ARTICLE TYPE

Spiro-Lactams as Novel Antimicrobial Agents

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ARTICLE HISTORY

Received:

Revised:

Accepted:

DOI:

Abstract: Structural modulation of previous identified lead spiro-β-lactams with antimicrobial activity was carried out. The target chiral spiro-γ-lactams, were synthesized via 1,3-dipolar cycloaddition reaction of a diazo-γ-lactam with electron-deficient dipolarophiles. *In vitro* activity against HIV and *Plasmodium* of a wide range of spiro-β-lactams and spiro-γ-lactams was evaluated. Among these compounds, one derivative with good anti-HIV activity and two with promising antiplasmodial activity (IC_{50} < 3.5 μM) were identified.

- **Objective:** The main objective of this work was to synthesize and evaluate the biologic activity of novel spiro-lactams based on previous identified lead compounds with antimicrobial activity.
- **Results:** A novel synthetic route to chiral spiro-γ-lactams has been established. The studied β- and γ-lactams were not cytotoxic, and three compounds with promising antimicrobial activity were identified, whose structural modulation may lead to new and more potent drugs.
- **Conclusion:** The designed structural modulation of biological active spiro-β-lactams involved the replacement of the four-membered β-lactam ring by a

1873-4294 /19 $58.00+.00

five membered γ-lactam ring. Although, conformational and superimposition computational studies revealed no significant differences between β- and γ-lactam pharmacophoric features, the studied structural modulation did not lead to compounds with similar biological profile. The observed results suggest that the β-lactamic core is a requirement for the activity against both HIV and *Plasmodium*.

Keywords: Anti-HIV agents; Antiplasmodial agents; Spiro-γ-lactams; Spiro-penicinallate; 5-Oxohexahydropyrrolo[2,1-*b*]thiazoles; Dipolar cycloaddition; Diazo compounds.

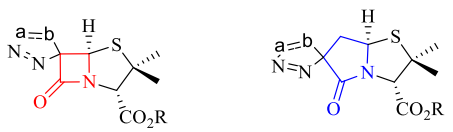
1. INTRODUCTION

Since the discovery of benzylpenicillin in 1928, β-lactams emerged as one of the most important and well-studied class of compounds in both the organic and the medicinal chemistry fields [1-10]. Although the huge impact of β-lactams on public health has been mainly associated with its antibiotic activity, several molecules containing the β-lactam core showed potential activity against other diseases, namely as cholesterol absorption inhibitors, β-lactamase inhibitors, antitumoral and antiviral agents [6, 11-15].

More recently, the interest on γ-lactams has grown, because this structural core is present in a wide range of compounds with biological activity. Molecules containing the γ-lactam moiety were already reported as having anti-inflammatory activity, as CCR4 antagonists, and present in cytotoxic and antineoplastic drugs [16-21].

We have previously reported the synthesis of chiral spirocyclic-β-lactam derivatives **1** via 1,3-dipolar cycloaddition of 6-diazopenicillanates with dipolarophiles [22]. Spiro-2-pyrazoline-β-lactams were obtained from the reaction with acrylonitrile, acrylates or methyl vinyl ketone, whereas the reaction with *N*-substituted-maleimides afforded spiro-1-pyrazoline-β-lactams. Those studies led to the discovery of a lead compound with remarkable anti-HIV (human immunodeficiency virus) and antiplasmodial properties [22-25].

In this work, we extended our previous studies to new spiro-lactams, in order to further explore structure-activity relationships. Life-threatening hypersensitivity reactions are a major problem in the use of β-lactams, therefore the manufacture of these drugs is subjected to ever increasing demanding requirements to avoid cross-contamination of other drugs [26-27]. In order to overcome the stability- and toxicity-related problems of β-lactams, we sought to produce spirocyclic-γ-lactams **2**, corresponding to the replacement of the four-membered β-lactam ring by the five membered γ-lactam ring (Figure 1). We now describe a synthetic strategy to produce these novel γ-lactams derivatives, as well as their evaluation as antimicrobial agents.



Spiro-β-Lactams **1** Spiro-γ-Lactams **2**

Figure 1. General molecular structure of spiro-β-lactams **1** and spiro-γ-lactams **2**.

2. MATERIALS AND METHODS

2.1. Chemistry

Thin-layer chromatography (TLC) analyses were performed using precoated silica gel plates. Flash column chromatography was performed with silica gel 60 as the stationary phase. ^{1}H Nuclear magnetic resonance (NMR) spectra (400 MHz) and ^{13}C NMR spectra (100 MHz) were recorded in $CDCl_3$, CD_3OD or hexadeuterated dimethylsulfoxide (DMSO) as solvents. Chemical shifts are expressed in parts per million (ppm) relatively to internal tetramethylsilane (TMS) and coupling constants (*J*) are in hertz. Infrared spectra (IR) were recorded in a Fourier Transform spectrometer coupled with a diamond Attenuated Total Reflectance (ATR) sampling accessory. Elemental analyses were carried out with an Elemental Vario Micro Cube analyser. High-resolution mass spectra (HRMS) were obtained on a TOF VG Autospect M spectrometer with electrospray ionization (ESI). Melting points (m.p.) were determined in open glass capillaries. Optical rotations were measured on an Optical Activity AA-5 electrical polarimeter. Benzyl (2*S*)-2-(*tert*-butoxycarbonylamino)-4-oxobutanoate **5** [28] was prepared as previously described [29].

2.1.1. (R)-Benzhydryl 2-amino-3-mercapto-3-methylbutanoate hydrochloride 7

To a stirred solution of *D*-penicillamine (1.0 g, 9.2 mmol) in dry methanol (9.2 mL), a solution of diphenyldiazomethane (1.7 g, 9.2 mmol) in dry dichloromethane (28 mL) was added dropwise. After 24 h at room temperature, an additional portion of diphenyldiazomethane (0.7 g, 4.6 mmol) was added and the reaction mixture was stirred for further 96 h. The solvent was removed under reduced pressure and diethyl ether was added (30 mL). The solution was cooled in an ice bath and HCl (1 M) was added dropwise until pH 1 was reached. The product precipitates as a white solid which is then filtered. Yield: 55% (1.78 g). m.p. 133.6-135.0 ºC. $[\alpha]_D^{25}$ = +15 (*c* 1 in MeOH). IR (ATR) ν 747, 906, 1077, 1176, 1222, 1449, 1497, 1513, 1577, 1731, 2486 and 2874 cm^{-1}. NMR ^{1}H (DMSO-d_6) δ= 1.21 and 1.46 (s, 3H), 1.36 and 1.40 (s, 3H), 4.12 and 4.30 (s, 1H), 6.92 and 6.94 (s, 1H), 7.29-7.39 (m, 6H), 7.50 (d, *J* = 7.4 Hz, 4H) and 8.91 (br s, 3H). NMR ^{13}C (DMSO-d_6) δ = 24.9, 25.6, 28.6, 60.5, 44.1, 49.9, 59.4, 62.3, 78.6, 78.8, 126.6, 126.7, 127.2, 127.9, 128.0, 128.1, 128.5, 139.2, 139.4, 139.5, 139.5, 166.2, 166.3. HRMS (ESI-TOF) *m/z*: Calcd for $C_{18}H_{22}NO_2S$ $[M\text{-}Cl]^+$ 316.1366; found 316.1366.

