoquinolin-2yl)-2-hydroxypropyl]-2-[(2-oxo-2*H*-chromene-3-carbonyl)amino succi- namide analogs (**7a-h**) as potent anti-viral and anti-HIV agents. The present investigation suggests the] importance of the various substituents on the coumarin moiety in the observed anti-viral and anti-HIV activity. This work has led to the identification of potent anti-viral compounds i.e., (**7d, 7e, 7f** and **7h**) against HEL cells, (**7d** and **7h**) against Hela cells, (**7d, 7e** and **7h**) against Vero cells, and (**7b, 7d, 7f** and **7g**) against both HIV-1 (111B) and HIV-2 (ROD). Further structure-activity relationship (**SAR**) studies on this interesting series of compounds will be presented in future publications.

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