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Synthesis and anti-HIV activity of new C_2 symmetric derivatives designed as HIV-1 protease inhibitors

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Abstract

The synthesis of several new anti-HIV-1 compounds is described. The new compounds contain a C_2 symmetry axis and a dihidroxyethylene moiety based on the D-tartaric acid back bone. The synthesis of these compounds was achieved in 36–69% overall yields from D-tartaric acid. The protocol included: acetylation of hydroxyl groups, followed by diamide formation and deacetylation or reduction with $LiAlH_4$. The anti-HIV 1 activities of these substances were evaluated in PM-1 cells, using Indinavir® as standard ($IC_{50} = 0.2$ μM). Two amino alcohol derivatives showed good inhibitory activity against the virus, with $IC_{50} = 2.0$ and 4 μM.

Keywords: HIV-1 protease; D-tartaric acid derivatives; Anti-HIV-1 activity

1. Introduction

The acquired immunodeficiency syndrome (AIDS) pandemic continues to be a medical, social and economic problem of staggering dimensions. The human immunodeficiency virus (HIV) has been identified as the etiologic agent of this disease [1–3]. HIV protease (HIV PR) processes the viral polyproteins $p55_{gag}$ and $p160_{gag\text{-}pol}$ into structural proteins and enzymes, including the protease itself, playing an essential role in the assembly and maturation of the virus [4]. Since inhibition of this aspartic protease leads to formation of non-infectious virions, the enzyme was identified as one of the major targets for intervention in HIV infection [5]. In fact, the introduction of a number of HIV PR inhibitors in clinical practice has become the main advance in AIDS therapy [6]. Despite the high potency and selectivity of these drugs, selection after long-term treatment led to the occurrence of HIV PR inhibitors resistant mutants [7]. On the other hand, the high cost of the clinically available HIV PR inhibitors consists in an economical drawback to the widespread implementation of these as AIDS therapies, specially in less developed countries. Thus, it is important to develop new HIV PR inhibitors, effective against resistant viruses and at the same time, available at low cost.

Our strategy in the development of new anti-HIV compounds is based on the development of analogues of known HIV PR inhibitors having C_2 symmetry. This strategy has been previously proved to be efficient for the design of compounds with desired pharmacological properties. Compounds which contain a C_2 symmetry or a pseudo-C_2 symmetry axis would present a good molecular complement within the enzyme and would be less capable to be recognized by the endogenous proteases, what should result in good bioavailability [8]. Guided by this approach we selected tartaric acid as our starting material.

The use of tartaric acid backbones as the core of HIV-1 protease inhibitors has been reported in literature [9,10]. The compounds described, e.g. compound **1**, lacked P1 and P1′ hydrophobic residues. Therefore, at

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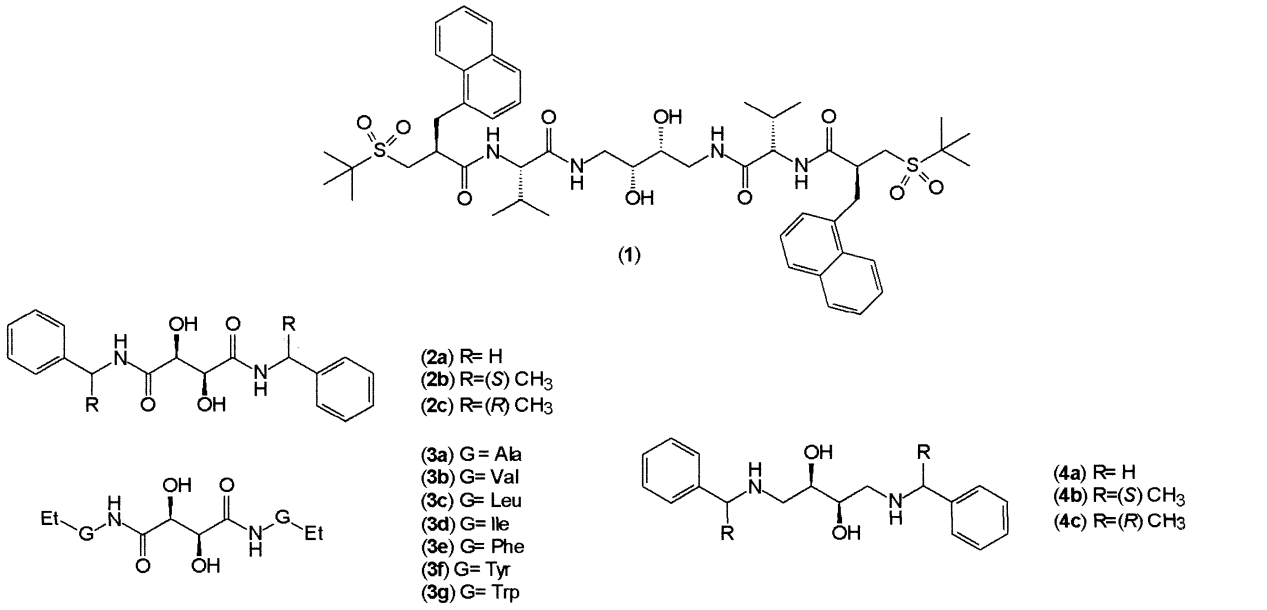
least, conceptually, they would not be able to interact to S1 and S1′ pockets in the catalytic site of the target enzyme. Despite this, such compounds have high potency in inhibiting in vitro isolated HIV-1 protease. Unfortunately, however, these compounds [9,10] have not proven to be effective in preventing HIV-1 infection in living cells [10], probably due to their low ability to penetrate the cellular membranes.

Compounds **2a**–**2c** and **4a**–**4c**, were designed as simple analogues of the known HIV-1 inhibitors by molecular simplification. These compounds present low molecular weight and thus should be more effective in penetrating biological membranes. In addition, due to their structures, they should have good in vivo metabolic and pharmacokinetic profile.



2. Results and discussion

The initial step in the synthesis of inhibitors **2a**–**2c** and **3a**–**3g** from D-tartaric acid was the protection of secondary hydroxyl groups, employing acetyl chloride under reflux for 48 h [11–13]. This furnish the key intermediate (**5**) in 85% yield. The coupling step of derivative (**5**) with benzylamine, (*S*)-α-methyl-benzylamine, (*R*)-α-methyl-benzylamine and the ethyl esters of alanine, valine, leucine, isoleucine, phenylalanine, tyrosine and tryptophan were performed through the formation of diacyl chloride intermediate (**6**), generated in situ using oxalyl chloride [14], and its addition over the amines, in presence of triethylamine in dichloromethane at room temperature. The desired diamides (**7a**–**7c**) and (**8a**–**8g**) were isolated in yields ranging from 78 to 95%. The selective de-esterification of the acetyl group of derivatives (**7a**–**7c**) and (**8a**–**8g**), using EtOH in the presence of catalytic H_2SO_4 under reflux furnished the inhibitors (**2a**–**2c**) and (**3a**–**3c**) in 63–85% yield. Compounds (**4a**–**4c**) were obtained in 55–62% yield, by the reduction of derivatives (**2a**–**2c**) using $LiAlH_4$ in THF at reflux temperature for 72 h [15] (Fig. 1).