2.1.2. (2S,4S)-Benzhydryl 2-((S)-3-(benzyloxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl)-5,5-dimethylthiazolidine-4-carboxylate 9

D-penicillamine benzhydryl ester hydrochloride **7** (0.762 g, 2.41 mmol) was dissolved in distilled water (7.4 mL), and $KHCO_3$ (0.241 g, 2.41 mmol) was added followed by addition of a solution of aldehyde **5** (0.740 g, 2.41 mmol) in ethanol (7.4 mL). The reaction mixture was stirred at room temperature for 18 h. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography [ethyl acetate/hexane (1:3)] to give thiazolidine **9** as a white solid. Yield: 64% (931 mg). m.p. 168.3-170.0 ºC. $[\alpha]_D^{25}$ = +50 (*c* 1 in CH_2Cl_2). IR (ATR) ν 1170, 1200, 1360, 1418, 1517, 1710, 1746, 2632, 2794, 2841 and 3342 cm^{-1}. NMR ^1H ($CDCl_3$) δ 0.98 (s, 3H), 1.42 (s, 9H), 1.62 (s, 3H), 2.28-2.30 (m, 2H), 2.82 (t, *J* = 12.8 Hz, N*H*), 3.65 (d, *J* = 13.2 Hz 1H), 4.44 (d, *J* = 5.6 Hz, 1H), 4.60-4.67 (m, 1H), 5.15 (dd, *J* = 32 and 12.4 Hz, 2H), 5.25 (d, *J* = 6.8 Hz, N*H*), 6.98 (s, 1H), 7.31-7.35 (m, 15H). NMR ^{13}C ($CDCl_3$) δ 28.2, 28.3, 29.3, 38.4, 52.3, 59.2, 63.8, 67.5, 74.3, 80.1, 126.9, 127.8, 128.5, 128.6, 128.6, 135.2, 139.3, 139.4, 155.2, 168.4, 171.6. HRMS (ESI-TOF) *m/z*: Calcd for $C_{34}H_{41}N_2O_6S$ $[M+H]^+$ 605.2651; found 605.2680.

2.1.3. (3S,6S,7aR)-Benzhydryl 6-((tert-butoxycarbonyl)amino)-2,2-dimethyl-5-oxohexahydropyrrolo[2,1-b]thiazole-3-carboxylate 8

***Method a*:** A solution of compound **7** (1.08 g, 3.41 mmol) and aldehyde **5** (1.05 g, 3.41 mmol) in pyridine (6 mL) was stirred under reflux over 18 h. The pyridine was removed under reduced pressure, the reaction mixture was diluted with ethyl acetate and washed with water. The organic phase was separated off, and the aqueous phase was extracted with ethyl acetate. The organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to give compound **8**. Yield: 24% (405.5 mg)

Method b: To a solution of thiazolidine **9** (119 mg, 0.2 mmol) in toluene (20 mL) a catalytic amount of *p*-toluenesulfonic acid monohydrate was added. The reaction mixture was refluxed over 70 h under N_2 atmosphere. The solvent was removed under reduced pressure and crystallization with ethyl acetate/hexane gave compound **8** as a white solid. Yield: 69% (67 mg). m.p. 203.5-204.8 ºC. $[\alpha]_D^{25}$ = +210 (*c* 0.5 in MeOH). IR (ATR) ν 1157, 1180, 1283, 1695, 2976 and 3307 cm^{-1}. NMR ^1H ($CDCl_3$, 400 MHz) δ = 1.26 (s, 3H), 1.45 (s, 9H), 1.55 (s, 3H), 1.97-2.05 (m, 1H), 3.17-3.20 (m, 1H), 4.60 (br s, 1H), 4.68 (s, 1H), 5.15 (br s, 1H), 5.38 (dd, *J* = 6.0 and 8.0 Hz, 1H), 6.93 (s, 1H), 7.33-7.35 (m, 10H). NMR ^{13}C ($CDCl_3$, 100 MHz) δ = 25.7, 28.3, 31.2, 39.8, 54.2, 57.9, 62.4, 67.4, 78.5, 80.3, 126.9, 127.7, 128.1, 128.4, 128.6, 128.6, 139.2, 167.2, 171.4, 173.1. HRMS (ESI-TOF) *m/z*: Calcd for $C_{27}H_{33}N_2O_6S$ $[M+H]^+$ 497.2098; found 497.2105.

2.1.4. (2S,4R)- and (2S,4S)-2-((S)-3-(Benzyloxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl)-5,5-dimethylthiazolidine-4-carboxylic acid 11

To a solution of *D*-penicillamine (73 mg, 0.49 mmol) in methanol (10 mL), aldehyde **5** (200 mg, 0.65 mmol) was added. After stirring 18 h at room temperature, the solvent was removed under reduced pressure and petroleum ether was added. The product was filtered as a white solid. Yield: 92% (199 mg). The ^{1}H NMR spectrum showed the presence of two diastereoisomers (ratio 64:36). m.p. 87.6-89.1 ºC. IR (ATR) ν 1157, 1366, 1686, 1702, 1734 and 2973 cm^{-1}. NMR ^{1}H (CD_3OD, 400 MHz) *Major isomer*: δ = 1.33 (s, 3H), 1.44 (s, 9H), 1.66 (s, 3H), 2.25-2.37 (m, 2H), 3.64 (s, 1H), 4.21 (dd, *J* = 4.8 and 9.6 Hz), 4.66 (dd, *J* = 5.6 and 8.0 Hz), 5.14-5.23 (m, 2H), 7.36-7.39 (m, 5H). HRMS (ESI-TOF) *m/z*: Calcd for $C_{21}H_{31}N_2O_6S$ $[M+H]^+$ 439.1883; found 439.1897.

2.1.5. (3S,6S,7aR)-6-((tert-Butoxycarbonyl)amino)-2,2-dimethyl-5-oxohexahydropyrrolo[2,1-b]thiazole-3-carboxylic acid 12

A solution of thiazolidine **11** (3.16 g, 7.21 mmol) in toluene (150 mL) was refluxed over 24 h under N_2 atmosphere. The solvent was removed under reduced pressure and diethyl ether was added. The product precipitates as a white solid. Yield: 83% (1.97 g). m.p. decomposes at 225 ºC. $[\alpha]_D^{25}$ = +145 (*c* 1 in MeOH). IR (ATR) ν 1161, 1279, 1513, 1671, 1708, 2961 and 3399 cm^{-1}. NMR ^{1}H (DMSO-d_6, 400 MHz) δ = 1.39 (s, 9H), 1.45 (s, 3H), 1.53 (s, 3H), 2.00-2.08 (m, 1H), 2.75-2.81 (m, 1H), 4.31 (s, 1H), 4.49-4.56 (m, 1H), 5.31 (t, *J* = 8 Hz, 1H), 7.30 (d, *J* = 12 Hz, 1H). NMR ^{13}C (DMSO-d_6 ,100 MHz) δ = 30.9, 33.4, 36.4, 41.9, 58.7, 62.1, 66.5, 72.4, 83.4, 160.3, 174.6, 176.1. HRMS (ESI-TOF) *m/z*: Calcd for $C_{14}H_{23}N_2O_5S$ $[M+H]^+$ 331.1319; found 331.1322.

2.1.6. (3S,6S,7aR)-6-Amino-2,2-dimethyl-5-oxohexahydropyrrolo[2,1-b]thiazole-3-carboxylic acid hydrochloride 10

Method a: To a solution of lactam **8** (405 mg, 0.82 mmol) in dichloromethane (15 mL), HCl (2 M in diethyl ether, 2.05 mL, 4.10 mmol) was added. The reaction mixture was stirred 22 h at room temperature. The solvent was removed under reduced pressure without heating and the product was obtained as a white solid. Yield: 70% (153 mg).

Method b: To a solution of lactam **12** (1.07 g, 3.24 mmol) in *tert*-butanol (122 mL), a solution of HCl (2 M in diethyl ether, 24 mL) was added. The reaction mixture was stirred over 6 days at room temperature. The solvent was removed under reduced pressure without heating, and diethyl ether was added. The product was filtered and obtained as a white solid. Yield: 93% (800 mg). m.p. decomposes at 230 ºC. $[\alpha]_D^{25}$ = +150 (*c* 0.5 in MeOH). IR (ATR) ν 738, 1206, 1412, 1504, 1681, 1733, 2405 and 2935 cm^{-1}.

NMR ^{1}H (DMSO-d_6, 400 MHz) δ = 1.46 (s, 3H), 1.55 (s, 3H), 2.14-2.17 (m, 1H), 2.96-3.01 (m, 1H), 4.42 (s, 1H), 4.45 (t, *J* = 8.4 Hz, 1H), 5.41 (dd, *J* = 8.0 and 6.0 Hz, 1H), 8.85 (s, NH_3). NMR ^{13}C (DMSO-d_6 ,100 MHz) δ = 25.6, 31.3, 35.8, 51.8, 57.3, 62.2, 66.8, 168.2, 168.8. HRMS (ESI-TOF) *m/z*: Calcd for $C_9H_{15}N_2O_3S$ $[M\text{-}Cl]^+$ 231.0796; found 231.0798.

2.1.7. (3S,6S,7aR)-3-Carboxy-2,2-dimethyl-5-oxohexahydropyrrolo[2,1-b]thiazol-6-aminium 4-methylbenzenesulfonate 13

To a solution of lactam **12** (2.23 g, 6.75 mmol) in acetonitrile (34 mL), *p*-toluenesulfonic acid monohydrate (2.57 g, 13.50 mmol) was added. The reaction mixture was stirred overnight at room temperature. The product precipitates as a white solid which was filtered and washed with cold acetonitrile. Yield: 99% (2.69 g). m.p. decomposes at 253 ºC. $[\alpha]_D^{25}$ = +130 (*c* 0.5 in MeOH). IR (ATR) ν 813, 1006, 1124, 1152, 1224, 1411, 1517, 1617, 1707, 2931 and 3412 cm^{-1}. NMR ^{1}H (DMSO-d_6, 400 MHz) δ = 1.46 (s, 3H), 1.55 (s, 3H), 2.04 (dd, *J* = 3.4 and 11.6 Hz, 1H), 2.29 (s, 3H), 2.96-3.01 (m, 1H), 4.37 (s, 1H), 4.49 (dd, *J* = 8.0 and 11.4 Hz, 1H), 5.42 (dd, *J* = 5.8 and 8.0 Hz, 1H), 7.11 (d, *J* = 7.8 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 1H), 8.49 (br s, NH_3). NMR ^{13}C (DMSO-d_6 ,100 MHz) δ = 20.8, 25.7, 31.1, 35.6, 52.0, 57.4, 62.2, 67.0, 125.5, 128.1, 137.6, 145.8, 168.2, 168.9. Anal. Calcd for $C_{16}H_{22}N_2O_6S_2$: C, 47.75; H, 5.51; N, 6.96; S, 15.93. Found: C, 47.47; H, 5.28; N, 6.85; S, 15.01.

2.1.8. (3S,6S,7aR)-3-((Benzhydryloxy)carbonyl)-2,2-dimethyl-5-oxohexahydropyrrolo[2,1-b]thiazol-6-aminium 4-methylbenzenesulfonate 14

To a stirred solution of compound **13** (1 g, 2.49 mmol) in methanol (2.5 mL) a solution of diphenyldiazomethane (483 mg, 2.49 mmol) in dichloromethane (10 mL) was added dropwise. After 24 h at room temperature, an additional portion of diphenyldiazomethane (242 mg, 1.25 mmol) was added and the reaction mixture was stirred for further 20 h. The solvent was removed under reduced pressure and diethyl ether was added. The product precipitates as a beige solid. Yield: 95% (1.34 g). m.p. 117.3-118.7 ºC. $[\alpha]_D^{25}$ = –120 (*c* 0.5 in MeOH). IR (ATR) ν 812, 1007, 1033, 1123, 1154, 1363, 1420, 1495, 1717 and 2964 cm^{-1}. NMR ^{1}H ($CDCl_3$, 400 MHz) δ = 1.11 (s, 3H), 1.32 (s, 3H), 2.28 (s, 3H), 2.80-2.86 (m, 1H), 4.25 (br s, 1H), 4.56 (s, 1H), 5.17 (dd, *J* = 6.0 and 7.9 Hz, 1H), 6.87 (s, 1H), 7.05 (d, *J* = 7.8 Hz, 2H), 7.21-7.31 (m, 10H), 7.73 (d, *J* = 8.0 Hz, 1H), 8.37 (br s, NH_3). NMR ^{13}C ($CDCl_3$, 100 MHz) δ = 21.5, 25.7, 30.8, 35.8, 53.1, 58.2, 62.6, 67.2, 78.7, 126.4, 127.0, 127.8, 128.2, 128.5, 128.7, 128.7, 129.1, 139.3,

139.4, 140.5, 141.3, 167.0, 168.5. HRMS (ESI-TOF) *m/z*: Calcd for $C_{22}H_{25}N_2O_6S$ [M-TsO]$^+$ 397.1580; found 397.1577.