Several procedures for the amide formation, the esters hydrolysis (ethanolysis) and reduction were tested before selecting the above methodologies. No epimerization of any chiral center occurred using the procedures described here. In particular, the ethanolysis step, apparently simple, was found to be crucial in maintaining the chiral integrity.

The preliminary anti-HIV profile of the derivatives (**2a**–**2c**), (**3a**–**3g**), (**4a**–**4c**), (**7a**) and (**8e**) was performed in a PM-1 cell culture plate assay [16]. We used standard HIV-1 isolate Z2Z6 (subtype D, highly infectious in PM-1 culture) with 3.96×10^3 $TCID_{50}$ ml^{-1} in a 96 holes plate, 10^4 cell per hole, and MOI $\sim 2 \times 10^{-3}$. Indinavir (Crixivan®) was employed as standard; one plate line was maintained as infection control. The infection was maintained under 5% CO_2 at 37 °C into a 5% CO_2 incubator, and daily verified by optical microscopy for 6 days. After this period the plates were revealed with 3-(4,5-dimethylthiazol-2-yl)-2,5-di-

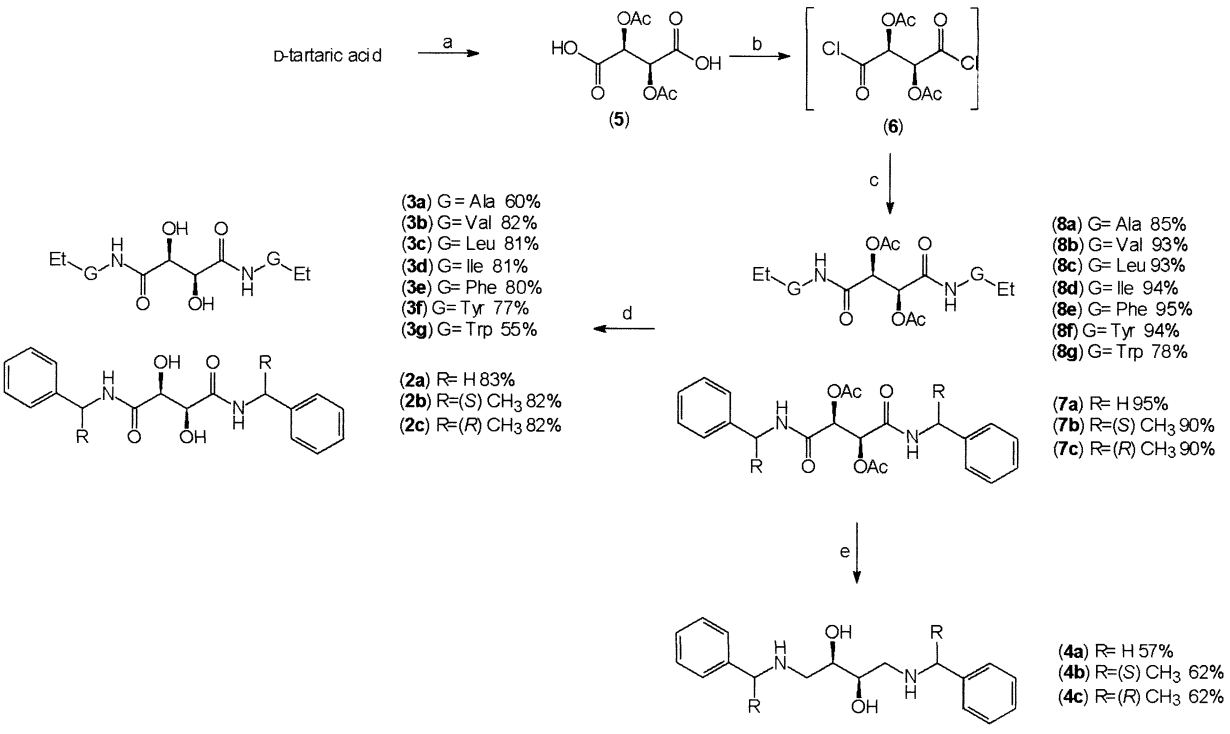


Fig. 1. (a) AcCl, reflux, 48 h, 85%; (b) oxalyl chloride, dichloromethane, DMF, r.t. 2 h; (c) primary amines, dichloromethane, triethylamine, r.t., 30 min; (d) EtOH, H_2SO_4, reflux, 4 h; (e) $LiAlH_4$, THF, reflux, 72 h.

Table 1
Anti-HIV activity of derivatives (**2a**–**2c**), (**3a**–**3g**), (**4a**–**4c**), (**7a**) and (**8e**) in PM-1 cells

Comp.	IC_{50}(μM)
Indinavir	0.2
3c	10
3f	50
4b	2
4c	4
8e	50
2a, **2b**, **2c**, **3a**, **3b**, **3d**, **3e**, **3g**, **4a** and **7a**	> 100 μM

phenyl-tetrazolium bromide (MTT) and read on ELISA reader with 490λ filter for the measurement of cellular viability [17]. Each plate was made in triplicate for statistical treatment.

Initial screening was performed at 100 μM and compounds (**3c**), (**3f**), (**4b**), (**4c**) and (**8e**) which demonstrated no inhibitory effect on cell viability at this concentration were submitted to the same assay in two different experiments using decreasing drug concentrations, starting with 100–6.25 μM (by 2 × decreasing steps) and with 10–0.039 μM (also 2 × decreasing steps) in order to determine the IC_{50}, as described elsewhere [1,16]. The results of these experiments are shown in Table 1.

As shown in Table 1, the new compounds are able to penetrate cellular membranes, do not exhibit any drastic cytotoxic effects and are able to prevent cell infections by HIV-1. In particular, compounds (**4b**) and (**4c**) ($IC_{50} = 2.0$ and 4 μM) have shown activities in the log range of Indinavir (Crixivan®), $IC_{50} = 0.2$ μM. The anti-HIV activities of these compounds can be attributed to a series of factors, including their simple design and their low molecular weight. The concept of low molecular weight compounds is well exemplified in literature and here again is successful.

3. Conclusions

The pharmacological data obtained from PM1 cells have demonstrated the relevant anti-HIV-1 activity for this new class of compounds. Derivatives (**4b**) and (**4c**) have shown activity in the log range of Indinavir (Crixivan®) [15]. These results confirm the utility of tartaric acid as an abundant chiral building block in the synthesis of anti-HIV compounds. In view of the fact that these compounds were synthesized as analogous of HIV PR inhibitors, and regarding their structural similarity, kinetic studies using HIV PR are underway in order to determine the K_i of these compounds against HIV-1 PR, and thereby confirm the probable mechanism of action of these new compounds. The present and other similar synthesized compounds will be tested against mutant HIV-1 strains.