2.1.9. (3S,6S,7aR)-Benzhydryl 6-amino-2,2-dimethyl-5-oxohexahydropyrrolo[2,1-b]thiazole-3-carboxylate 4

To a solution of compound **14** (300 mg, 0.528 mmol) in dichloromethane (16 mL) a saturated aqueous solution of $NaHCO_3$ (16 mL) was added. The reaction mixture was stirred for 30 min at room temperature. The organic phase was separated off, and the aqueous phase was extracted with dichloromethane. The organic extracts were combined, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to give compound **4** as a yellowish oil. Yield: 96% (201 mg). $[\alpha]_D^{25}$ = +128.75 (*c* 4 in CH_2Cl_2). IR (ATR) ν 732, 976, 1172, 1264, 1399, 1450, 1495, 1705, 1735, 2976 and 3377 cm^{-1}. NMR ^{1}H (DMSO-d_6, 400 MHz) δ = 1.24 (s, 3H), 1.52 (s, 3H), 1.73-1.81 (m, 1H), 1.99 (br s, NH_2), 2.84-2.90 (m, 1H), 3.80 (dd, *J* = 8.0 and 11.2 Hz, 1H), 4.58 (s, 1H), 5.34 (dd, *J* = 6.0 and 8.0 Hz, 1H), 6.91 (s, 1H), 7.30-7.46 (m, 10H). NMR ^{13}C (DMSO-d_6, 100 MHz) δ = 25.4, 30.7, 39.8, 54.9, 57.4, 61.7, 66.9, 77.5, 126.4, 126.9, 127.9, 128.1, 128.3, 128.5, 128.5, 139.8, 139.9, 167.1, 175.2. HRMS (ESI-TOF) *m/z*: Calcd for $C_{22}H_{25}N_2O_3S$ [M+H$^+$] 397.1576; found 397.1580.

2.1.10. (3S,7aR)-Benzhydryl 6-diazo-2,2-dimethyl-5-oxohexahydropyrrolo[2,1-b]thiazole-3-carboxylate 3

To an ice-cold solution of freshly prepared amino-γ-lactam **4** (250 mg, 0.63 mmol) in dichloromethane (40 mL), cold water (40 mL) was added followed by $HClO_4$ (1 M, 1.3 mL) and $NaNO_2$ (109 mg, 1.58 mmol). The reaction mixture was stirred at 0 ºC for 1 h. The organic phase was separated off, and the aqueous phase was extracted with dichloromethane. The combined organic extracts were washed with cold saturated NaCl, dried (Na_2SO_4) and concentrated under reduced pressure (no heat) to give compound **3** as a yellow oil which was used without further purification in cycloaddition reactions with dipolarophiles. Yield: 97% (249 mg). IR (ATR) ν 696, 1157, 1262, 1701 and 3286 cm^{-1}. NMR ^{1}H ($CDCl_3$, 400 MHz) δ = 1.28 (s, 3H), 1.56 (s, 3H), 3.20 (dd, *J* = 2.0 and 14.4 Hz, 1H), 3.54 (dd, *J* = 7.6 and 14.4 Hz, 1H), 4.85 (s, 1H), 5.59 (dd, *J* = 1.6 and 7.6 Hz, 1H), 6.94 (s, 1H), 7.29-7.35 (m, 10H). NMR ^{13}C ($CDCl_3$, 100 MHz) δ = 26.3, 26.5, 33.1, 52.3, 53.4, 58.8, 64.4, 69.9, 78.3, 126.9, 127.7, 128.1, 128.4, 128.6, 128.6, 139.2, 168.0, 170.7.

2.1.11. (2R,4S)-Methyl 2-((S)-3-(benzyloxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl)-5,5-dimethylthiazolidine-4-carboxylate 15a and (2S,4S)-methyl 2-((S)-3-

(benzyloxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl)-5,5-dimethylthiazolidine-4-carboxylate 15b

To a solution of thiazolidine **11** (730 mg, 1.51 mmol) in dichloromethane (10 mL) at 0 ºC was added an excess of ethereal diazomethane. The reaction mixture was manually stirred and monitored by TLC. Excess of diazomethane was purged with nitrogen and purification of the crude product by flash chromatography [ethyl acetate/hexane (1:3)] gave, in order of elution, compound **15a** as a yellow oil and compound **15b** as a white solid.

Compound **15a:** Yield: 27% (180 mg). $[\alpha]_D^{25}$ = +45 (*c* 1 in CH_2Cl_2). IR (ATR) ν 1152, 1495, 1695, 1735, 1751, 3281 and 3358 cm^{-1}. NMR ^1H ($CDCl_3$, 400 MHz) δ = 1.16 (s, 3H), 1.43 (s, 9H), 1.60 (s, 3H), 1.91-1.95 (m, 1H), 2.12-2.18 (m, 1H), 3.38 (br s, 1H), 3.69 (br s, 1H), 3.77 (s, 3H), 4.48 (br s, 1H), 4.62 (dd, *J* = 2.4 and 10.4 Hz, 1H), 4.70 (s, 1H), 5.13 (d, *J* = 12.0 Hz, 1H), 5.19 (d, *J* = 12.0 Hz, 1H), 5.83 (d, *J* = 8.0 Hz, 1H), 7.35-7.38 (m, 5H). NMR ^{13}C ($CDCl_3$, 100 MHz) δ = 27.2, 27.8, 28.3, 40.9, 52.2, 52.9, 59.1, 62.8, 67.1, 71.7, 79.9, 128.2, 128.4, 128.5, 128.6, 135.5, 155.6, 169.6, 172.2.HRMS (ESI-TOF) *m/z*: Calcd for $C_{22}H_{33}N_2O_6S$ $[M+H]^+$ 453.2035; found 453.2054.

Compound **15b:** Yield: 35% (241 mg). m.p. 104.5-106.0 ºC. $[\alpha]_D^{25}$ = +50 (*c* 1 in CH_2Cl_2). IR (ATR) ν 1057, 1153, 1202, 1702, 1711, 1746, 2977 and 3322 cm^{-1}. NMR ^1H ($CDCl_3$, 400 MHz) δ = 1.19 (s, 3H), 1.43 (s, 9H), 1.62 (s, 3H), 2.28-2.36 (m, 2H), 2.78 (br s, 1H), 3.55 (d, *J* = 9.2 Hz, 1H), 3.76 (s, 3H), 4.44 (br s, 1H), 4.62 (br s, 1H), 5.19 (dd, *J* = 12.0 and 29.6 Hz, 2H), 5.27 (br s, N*H*), 7.35-7.38 (m, 5H). NMR ^{13}C ($CDCl_3$, 100 MHz) δ = 28.3, 28.4, 29.3, 38.3, 52.1, 52.3, 59.1, 63.9, 67.5, 74.4, 80.1, 128.5, 128.6, 135.2, 155.2, 169.7, 171.7. HRMS (ESI-TOF) *m/z*: Calcd for $C_{22}H_{33}N_2O_6S$ $[M+H]^+$ 453.2041; found 453.2054.

2.1.12. General procedure for the cycloaddition reactions of diazo-γ-lactam 3 and dipolarophiles

To an ice-cold solution of freshly prepared diazo-γ-lactam **3** in dry dichloromethane (7 mL), the appropriate dipolarophile was added. The reaction mixture was stirred at room temperature or at 0 ºC and under N_2 atmosphere for the time indicated in each case. After removal of the solvent under reduced pressure (no heat), the products were isolated by flash chromatography.

2.1.12.1. (3S,3'S,7a'R)-3'-Benzhydryl 4,5-dimethyl 2',2'-dimethyl-5'-oxo-3',5',7',7a'-tetrahydro-2'H-spiro[pyrazole-3,6'-pyrrolo[2,1-b]thiazole]-3',4,5-tricarboxylate 16a

Obtained from compound **3** (204 mg, 0.50 mmol) and dimethyl acetylenedicarboxylate (0.12 mL, 1.0 mmol) as described in the general procedure (reaction time: 4 h) at 0 ºC. Purification by flash chromatography [hexane/ethyl acetate (3:1)] gave compound **16a** as a brown oil. Yield: 72% (0.197 mg). IR (ATR) ν 1125, 1172, 1719, 1735, 2853, 2923 and 3395 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.33 (s, 3H), 1.70 (s, 3H), 3.23 (dd, *J* = 12.8 and 16.8 Hz, 1H), 3.87 (s, 3H), 3.91 (d, *J* = 4.4 Hz, 1H), 3.95 (s, 3H), 4.94 (s, 1H), 5.60 (dd, *J* = 4.4 and 13.2 Hz), 6.97 (s, 1H), 7.31-7.35 (m, 10H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ = 25.6, 30.8, 33.5, 52.3, 52.9, 53.8, 59.9, 71.2, 79.0, 112.0, 126.9, 127.9, 128.1, 128.5, 128.6, 128.7, 138.8, 138.9, 144.0, 144.8, 147.1, 161.5, 161.8, 167.4. HRMS (ESI-TOF) *m/z*: Calcd for $C_{28}H_{28}N_3O_7S$ $[M+H]^+$ 550.1636; found 550.1642.

2.1.12.2. (3S,3'S,7a'R)-3'-Benzhydryl 5-methyl 2',2'-dimethyl-5'-oxo-3',5',7',7a'-tetrahydro-2'H-spiro[pyrazole-3,6'-pyrrolo[2,1-b]thiazole]-3',5-dicarboxylate 16b

Obtained from compound **3** (402 mg, 0.99 mmol) and methyl propiolate (0.18 mL, 1.98 mmol) as described in the general procedure (reaction time: 19 h) at room temperature. Purification by flash chromatography [hexane/ethyl acetate (2:1)] gave compound **16b** as a brown oil. Yield: 38% (189 mg). IR (ATR) ν 696, 1172, 1218, 1720, 2964 and 3407 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.25 (s, 3H), 1.62 (s, 3H), 3.36 (dd, *J* = 4.0 and 16.0 Hz, 2H), 3.86 (s, 3H), 4.90 (s, 1H), 5.52 (dd, *J* = 4.0 and 12.8 Hz, 1H), 6.60 (s, 1H), 6.89 (s, 1H), 7.25-7.28 (m, 10H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ = 24.6, 29.9, 32.6, 52.7, 59.3, 70.1, 77.9, 106.2, 125.9, 125.9, 126.6, 126.7, 126.9, 127.5, 127.6, 139.2, 144.5, 146.1, 161.1, 166.3, 166.7, 173.8. HRMS (ESI-TOF) *m/z*: Calcd for $C_{26}H_{26}N_3O_5S$ $[M+H]^+$ 492.1585; found 492.1588.

2.1.12.3. (1'R,3S,6a'S,7aR)-Benzhydryl 2,2,5'-trimethyl-4',5,6'-trioxo-3,4',5,5',6',6a',7,7a-octahydro-2H,3a'H-spiro[pyrrolo[2,1-b]thiazole-6,1'-pyrrolo[3,4-c]pyrazole]-3-carboxylate 17a

Obtained from compound **3** (210 mg, 0.52 mmol) and *N*-methylmaleimide (92 mg, 0.83 mmol) as described in the general procedure (reaction time: 1 h) at room temperature. Purification by flash chromatography [hexane/ethyl acetate (3:1)] gave compound **17a** as a brown oil with a yield of 12% (34 mg). $[\alpha]_D^{25}$ = −160 (*c* 0.5 in CH_2Cl_2). IR (ATR) ν 728, 1171, 1718, 2968 and 3265 cm^{-1}. NMR ^{1}H ($CDCl_3$, 400 MHz) δ = 1.34 (s, 3H), 1.65 (s, 3H), 2.97 (s, 3H), 3.12 (dd, *J* = 6.8 and 9.6 Hz, 1H), 3.27 (d, *J* = 7.6 Hz, 1H), 4.70 (s, 1H), 5.79 (t, *J* = 6.4 Hz, 1H), 5.87 (d, *J* = 7.6 Hz, 1H), 6.95 (s, 1H),

7.29-7.36 (m, 10H). NMR ^{13}C ($CDCl_3$, 100 MHz) δ = 25.4, 26.1, 31.7, 33.2, 43.1, 59.5, 63.7, 68.0, 78.8, 95.1, 101.1, 126.8, 127.7, 128.2, 128.5, 128.6, 128.7, 138.9, 139.0, 166.8, 168.0, 168.4, 172.4. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{27}H_{26}N_4O_5S$ $[M+H]^+$ 519.1691; found 519.1697.

2.1.12.4. (1'R,3S,6a'S,7aR)-Benzhydryl 4',5,6'-trioxo-5'-phenyl-3,4',5,5',6',6a',7,7a-octahydro-2H,3a'H-spiro[pyrrolo[2,1-b]thiazole-6,1'-pyrrolo[3,4-c]pyrazole]-3-carboxylate 17b

Obtained from compound **3** (210 mg, 0.52 mmol) and *N*-phenylmaleimide (144 mg, 0.83 mmol) as described in the general procedure (reaction time: 1 h) at room temperature. Purification by flash chromatography [hexane/ethyl acetate (3:1)] gave compound **17b** as white solid with a yield of 21% (65 mg). mp: 110.2-113.4 ºC $[\alpha]_D^{25}$ = −200 (*c* 0.5 in CH_2Cl_2). IR (ATR) ν 694, 1159, 1169, 1374, 1388 and 1713 cm^{-1}. NMR ^1H ($CDCl_3$, 400 MHz) δ = 1.34 (s, 3H), 1.67 (s, 3H), 3.18-3.21 (m, 2H), 3.44 (d, *J* = 8.0 Hz, 1H), 4.72 (s, 1H), 5.80 (t, *J* = 6.0 Hz, 1H), 6.01 (d, *J* = 8.0 Hz, 1H), 6.96 (s, 1H), 7.32-7.37 (m, 10H), 7.43-7.50 (m, 5H). NMR ^{13}C ($CDCl_3$, 100 MHz) δ = 26.1, 31.8, 33.3, 43.1, 59.6, 63.7, 68.0, 78.8, 94.8, 101.9, 126.1, 126.8, 127.8, 128.2, 128.5, 128.6, 128.7, 129.3, 129.4, 130.6, 138.9, 139.0, 166.8, 167.3, 167.9, 171.5. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{32}H_{29}N_4O_5S$ $[M+H]^+$ 581.1846; found 581.1853.