4. Experimental

Thin layer chromatography was performed using aluminum sheets precoated with silica gel 60 (HF-254, Merck) to a thickness of 0.25 mm and detected by ultraviolet light or iodine. Column chromatography was performed on silica gel 60, 230–400 mesh (Merck). NMR (^{1}H, ^{13}C) spectra were recorded in a Bruker AMX-200 or WX-200 Fourier transform spectrometer. Coupling constants (J) are reported in hertz (Hz), and chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (TMS, 0.00 ppm). Splitting patterns are as follows: br, broad; s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet. Infrared (IR) spectra were recorded with a Nicolet 505 Magna spectrophotometer by using sodium chloride cell or potassium bromide plates. High Resolution mass spectra were obtained in a Bruker Reflex III MALDI-ToF instrument. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter. Solvents used in the reactions were redistilled prior to use and stored over 3–4 Å molecular sieves.

4.1. 2,3-Diacetoxy-(2S,3S)-butanedioic (5) [13]

A refluxing solution of D-tartaric acid (20.00 g, 133 mmol) in acetyl chloride (200 ml) was stirred for 48 h. After this period, the reaction mixture was evaporated. Recrystallization of the residue AcOEt–hexane gave compound (**5**) (26.52 g, 113 mmols) at 85% yield as a white hygroscopic crystalline solid: melting point (m.p.) 109–110 °C; $[\alpha]_D = -95.0$ ($c = 1.00$, H_2O); ^{1}H NMR ($CDCl_3$) 200 MHz) δ 5.72 (s, 1H), 2.21 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.8, 163.4, 72.2, 20.2; IR (cm^{-1}) 3300, 2942, 1743, 1693, 1239, 1089.

4.2. 1N,4N-Dibenzyl-2,3-diacetoxy-(2S,3S)-butanediamide (7a) [14]

Oxalyl chloride (1.62 g, 12.8 mmols) was added for a period of 10 min to a solution of compound (**5**) (1.00 g, 4.27 mmols), and DMF (0.2 ml) of anhydrous dichloromethane at 0 °C under magnetic stirring in an argon atmosphere. After a period of 2 h, at room temperature (r.t.), the solution was evaporated under vacuum, and the yellowish solid residue was extracted with dichloromethane (20 ml), and added over a period of 20 min to a mixture of benzylamine (1.10 g, 10.3 mmols) and triethylamine (1.29 g, 12.8 mmols), at r.t. After 30 min of magnetic stirring, the mixture was concentrated under vacuum, extracted with AcOEt (100 ml) and treated with aqueous HCl (2 × 70 ml). The organic phase was washed with a saturated solution of sodium chloride (50 ml), dried with sodium sulfate, and evaporated under vacuum, providing the diamide (**7a**) (1.69 g, 4.10 mmols) at 96% yield, as a white solid. Analytically pure samples may be obtained by recrystallization in AcOEt–hexane: m.p. 184–185 °C; $[\alpha]_D = -4.8$ ($c = 1.12$, CH_3OH); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.26 (m, 5H), 6.43 (m, 1H), 5.69 (s, 1H), 4.52 (dd, $J = 6.5$, 14.8 Hz, 1H), 4.29 (dd, $J = 5.1$, 14.8 Hz, 1H), 2.05 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.1, 166.1, 137.6, 128.8, 127.7, 72.5, 43.5, 20.4; IR (cm^{-1}) 3321, 3088, 3033, 2942, 1474, 1683, 1660, 1533, 1239, 1089, 749, 702; HR MS m/z Calc. for $C_{22}H_{24}N_2O_6$: 412.44. Found: 412.39%.

4.3. 1N,4N-Di[1-phenyl-(1S)-ethyl]-2,3-diacetoxy-(2S,3S)-butanediamide (7b)

Compound **7b** (1.69 g, 3.84 mmols) was obtained from 2,3-diacetoxy-(2*S*,3*S*)-butanedioic acid (**5**) (1.00 g, 4.27 mmols) and (*S*)-α-methylbenzylamine (1.25 g, 10.3 mmols), by the same method described for obtaining **7a**, with 90% yield, as a white solid. Analytically pure samples may be obtained by recrystallization in acetone–water: m.p. 226–227 °C; $[\alpha]_D = -53.7$ ($c = 1.08$, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) 200 MHz) δ 7.25 (m, 5H), 7.08 (br s, 1H), 5.64 (s, 1H), 4.99 (m, 1H), 1.94 (s, 3H), 1.42 (d, $J = 6.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 168.2, 164.1, 141.4, 127.2, 126.0, 124.8, 71.3, 47.8, 20.0, 19.0; IR (cm^{-1}) 3255, 3063, 3029, 2978, 1755, 1649, 1544, 1208, 1055, 756, 698; HR MS m/z Calc. for $C_{24}H_{28}N_2O_6$: 440.49. Found: 440.71%.

4.4. 1N,4N-Di[1-phenyl-(1R)-ethyl]-2,3-diacetoxy-(2S,3S)-butanediamide (7c)

Compound **7c** (1.69 g, 3.84 mmols) was obtained from 2,3-diacetoxy-(2*S*,3*S*)-butanedioic acid (**5**) (1.00 g, 4.27 mmols) and (*R*)-α-methylbenzylamine (1.25 g, 10.3 mmols), by the same method described for obtaining (**7a**), with 90% yield, as a white solid. Analytically pure samples may be obtained by recrystallization in AcOEt–hexane: m.p. 212–213 °C; $[\alpha]_D = +41.8$ ($c = 0.98$, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.27 (m, 5H), 6.77 (d, $J = 8.0$ Hz, 1H), 5.70 (s, 1H), 5.05 (m, 1H), 1.94 (s, 3H), 1.44 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.4, 165.5, 142.5, 128.9, 127.7, 126.4, 72.7, 48.9, 21.6, 20.6; IR (cm^{-1}) 3359, 3087, 3031, 2986, 2942, 1756, 1660, 1529, 1208, 1057, 765, 701; HR MS m/z Calc. for $C_{24}H_{28}N_2O_6$: 440.49. Found: 440.71%.

4.5. 1N,4N-Di[1-carboethoxy-3-methyl-(1S)-butyl]-2,3-diacetoxy-(2S,3S)-butanediamide (8c)

Compound **8c** (2.05 g, 3.97 mmols) was obtained from 2,3-diacetoxy-(2*S*,3*S*)-butanedioic acid (**5**) (1.00 g, 4.27 mmols) and the ethyl ester of leucine (1.64 g, 10.3 mmols), by the same method described for obtaining (**7a**), with 93% yield, as a white solid. Analytically pure samples may be obtained by recrystallization in AcOEt–hexane: m.p. 156–157 °C; $[\alpha]_D = -14.0$ ($c = 1.00$,

CH_2Cl_2); ^1H NMR ($CDCl_3$, 200 MHz) δ 6.64 (d, $J = 8.4$ Hz, 1H), 5.65 (s, 1H), 4.59 (m, 1H), 4.19 (q, $J = 7.1$ Hz, 2H), 2.19 (s, 3H), 1.62 (m, 3H), 1.28 (t, $J = 7.1$ Hz, 3H), 0.95 (d, $J = 5.4$ Hz, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 172.7, 169.1, 165.9, 72.9, 61.8, 51.1, 41.9, 24.9, 23.0, 22.1, 20.7, 14.3; IR (cm^{-1}) 3308, 2961, 2873, 1758, 1656, 1541, 1203, 1058; HR MS *m/z* Calc. for $C_{24}H_{40}N_2O_{10}$: 516.58. Found: 516.32%.

*4.6. 1N,4N-Di[1-carboethoxy-3-methyl-(1S)-ethyl]-2,3-diacetoxy-(2S,3S)-butanediamide (**8a**)*

Compound **8a** (1.57 g, 3.63 mmols) was obtained from 2,3-diacetoxy-(2*S*,3*S*)-butanedioic acid (**5**) (1.00 g, 4.27 mmols) and the ethyl ester of L-alanine (1.21 g, 10.3 mmols), by the same method described for obtaining **7a**, with 85% yield, as a white solid. Analytically pure samples may be obtained by recrystallization in AcOEt–hexane: m.p. 170–171 °C; $[\alpha]_D = -2.5$ ($c = 0.99$, CH_2Cl_2); ^1H NMR ($CDCl_3$, 200 MHz) δ 6.97 (d, $J =$ 6.8 Hz, 1H), 5.63 (s, 1H), 4.48 (m, 1H), 4.17 (q, $J = 7.1$ Hz, 2H), 2.14 (s, 3H), 1.37 (d, $J = 7.1$ Hz, 3H), 1.24 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 172.6, 169.0, 165.6, 72.3, 61.9, 48.4, 20.6, 18.4, 14.2; IR (cm^{-1}) 3372, 3318, 2968, 1748, 17 390, 1663, 1537, 1261, 1202, 1032; HR MS *m/z* Calc. for $C_{18}H_{28}N_2O_{10}$:432.42. Found: 432.17%.

*4.7. 1N,4N-di[1-carboethoxy-2-(1H-3-indoil)-(1S)-ethyl]-2,3-diacetoxy-(2S,3S)-butanediamide (**8g**)*

Compound **8g** (2.20 g, 3.33 mmols) was obtained from 2,3-diacetoxy-(2*S*,3*S*)-butanedioic acid (**5**) (1.00 g, 4.27 mmols) and the ethyl ester of L-tryptophane (2.39 g, 10.3 mmols), by the same method described for obtaining **7a**, with 78% yield, after flash chromatography with silica gel (AcOEt–hexane 4:6), as a violet solid, m.p. 103–105 °C; $[\alpha]_D = -41.7$ ($c = 1.07$, CH_2Cl_2); ^1H NMR ($CDCl_3$, 200 MHz) δ 8.58 (s, 1H), 7.37 (d, $J = 7.3$ Hz, 1H), 7.08 (d, $J = 7.2$ Hz, 1H), 6.99 (m, 4H), 5.53 (s, 1H), 4.67 (m, 1H), 3.79 (q, $J = 7.0$ Hz, 2H), 3.11 (m, 2H), 1.62 (s, 3H), 0.91 (t, $J = 7.0$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 171.5, 169.7, 166.1, 136.2, 127.5, 123.6, 122.0, 119.4, 118.4, 111.5, 109.2, 72.2, 61.8, 53.0, 27.7, 20.2, 13.9; IR (cm^{-1}) 3406, 3058, 2981, 1740, 1673, 1525, 1205, 744; HR MS *m/z* Calc. for $C_{34}H_{38}N_4O_{10}$: 662.69. Found: 662.44%.

*4.8. 1N,4N-di[1-carboethoxy-2-phenyl-(1S)-ethyl]-2,3-diacetoxy-(2S,3S)-butanediamide (**8e**)*

Compound **8e** (2.37 g, 3.06 mmols) was obtained from 2,3-diacetoxy-(2*S*,3*S*)-butanedioic acid (**5**) (1.00 g, 4.27 mmols) and the ethyl ester of L-phenylalanine (1.99 g, 10.3 mmols), by the same method described for obtaining **7a**, with 95% yield, as a white solid. Analytically pure samples may be obtained by recrystallization in AcOEt–hexane: m.p. 159–160 °C; $[\alpha]_D = +55.2$ ($c =$ 1.16, CH_2Cl_2). ^1H NMR ($CDCl_3$, 200 MHz) δ 7.45 (m, 5H), 6.98 (d, $J = 7.5$ Hz, 1H), 5.86 (s, 1H), 5.02 (m, 1H), 4.32 (q, $J = 7.2$ Hz, 2H), 3.35 (m, 2H), 2.33 (s, 3H), 1.43 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.9, 169.1, 165.8, 136.8, 129.5, 128.7, 127.3, 72.2, 61.8, 53.5, 38.7, 20.6, 14.2; IR (cm^{-1}) 3353, 3333, 3086, 3033, 2983, 1755, 1663, 1536, 1211, 1066, 748, 701; HR MS *m/z* Calc. for $C_{30}H_{36}N_2O_{10}$: 584.62. Found: 584.13%.

*4.9. 1N,4N-di[1-carboethoxy-2-methyl-(1S)-propyl]-2,3-diacetoxy-(2S,3S)-butanediamide (**8b**)*

Compound **8b** (1.94 g, 3.97 mmols) was obtained from 2,3-diacetoxy-(2*S*,3*S*)-butanedioic acid (**5**) (1.00 g, 4.27 mmols) and the ethyl ester of L-valine (1.49 g, 10.3 mmols), by the same method described for obtaining **7a**, with 93% yield, as a white solid. Analytically pure samples may be obtained by recrystallization in AcOEt–hexane: m.p. 147–148 °C; $[\alpha]_D = +5.5$ ($c = 0.99$, CH_2Cl_2). ^1H NMR ($CDCl_3$) 200 MHz) δ 6.85 (d, $J =$ 7.5 Hz, 1H), 5.56 (s, 1H), 4.44 (m, 1H) 4.14 (q, $J = 7.1$ Hz, 2H), 2.15 (s, 3H), 2.13 (m, 1H), 1.24 (t, $J = 7.1$ Hz, 3H), 0.89 (m, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 171.4, 169.1, 166.1, 72.5, 71.0, 61.6, 57.4, 31.6, 20.7, 19.0, 17.9 14.3; IR (cm^{-1}) 3373, 3318, 2966, 1747, 1740, 1666, 1537, 1261, 1202, 1032; HR MS *m/z* Calc. for $C_{22}H_{36}N_2O_{10}$: 488.53. Found: 488.62%.