2.1.13. General procedure for the conversion to carboxylic acids

To a solution of the appropriate spiro-γ-lactam in anhydrous dichloromethane (7 mL) at -5 °C, anisole (7 equiv) and trifluoroacetic acid (TFA) (25 equiv) were added. The reaction mixture was stirred for 4 h at -5 ºC. The mixture was diluted with cold diethyl ether, and the solvent was evaporated. The residue was dissolved in tetrahydrofuran, saturated aqueous solution of $NaHCO_3$ was added and the mixture was stirred at 0 °C for 15 min. Ethyl acetate was added, the organic phase was separated and the aqueous phase was extracted twice with ethyl acetate. The aqueous layer was then acidified to pH 3 in an ice bath with HCl (1 M) and extracted with ethyl acetate. The organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to give the desired acid.

2.1.13.1. (3S,3'S,7a'R)-4,5-Bis(methoxycarbonyl)-2',2'-dimethyl-5'-oxo-3',5',7',7a'-tetrahydro-2'H-spiro[pyrazole-3,6'-pyrrolo[2,1-b]thiazole]-3'-carboxylic acid 18a

Obtained from compound **16a** (105 mg, 0.191 mmol). Crystallization with ethyl acetate/hexane gave compound **18a** as a grey solid. Yield: 97% (71 mg). mp: decomposes at 184 ºC. $[\alpha]_D^{25}$ = +140 (*c* 0.5 in MeOH). IR (ATR) ν 807, 1069, 1124, 1210, 1387, 1560,

1710, 1741, 2960 and 3357 cm^{-1}. NMR ^{1}H ($CDCl_3$, 400 MHz) δ = 1.57 (s, 3H), 1.72 (s, 3H), 3.23 (dd, *J* = 13.0 and 16.9 Hz, 1H), 3.86 (s, 3H), 3.89-3.96 (m, 4H), 4.76 (s, 1H), 5.56 (dd, *J* = 4.2 and 12.9 Hz, 1H). NMR ^{13}C ($CDCl_3$, 100 MHz) δ = 26.0, 30.3, 32.9, 52.5, 53.1, 53.4, 59.9, 71.3, 112.1, 144.3, 145.0, 147.0, 161.6, 161.9, 170.8. HRMS (ESI-TOF) *m/z*: Calcd for $C_{15}H_{17}N_3O_7S$ $[M+H]^+$ 384.0860; found 384.0865.

2.1.13.2. (3S,3'S,7a'R)-5-(methoxycarbonyl)-2',2'-dimethyl-5'-oxo-3',5',7',7a'-tetrahydro-2'H-spiro[pyrazole-3,6'-pyrrolo[2,1-b]thiazole]-3'-carboxylic acid 18b

Obtained from compound **16b** (522 mg, 1.06 mmol). Crystallization with ethyl acetate/hexane gave compound **18b** as off-white solid. Yield: 86% (30 mg). mp: decomposes at 105 ºC. $[\alpha]_D^{25}$ = +200 (*c* 0.5 in MeOH). IR (ATR) ν 807, 986, 1136, 1188, 1222, 1395, 1641, 1716, 2975 and 3362 cm^{-1}. NMR ^{1}H ($CDCl_3$, 400 MHz) δ = 1.57 (s, 3H), 1.72 (s, 3H), 3.14-3.23 (m, 1H), 3.41-3.48 (m, 1H), 3.92 (s, 3H), 4.79 (s, 1H), 5.56 (dd, 1H, J = 3.9 and 12.7 Hz), 6.66 (s, 1H). NMR ^{13}C ($CDCl_3$, 100 MHz) δ = 26.1, 30.6, 33.2, 52.6, 53.3, 60.2, 71.3, 107.3, 140.6, 145.7, 146.9, 162.1, 170.7. HRMS (ESI-TOF) *m/z*: Calcd for $C_{13}H_{16}N_3O_5S$ $[M+H]^+$ 326.0805; found 326.0807.

2.2. Biological Evaluation

2.2.1. Anti-HIV activity

2.2.1.1.Cell lines

TZM-bl cells (AIDS Research and Reference Reagent Program, National Institutes of Health, USA) were cultured in complete growth medium that consists of Dulbecco's minimal essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml of penicillin-streptomycin (Gibco/Invitrogen, USA), 1 mM of sodium pyruvate (Gibco/Invitrogen, USA), 2 mM of *L*-glutamine (Gibco/Invitrogen, USA) and 1 mM of non-essential amino acids (Gibco/Invitrogen, USA). Cell cultures were maintained at 37ºC in 5% CO_2.

2.2.1.2.Viruses and titration

The HIV-1 SG3.1 subtype B X4, which uses the XCR4 coreceptor, lab-adapted strain was obtained by transfection of HEK293T cells with pSG3.1 plasmid using jetPrime transfection reagent (Polyplus-tranfection SA, Illkirch, France) according to manufacturer's instructions. The 50% tissue culture infectious dose ($TCID_{50}$) of each virus was determined in a single-round viral infectivity assay using a luciferase reporter

gene assay in TZM-bl cells [30-31] and calculated using the statistical method of Reed and Muench.

2.2.1.3.Cellular viability assays

The *in vitro* cytotoxicity of test compounds was evaluated in TZM-bl cells using alamarBlue cell viability reagent (Life Technologies, USA). Cells were cultured in the presence and absence of serial-fold dilutions of the test compounds. Each dilution of each compound was performed in triplicate wells. Medium controls (only growth medium), cell controls (cells without test compound) and cytotoxicity controls (a compound that kill cells) were employed in each assay. The cytotoxicity of each test compound was expressed by the 50% cytotoxic concentration (CC_{50}), corresponding to the concentration of compound causing 50% decrease of cellular viability.

2.2.1.4. Antiviral assays

The antiviral activity of test compounds was determined in a single-round viral infectivity assay using TZM-bl reporter cells, as previously described [30, 32]. Briefly, TZM-bl cells were infected with 200 $TCID_{50}$ of HIV-1 in the presence of serial fold dilutions of the compounds in growth medium, supplemented with diethylaminoethyl-dextran (DEAE-dextran). After 48 h of infection, luciferase expression was quantified with Pierce Firefly Luc One-Step Glow Assay Kit (ThermoFisher Scientific, Rockford, USA) according to the manufacturer's instructions. For each virus and compound dilution, the assay was set up in triplicate wells. Virus controls, cell controls and inhibitors controls (drugs with a known action against each virus) were employed.

2.2.1.5. Statistical analysis

Statistical analysis was performed using Prism version 5.01 for Windows (GrahPad Software, San Diego, California USA, www.graphpad.com) with a level of significance of 5%.

2.2.2. Anti-Plasmodial activity against P. berghei hepatic stages

Inhibition of hepatic stage *Plasmodium* infection by test compounds was determined by measuring the luminescence intensity in Huh-7 cells infected with a firefly luciferase-expressing *P. berghei* sporozoites (Pb-Luc), as previously described [33-34]. Briefly, Huh-7 cells, a human hepatoma cell line, were cultured in 1640 RPMI medium supplemented with 10% v/v fetal bovine serum, 1% v/v nonessential amino acids, 1% v/v penicillin/streptomycin, 1% v/v glutamine, and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7, and maintained at 37 °C with 5% CO_2.

For infection assays, 1.0×10^4 Huh-7 cells (per well) were seeded in 96-well plates the day before drug treatment and infection. Serial dilutions of each compound were then prepared in infection medium. On the day of infection, culture medium was replaced by the appropriate compound concentration and incubated for 1 h. Next, 1.0×10^4 firefly luciferase-expressing *P. berghei* sporozoites, freshly obtained through disruption of salivary glands of infected female *Anopheles stephensi* mosquitoes, were added to each well. An amount of the DMSO solvent equivalent to that present in the highest compound concentration was diluted in infection medium and used as control. Sporozoite addition was followed by centrifugation at 1700×g for 5 min and subsequent incubation for 48 h at 37 ºC with 5% CO_2. The effect of the compounds on the viability of Huh-7 cells was assessed by the Alamar Blue assay (Invitrogen, U.K.) according to the manufacturers protocol, followed by measurement of parasite infection load by a bioluminescence assay (Biotium, USA). Nonlinear regression analysis was employed to fit the normalized results of the dose-response curves, and half maximal inhibitory concentration (IC_{50}) values were determined using Prism version 5.0 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com).

2.3. Computational Methodology

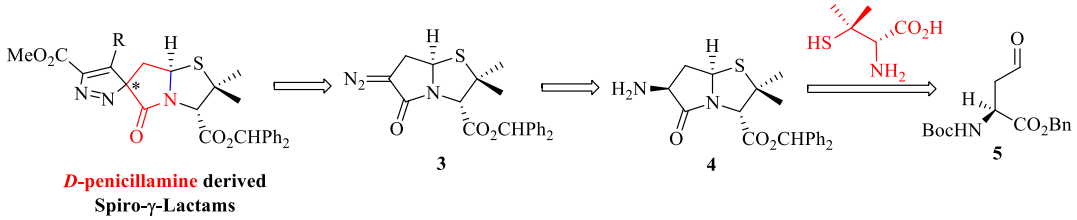
Quantum chemical calculations were carried out in order to explore the structure and the preferred conformations of molecules **18b**, **19**, **BSS-930A** and **BSS-939**. The structures were optimized at the density functional (DFT) level of theory, using the B3LYP hybrid functional [35-37] and the standard 6-31G(d) basis set. All calculations were performed using the GAMESS program package [38] and graphical representations were produced with Gaussview. The optimized structures are depicted in Figure 3 and Figure 5, and in Figures S35 to S38 of the Supporting Information. Energy values and Cartesian coordinates are given in Supporting Information. Molecule superimposition studies were performed with LigandScout 4.3 software [39], and are represented in Figure 4, and in Figures S39 to S50 of the Supporting Information.

3. RESULTS AND DISCUSSION

3.1. Chemistry

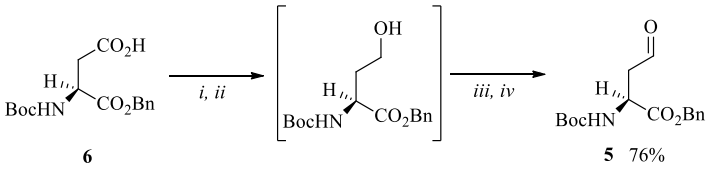
The retrosynthetic analysis of chiral spirocyclic-γ-lactams is outlined in Scheme 1. The pyrazole ring could be formed via 1,3-dipolar cycloaddition of 6-diazo-γ-lactam **3** with electron-deficient dipolarophiles. In order to obtain this chiral 6-diazo-γ-lactam a

synthetic route starting from a natural occurring amino acid was considered. Thus, following the methodology developed by Baldwin *et al.* for related systems, amine **4** could be obtained through a multi-step strategy from *D*-penicillamine. Diazo-γ-lactam **3** could be prepared by reacting amine **4** with sodium nitrite and perchloric acid, as described for the synthesis of 6-diazopenicinallate from 6-aminopenicinallate [40-41].



Scheme 1. Retrosynthetic analysis for the synthesis of chiral spiro-γ-lactams.

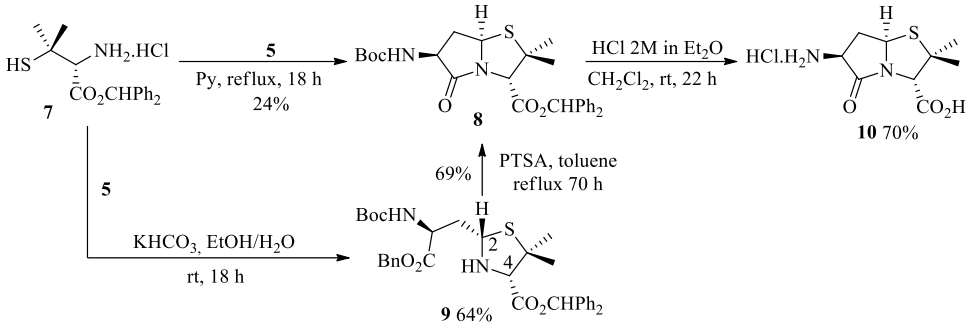
L-Aspartic acid derived aldehyde, benzyl (2*S*)-2-(*tert*-butoxycarbonylamino)-4-oxobutanoate **5** [28] was synthesized by using a method described for the preparation of the *R* enantiomer which involves the initial reduction of compound **6** to the corresponding alcohol, followed by a Swern oxidation (Scheme 2) [29]. The use of the alcohol without purification by flash chromatography afforded aldehyde **5** in 76% overall yield.



Scheme 2. Synthesis of benzyl (*S*)-2-(*tert*-butoxycarbonylamino)-4-oxobutanoate **5**. *i*. *N*-methylmorpholine, ethyl chloroformate, tetrahydrofuran, -15 ºC; *ii*. $NaBH_4$, H_2O, -10 ºC; *iii*. $(COCl)_2$, DMSO, CH_2Cl_2, -60 ºC; *iv*. triethylamine.