*4.10. 1N,4N-di[1-carboethoxy-2-methyl-(1S, 2S)-butyl]-2,3-diacetoxy-(2S,3S)-butanediamide (**8d**)*

Compound **8d** (2.07 g, 4.01 mmols) was obtained from 2,3-diacetoxy-(2*S*,3*S*)-butanedioic acid (**5**) (1.00 g, 4.27 mmols) and the ethyl ester of L-isoleucine (1.64 g, 10.3 mmols), by the same method described for obtaining **7a**, with 94% yield, as a white solid. Analytically pure samples may be obtained by recrystallization in AcOEt–hexane: m.p. 137–138 °C; $[\alpha]_D = -8.53$ ($c =$ 0.98, CH_2Cl_2); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.50 (d, $J = 8.0$ Hz, 1H), 4.80 (d $J = 7.9$, 1H), 4.48 (m, 1H), 4.35 (d, $J = 7.9$, 1H), 4.19 (q, $J = 7.1$ Hz, 2H), 2.0 (s, 3H), 1.88 (m, 1 H), 1.30 (m, 5H), 0.89 (m, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.7, 171.0, 169.8, 70.9, 61.5, 56.4, 37.9, 25.1, 15.5, 14.3, 11.7; IR (cm^{-1}) 3331, 2970, 2937, 1759, 1747, 1665, 1536, 1260, 1202, 1056; HR MS *m/z* Calc. for $C_{24}H_{40}N_2O_{10}$: 516.58. Found: 516.62%.

*4.11. 1N,4N-di[2-(hydroxyphenyl)-1-carboethoxy-(1S)-ethyl]-2,3-diacetoxy-(2S, 3S)-butanediamide (**8f**)*

Compound **8f** (2.34 g, 3.80 mmols) was obtained from 2,3-diacetoxy-(2*S*,3*S*)-butanedioic acid (**5**) (1.00 g, 4.27 mmols) and the ethyl ester of L-tyrosine (2.15 g, 10.3 mmols), by the same method described for obtaining **7a**,

with 94% yield, after flash chromatography with silica gel (CH_2Cl_2:CH_3OH 96:4), as a crystalline white solid, m.p. 210–211 °C; $[\alpha]_D = -35.8$ ($c = 1.02$, CH_3OH); ^1H NMR(DMSO-d_6, 200 MHz) δ 9.24 (s, 1H), 8.35 (d, $J =$ 7.7, 1H), 6.97 (d, $J = 8.2$ Hz, 2H), 6.64 (d, $J = 8.2$, 2H), 5.50 (s, 1H), 4.36 (m, 1H), 4.00 (q, $J = 7.0$ Hz, 2H), 2.85 (m, 2H), 1.94 (s, 3H), 1.09 (t, $J = 7.0$ Hz); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 171.4, 169.7, 166.2, 156.5, 130.4, 127.4, 115.5, 72.1, 61.2, 54.3, 36.9, 21.0, 14.5; IR (cm^{-1}) 3353, 3333, 3301, 3086, 3033, 2983, 1755, 1663, 1536, 1211, 1066, 748, 701; HR MS *m/z* Calc. for $C_{30}H_{36}N_2O_{12}$: 616.62. Found: 616.68%.

*4.12. 1N,4N-dibenzyl-2,3-dihydroxy-(2S,3S)-butanediamide (**2a**)*

Sulfuricacid (0.5 ml) was added to a diamide solution (**7a**) (0.52 g, 1.25 mmol) in absolute etnanol (50 ml) at r.t. and the mixture was kept under magnetic stirring at reflux temperature for 4 h. After cooling, the solvent was concentrated under vacuum to half the original volume, and had AcOEt (70 ml) added. The mixture was extracted with aqueous NaOH at 5% (20 ml) and aqueous 1 N HCl (20 ml). The organic phase was washed with NaCl, dried with anhydrous sodium sulfate and evaporated under vacuum, providing the diol (**2a**) (0.34 g, 10.0 mmol) at 83% yield, as a crystalline solid, m.p. 198–200 °C; $[\alpha]_D = +15.2$ ($c = 1.20$, CH_3OH); ^1H NMR (DMSO-d_6, 200 MHz) δ 8.04 (m, 1H), 7.08 (m, 5H), 5.52 (d, $J = 7.0$ Hz), 4.16 (m, 2H), 3.13 (s, 1H); ^{13}C NMR (DMSO-d_6) 50 MHz) δ 172.6, 139.9, 128.6, 127.6, 127.1, 73.2, 42.4; IR (cm^{-1}) 3362, 3316, 3086, 3034, 2927, 1628, 1546, 1095, 742, 697; HR MS *m/z* Calc. for $C_{18}H_{20}N_2O_4$: 328.36. Found: 328.10%.

*4.13. 1N,4N-di[1-phenyl-(1S)-ethyl]-2,3-dihydroxy-(2S,3S)-butanediamide (**2b**)*

Compound **2b** (0.36 g, 1.03 mmol) was obtained from the derivative (**7b**) (0.55 g, 1.25 mmol), by the same procedure described for obtaining **2a** with 82% yield as a crystalline solid, m.p. 130–131 °C; $[\alpha]_D = -91.3$ ($c =$ 1.03, CH_2Cl_2); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.29 (m, 6H), 5.20 (br s, 1H), 5.06 (dq, $J = 7.4$, 7.0 Hz, 1H), 4.23 (s, 1H), 1.51 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.0, 142.3, 128.8, 127.7, 126.1, 70.3, 49.0, 21.9; IR (cm^{-1}) 3413, 3332, 3087, 3032, 2973, 1658, 1526, 1140, 1063, 763, 698; HR MS *m/z* Calc. for $C_{20}H_{24}N_2O_4$: 356.42. Found: 356.18%.

*4.14. 1N,4N-Di[1-phenyl-(1R)-ethyl]-2,3-dihydroxy-(2S,3S)-butanediamide (**2c**)*

Compound **2c** (0.36 g, 1.03 mmol) was obtained from the derivative (**7c**) (0.55 g, 1.25 mmol), by the same procedure described for obtaining **2a** with 82% yield as a crystalline solid, m.p. 144–146 °C; $[\alpha]_D = -46.5$ ($c =$ 1.12, CH_2Cl_2), ^1H NMR ($CDCl_3$, 200 MHz) δ 7.34 (d, $J = 7.8$ Hz, 1H), 7.21 (m, 5H), 5.32 (br s, 1H), 5.04 (m, 1H), 4.31 (s, 1H), 1.50 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.1, 142.3, 128.9, 127.6, 126.0, 70.5, 48.8, 22.0; IR (cm^{-1}) 3386, 3342, 3194, 2985, 2964, 1661, 1642, 1532, 1443, 1139, 1077, 763, 700; HR MS *m/z* Calc. for $C_{20}H_{24}N_2O_4$: 356.42. Found: 356.81%.

*4.15. 1N,4N-di[1-carboethoxy-3-methyl-(1S)-butyl]-2,3-dihydroxy-(2S,3S)-butanediamide (**3c**)*

Compound **3c** (0.64 g, 1.01 mmol) was obtained from the derivative (**8c**) (0.55 g, 1.25 mmol), by the same procedure described for obtaining **2a** with 81% yield as a colorless oil. $[\alpha]_D = -21.6$ ($c = 1.76$, CH_2Cl_2); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.35 (d, $J = 8.0$ Hz, 1H), 4.80 (br s, 1H), 4.51 (m, 1H), 4.33 (s, 1H), 4.17 (q, $J = 7.1$ Hz, 2H), 1.62 (m, 3H), 1.25 (t, $J = 7.1$ Hz, 3H), 0.92 (m, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.4, 172.0, 70.7, 61.5, 50.6, 41.3, 24.8, 22.7, 21.9, 14.1; IR (cm^{-1}) 3388, 2960, 2872, 1724, 1651, 1533, 1357, 1202, 1154; HR MS *m/z* Calc. for $C_{20}H_{36}N_2O_8$: 432.51. Found: 432.85%.

*4.16. 1N,4N-di[1-carboethoxy-(1S)-ethyl]-2,3-dihydroxy-(2S,3S)-butanediamide (**3a**)*

Compound **3a** (0.26 g, 0.75 mmol) was obtained from the derivative (**8a**) (0.54 g, 1.25 mmol), by the same procedure described for obtaining **2a** with 60% yield as a crystalline solid, m.p. 102–103 °C; $[\alpha]_D = -2.53$ ($c =$ 0.98, CH_2Cl_2); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.58 (d, $J = 7.4$ Hz, 1H), 4.48 (m, 1H), 4.39 (m, 1H), 4.17 (q, $J =$ 7.1 Hz, 2H), 3.92 (br s, 1H), 1.38 (d, $J = 7.1$ Hz, 3H), 1.24 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.0, 172.3, 71.1, 61.8, 48.2, 18.2, 14.2; IR (cm^{-1}) 3388, 2960, 2872, 1724, 1651, 1533, 1357, 1202, 1154; HR MS *m/z* Calc. for $C_{14}H_{24}N_2O_8$: 348.35. Found: 340.00%.

*4.17. 1N,4N-di[1-carboethoxy-2-phenyl-(1S)-ethyl]-2,3-dihydroxy-(2S,3S)-butanediamide (**3e**)*

Compound **3e** (0.51 g, 1.02 mmol) was obtained from the derivative (**8e**) (0.75 g, 1.28 mmol), by the same procedure described for obtaining **2a** with 80% yield as a crystalline solid, m.p. 138–140 °C; $[\alpha]_D = +78.5$ ($c =$ 1.08, CH_2Cl_2); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.41 (d, $J = 7.5$ Hz, 1H), 7.20 (m, 5H), 4.75 (m, 1H), 4.57 (br s, 1H), 4.26 (br s, 1H) 4.07 (q, $J = 7.2$ Hz, 2H), 3.00 (m, 2H), 1.14 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.2, 170.8, 135.7, 129.6, 128.8, 127.4, 71.0, 61.9, 53.2, 38.3, 14.3; IR (cm^{-1}) 3448, 3384, 3347, 3062, 3030, 2979, 1729, 1658, 1625, 1531, 1209, 1138, 700, 609; HR MS *m/z* Calc. for $C_{26}H_{32}N_2O_8$: 500.54. Found: 500.72%.

*4.18. 1N,4N-Di[1-carboethoxy-2-(1H-3-indoil)-(1S)-ethyl]-2,3-dihydroxy-(2S,3S)-butanediamide (**3g**)*

Compound **3g** (0.42 g, 0.73 mmol) was obtained from the derivative (**8g**) (0.83 g, 1.25 mmol), by the same procedure described for obtaining **2a** with 55% yield as a crystalline solid, m.p. 114–116 °C; $[\alpha]_D = +17.9$ ($c =$ 0.89, CH_3OH); ^1H NMR ($CDCl_3$, 200 MHz) δ 10.92 (s, 1H), 7.78 (d, $J = 7.7$ Hz, 1H), 7.50 (d, $J = 7.2$Hz, 1H), 6.99 (m, 4H), 5.99 (d, $J = 7.1$ Hz, 1H), 4.63 (m, 1H), 4.33 (d, $J = 7.1$ Hz, 1 H), 3.97 (q, $J = 7.0$ Hz, 2H), 3.21 (m, 2H), 1.07 (t, $J = 7.0$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 172.2, 171.8, 136.5, 127.7, 124.5, 121.5, 118.9, 118.6, 111.9, 109.0, 73.0, 61.2, 52.9, 27.8, 14.3; IR (cm^{-1}) 3389, 3316, 3060, 2977, 2928, 1727, 1650, 1538, 1220, 1106, 736; HR MS *m/z* Calc. for $C_{30}H_{34}N_4O_8$: 578.62. Found: 578.60%.

*4.19. 1N,4N-di[1-carboethoxy-2-methyl-(1S)-propyl]-2,3-dihydroxy-(2S,3S)-butanediamide (**2b**)*

Compound **3b** (0.41 g, 1.03 mmol) was obtained from the derivative (**8b**) (0.61 g, 1.25 mmol), by the same procedure described for obtaining **2a** with 82% yield as a colorless oil. $[\alpha]_D = -2.3$ ($c = 1.29$, CH_2Cl_2); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.51 (d, $J = 7.5$ Hz, 1H), 4.96 (br s, 1H), 4.41 (m, 2H), 4.07 (q, $J = 7.1$ Hz, 2H), 2.13 (m, 1H), 1.24 (t, $J = 7.1$ Hz, 3H), 0.87 (m, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.4, 171.3, 71.5, 61.5, 57.1, 31.2, 19.0, 17.8, 14.2; IR (cm^{-1}) 3404, 2968, 2938, 1737, 1662, 1530, 1206, 1150, 1025; HR MS *m/z* Calc. for $C_{18}H_{32}N_2O_8$: 404.46. Found: 405.10%.

*4.20. 1N,4N-di[l-carboethoxy-2-methyl-(1S, 2S)-butyl]-2,3-dihydroxy-(2S,3S)-butanediamide (**2d**)*

Compound **3d** (0.44 g, 1.01 mmol) was obtained from the derivative (**8d**) (0.64 g, 1.25 mmol), by the same procedure described for obtaining **2a** with 81% yield as a colorless oil. $[\alpha]_D = 67.3$ ($c = 0.98$, CH_2Cl_2); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.50 (d, $J = 8.0$ Hz, 1 H), 4.80 (d, $J = 7.9$, 1H), 4.48 (m, 1H), 4.35 (d, $J = 7.9$, 1H), 4.19 (q, $J = 7.1$ Hz, 2H), 1.88 (m, 1H), 1.30 (m, 5H), 0.89 (m, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.7, 171.0, 70.9, 61.5, 56.4, 37.9, 25.1, 15.5, 14.3, 11.7; IR (cm^{-1}) 3404, 2968, 1736, 1660, 1530, 1203, 1148, 1024; HR MS *m/z* Calc. for $C_{20}H_{36}N_2O_8$: 432.51. Found: 432.72%.

*4.21. 1N,4N-di[2-(4-hydroxyphenyl)-1-carboethoxy-1-(1S)-ethyl]-2,3-dihydroxy-(2S,3S)-butanediamide (**2f**)*

Compound **3f** (0.51 g, 0.96 mmol) was obtained from the derivative (**8f**) (0.77 g, 1.25 mmol), by the same procedure described for obtaining **2a** with 77% yield as a crystalline solid, m.p. 104–105 °C; $[\alpha]_D = -13.7$ ($c =$ 0.95, CH_3OH); ^1H NMR (DMSO-d_6, 200 MHz) δ 9.27 (s, 1H), 7.70 (d, $J = 7.7$, 1H), 6.99 (d, $J = 8.3$ Hz, 2H), 6.67 (d, $J = 8.3$, 2H), 5.87 (d, $J = 7.0$ Hz, 1H), 4.51 (m, 1H),4.27 (d, $J = 7.0$, 1H), 4.04 (q, $J = 7.0$ Hz, 2H), 2.92 (m, 2H), 1.12(t, $J = 7.0$ Hz); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 172.2, 1716, 156.7, 130.8, 126.9, 115.7, 73.0, 61.2, 53.8, 36.9, 14.5; IR (cm^{-1}) 3353, 3333, 3302, 3086, 3033, 2983, 1664, 1532, 1211, 1066, 748, 701; HR MS *m/z* Calc. for $C_{26}H_{32}N_2O_{10}$: 532.54. Found: 532.72%.

*4.22. 1,4-di(benzylamine)-(2R,3R)-butane-2,3-diol (**4a**) [15]*

A solution of compound **7a** (1.20 g, 2.91 mmols) in anhydrous THF (20 ml), was added to a suspension of $LiAlH_4$ (1.30 g, 34.0 mmols) in anhydrous THF (20 ml), under argon at r.t. The reaction mixture was kept under magnetic stirring at reflux temperature for 48 h. After cooling at 0 °C, water (2 ml) and aqueous NaOH at 10% (3 ml) were carefully added, and the mixture was maintained under stirring at r.t. for 1 h, when it was then evaporated under vacuum. The solid residue obtained was dissolved in HCl 1 N (100 ml), extracted with AcOEt (2 × 20 ml). Aqueous NaOH at 50% was added to the aqueous phase, until pH 10, and was extracted with AcOEt (5 × 100 ml). This organic phase was washed with saturated NaCl (50 ml), dried with anhydrous sodium sulfate and evaporated under vacuum. Flash column chromatography with silica gel (NH_4OH conc.:CH_3OH:CH_2Cl_2 0.25:4.75:95) of the residue obtained the amino alcohol (**4a**) (0.48 g, 1.60 mmol) at 55% yield, as a crystalline solid, m.p. 77–79 °C; $[\alpha]_D = -42.3$ ($c = 1.08$, CH_2Cl_2); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.27 (m, 5H), 3.82 (d, $J = 2.3$ Hz, 1H), 3.76 (d, $J = 5.9$ Hz, 1H), 3.08 (dd, $J = 3.4$, 11.2 Hz, 1H), 3.05 (br s, 1H), 2.71 (d, $J = 11.2$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 139.5, 128.7, 128.4, 127.4, 73.1, 54.1, 53.3; IR (cm^{-1}) 3334, 3289, 3086, 3028, 2928, 2873, 1644, 1454, 1254, 1055, 740, 701 HR MS *m/z* Calc. for $C_{18}H_{24}N_2O_2$: 300.40. Found: 300.54%.

*4.23. 1,4-di[1-phenyl-(1S)-ethylamine]-(2R,3R)-butane-2,3-diol (**4b**)*

Compound **4b** (0.55 g, 1.69 mmol) was obtained from the derivative (**7b**) (1.20 g, 2.73 mmol), by the same procedure described for obtaining **4a** with 62% yield as a colorless oil. $[\alpha]_D = +81.3$ ($c = 1.13$, CH_2Cl_2); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.09 (m, 5H), 4.03 (br s, 1H), 3.74 (s, 1H), 4.50 (q, $J = 6.6$ Hz, 1H), 3.08 (dd, $J = 3.4$, 12.0 Hz, 1H), 2.71 (d, $J = 12.0$ Hz, 1H), 1.17 (d, $J = 6.6$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 144.6, 128.7, 127.3, 126.5, 73.1, 58.3, 51.7, 23.6; IR (cm^{-1}) 3302, 3084, 3027, 2967, 2858, 1493, 1452, 1117, 1078, 763, 701; HR MS *m/z* Calc. for $C_{20}H_{28}N_2O_2$: 328.45. Found: 328.30%.

*4.24. 1,4-di[1-phenyl-(1R)-ethylamine]-(2R,3R)-butane-2,3-diol (**4c**)*

Compound **4c** (0.55 g, 1.69 mmol) was obtained from the derivative (**7c**) (1.20 g, 2.73 mmol), by the same procedure described for obtaining **4a** with 62% yield as a colorless oil. $[\alpha]_D = -98.0$ ($c = 0.95$, CH_2Cl_2); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.19 (m, 5H), 4.06 (br s, 1H), 3.60 (m, 2H), 2.88 (dd, $J = 3.0$, 12.0 Hz, 1H), 2.49 (d, $J =$ 12.0 Hz, 1H), 1.31 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 144.5, 128.8, 127.4, 126.9, 73.1, 58.9, 51.9, 24.8; IR (cm^{-1}) 3310, 3084, 3027, 2966, 2854, 1493, 1452, 1118, 1079, 763, 701; HR MS *m/z* Calc. for $C_{20}H_{28}N_2O_2$: 328.45. Found: 328.70%.

5. Pharmacological evaluation [16,17]

The pharmacological evaluation of the derivatives obtained was undertaken using test plates with a PM1 strain cell culture, lymphocytic strain established in culture, expressing the receptors CD4+ and co-receptors C5 and R4 of the HIV-1 and producers of syncytium, incubated with isolated standard virus Z2Z6 purified by passage in cell culture PM-1, having a titter of $3{,}96 \times 10^2$ $TCID_{50}$ ml^{-1} The infection was done using plates having 96-wells, each containing 10^4 cells per well, infected with a multiplicity of infection (MOI) of 0.002. The compounds being evaluated were initially diluted in dimethylsulphoxide (DMSO) to a final concentration of 10 mM and subsequently diluted in base medium RPMI 1640–20 μM.

Nine-wells of the cells infected initially with the isolated HIV Z6 were exposed to decreasing concentrations of the compounds at 20 μM by a factor of 2 (base log 2). The culture medium employed was the RPMI 1640, added with 10% bovine fetal serum, the antibiotics streptavidine–penicicline and L-glutamine. The most concentrated well had a final concentration of 100 μM, with the subsequent dilutions being as follows: 10, 5, 1.25, 0.625, 0.312, 0.156, 0.078 and 0.039 μM.

The last and tenth well was kept as a control of the infection, without the presence of the drug. Each line of ten wells was produced in triplicate, for posterior statistical treatment. INDINAVIR was used as control, in the same dilutions as the compounds being tested, as described, for cytotoxic analysis. The infection was kept in an oven with CO_2 at 5%, at a temperature of 37 °C and verified daily by optical phase microscopy for the analysis of the occurrence of syncytia, which was confirmed on the 4th day after infection.

The technique used for revealing the assay was coloration by bromide of 3-(4,5-dimethylthyazole-2-il)-2,5-diphenyl-tetrazole to measure the cellular viability (MTT technique) [17], on the 6th day after infection. After coloration, the 96-well plate was read in an ELISE counter, with a 490λ, absorption filter. The results were analyzed using a Microsoft EXCEL matrix, with correction of the blanks, and plotting of the emission frequency graph of the assay (in percentage, using as the 100% standard the emission from the viable cells of the wells without infection) as measurement of cellular viability. The value of 50% of emission of the standard was considered as cut-off point for the IC_{50} calculation. This value was attained, after plotting on the graph of the logarithmic regression curve equation, the points obtained from the IC curve prior to the formation of the plateau of the curve.

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References

[1] F. Barré-Sinoussi, J.C. Chermann, M. Rey, M.T. Nugeyre, S. Chamaret, J. Gruest, C. Daugnet, C. Axier-Blin, F. Vezinet-Brum, C. Rouzioux, W. Rozenbaum, L. Moutagneir, Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS), Science 220 (1983) 868–871.
[2] R.C. Gallo, P.S. Sarin, E.P. Gelman, M. Robert-Guroff, E. Richardson, V.S. Kalyanaraman, D. Mann, G. Sidhu, R.E. Stahl, S. Zolla-Pazner, J. Leibowitch, M. Popovic, Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS), Science 220 (1983) 865–867.
[3] A.D. Frankel, J.A.T. Young, HIV-1: fifteen proteins and a RNA, Annu. Rev. Biochem. 67 (1998) 1–25.
[4] E.O. Freed, HIV-1 gag proteins: diverse functions in the virus life cycle, Virology 251 (1998) 1–15.
[5] T.D. Meek, D.M. Lambert, B.W. Metcalf, S.R. Petteway, G.B. Dreyer, HIV-1 protease inhibitors, in: E. De Clercq (Ed.), Design of Anti-AIDS Drugs, Elsevier, Amsterdam, 1990, pp. 225–256.
[6] C. Flexner, HIV-protease inhibitors, New Engl. J. Med. 338 (1998) 1281–1292.
[7] J.W. Erickson, S.K. Burt, Structural mechanisms of HIV drug resistance, Annu. Rev. Pharmacol. Toxicol. 36 (1996) 545–571.
[8] A. Wlodawer, J. Vondrasek, Inhibitors of HIV-1 protease: a major success of structure-assisted drug design, Annu. Rev. Biophys. Biomol. Struct. 27 (1998) 249–284.
[9] M. Marastoni, M. Bergonzoni, F. Bortolotti, R. Tomatis, Symmetry-based HIV protease inhibitors containing (*S*,*S*) or (*R*,*R*) tartaric acid core structure, Arzneim.-Forsch. 47 (1997) 889–892.
[10] K.-H. Budt, A. Peyman, J. Jansen, J. Knolle, C. Meichsner, A. Paessens, D. Ruppert, B. Stowassner, HIV protease inhibitor HOE/BAY 793, structure-activity relationships in a series of C_2-symmetric diols, Bioorg. Med. Chem. 3 (1995) 559–571.
[11] L.J.O. Figueiredo, O.A.C. Antunes, Chemometric classification of HIV-1 protease inhibitors, Int. J. Quantum Chem. 76 (2000) 744–755.
[12] PDB entry: 1B7V.
[13] J.F. Almeida, J. Anaya, N. Martin, M. Grande, J.R. Moran, M.C. Caballero, New enantioselective synthesis of 4-hydroxy-2-

oxopyrrolidine-*N*-acetamide (oxiracetam) from malic acid, Tetrahedron Asymmetry 3 (1992) 1431–1440.

[14] J.E. Lynch, S.M. Riseman, W.I. Laswell, D.M. Tschaen, R.P. Volante, G.B. Smith, I. Shinkai, Mechanism of an acid chloride imine reaction by low-temperature FT-IR-beta-lactam formation occurs exclusively through a ketene intermediate, J. Org. Chem. 54 (1989) 3782–3792.

[15] V.J. Gatto, K.A. Arnold, A.M. Viscariello, S.R. Miller, C.R. Morgan, G.W. Gokel, Syntheses and binding-properties of bibrachial lariat ethers (bibles)—survey of synthetic methods and cation selectivities, J. Org. Chem. 51 (1986) 5373–5384.

[16] H. Nakashima, R. Pauwels, M. Baba, D. Schols, J. Desmyter, E. De Clercq, Tetrazolium-based plaque assay for hiv-1 and hiv-2 and its use in the evaluation of antiviral compounds, J. Virol. Methods 26 (1989) 319–330.

[17] J.P. Vacca, B.D. Dorsey, W.A. Schleif, R.B. Levin, S.L. McDaniel, P.L. Darke, J. Zugay, J.C. Quintero, O.M. Blahy, E. Roth, V.V. Sardana, A.J. Schlabach, P.I. Graham, J.H. Condra, L. Gotlib, P.S. Anderson, E.A. Emini, J.R. Huff, L-735,524: an orally bioavailable human immunodeficiency virus type 1 protease inhibitor, Proc. Natl. Acad. Sci. (USA) 91 (1994) 4096–4100.