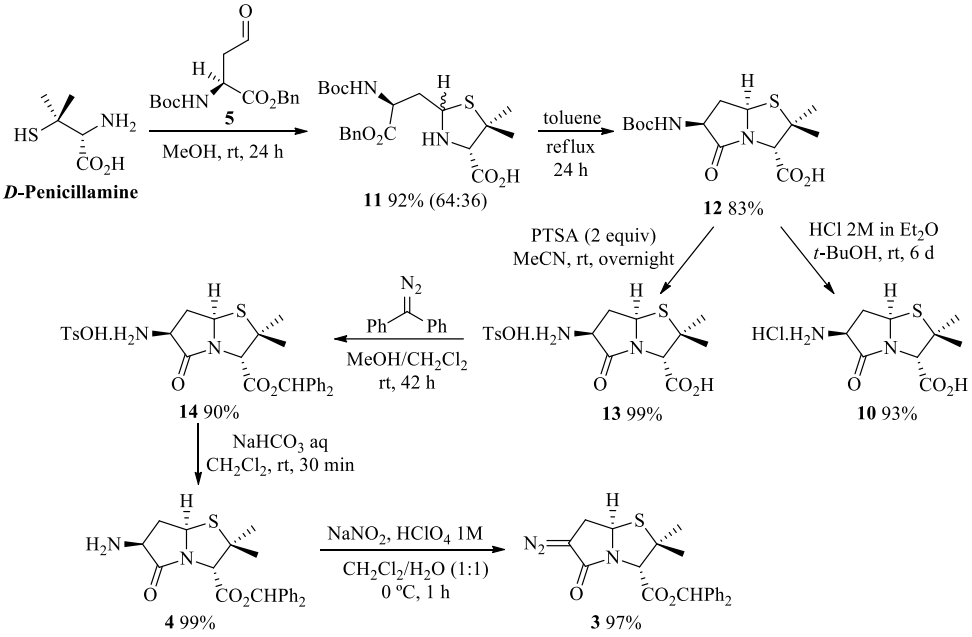
Our initial goal was the synthesis of bicyclic-γ-lactam **8** which was prepared by two distinct methodologies. First, we carried out the reaction of *D*-penicillamine benzhydryl ester hydrochloride **7** with aldehyde **5** in refluxing pyridine, leading to compound **8** in moderate yield (24%). We then explored a two-step approach, which comprised the synthesis of thiazolidine **9** followed by a cyclization step. Thus, the reaction of *D*-penicillamine benzhydryl ester hydrochloride **7** with aldehyde **5**, in presence of base, was carried out giving thiazolidine **9** diastereoselectively and in 64% yield. The stereochemistry of thiazolidine **9** was assigned based on its two-dimensional NOESY (Nuclear Overhauser Effect Spectroscopy) spectrum, in which cross peaks between protons H-2 and H-4 were observed. Thiazolidine **9** was then converted into

bicyclic-γ-lactam **8** (69% yield) by heating in refluxing toluene for 70 h in presence of catalytic amounts of *p*-toluenesulfonic acid (PTSA) (Scheme 3). In order to carry out the amino group deprotection, bicyclic-γ-lactam **8** was treated with an excess of HCl (2 M solution in diethyl ether) in dichloromethane at room temperature to afford bicyclic-γ-lactam **10** in 70% yield. However, together with the *N*-Boc deprotection, the cleavage of the benzhydryl group was also observed. At this stage, since the cleavage of this group was not desirable a different strategy was considered.



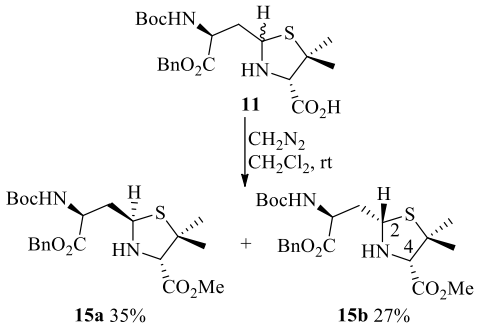
Scheme 3. Synthesis of bicyclic-γ-lactam **8**.

The alternative approach was the use of *D*-penicillamine and to carry out the ester protection with diphenyldiazomethane at a later stage (Scheme 4). Thus, *D*-penicillamine reacted with aldehyde **5** to give a mixture of diastereoisomers **11** (64:36) in 92% yield.



Scheme 4. Synthesis of diazo-γ-lactam **3**.

The structure of thiazolidines **11** was confirmed by their conversion into the corresponding methyl esters **15a** and **15b** by treatment with diazomethane, in 62% overall yield (Scheme 5). Isomers (2*S*,4*R*)-**15a** and (2*S*,4*S*)-**15b** were separated by flash chromatography allowing full characterization. The assigned stereochemistry was supported by the two-dimensional NOESY spectrum of compound **15b**, which showed cross peaks between proton H-2 and proton H-4.



Scheme 5. Esterification of the diastereosisomeric mixture of thiazolidine **11**.

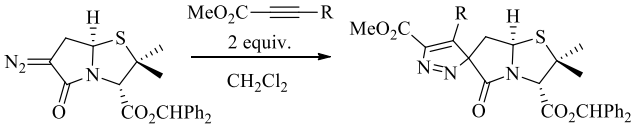
By refluxing the mixture of diastereoisomers **11** in toluene for 24 h, the bicyclic-γ-lactam **12** was obtained diastereoselectively in 83% yield. The deprotection of amino moiety of compound **12** was carried out using the strategy previously mentioned in Scheme 3. Treatment of **12** with an excess of HCl (2 M solution in diethyl ether) in *tert*-butanol at room temperature for 6 days, afforded γ-lactam **10** in 93% yield. However, a faster *N*-Boc deprotection could be achieved using *p*-toluenesulfonic acid in acetonitrile, as described by Chauvett *et al.* for other systems [42]. In fact, the reaction of compound **12** under those conditions led to compound **13** in quantitative yield. The reaction of γ-lactam **13** with diphenyldiazomethane afforded γ-lactam benzhydryl ester **14** in 90% yield. Subsequent treatment of **14** with an aqueous solution of $NaHCO_3$ gave the amino-γ-lactam **4** in quantitative yield. Finally, the treatment of amino-γ-lactam **4** in a biphasic solvent system, as described by Sheehan and Commons for β-lactamic systems [43], with sodium nitrite in presence of $HClO_4$ allowed the synthesis of the *D*-penicillamine-derived diazo-γ-lactam **3** in 97% yield.

Next, we studied the chemical behavior of diazo-γ-lactam **3** as 1,3-dipole in the presence of electron-deficient alkynes, in order to obtain the target spiro-γ-lactams **16** (Table 1). The cycloaddition of diazo-γ-lactam **3** with dimethyl acetylenedicarboxylate, performed at 45 ºC for 4 hours, led to spiro-γ-lactam **16a** in 30% yield (entry 1). Carrying out the cycloaddition reaction at room temperature did not significantly improve the

isolated yield of spiro-γ-lactam **16a** (entries 2 and 3). However, decreasing the temperature to 0 ºC and carrying out the reaction for 4 h afforded spiro-lactam **16a** in 72% yield (entry 4).

The 1,3-dipolar cycloaddition of diazo-γ-lactam **3** with methyl propiolate was also studied, leading to the desired spiro-γ-lactam **16b** in a regioselective fashion and in moderate yields, regardless of the reaction conditions studied (entries 5-7).

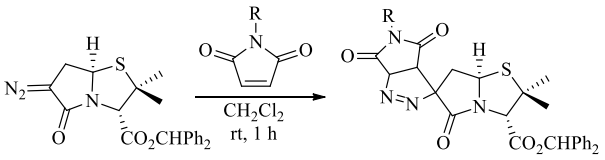
Table 1. 1,3-Dipolar cycloadditions of 6-diazo-γ-lactam **3** with electron-deficient alkynes.



3 **16a** R = CO_2Me **16b** R = H

entry	dipolarophile	reaction conditions	isolated yield (%)
1	R = CO_2Me	45 ºC, 4 h	**16a**, 30
2	R = CO_2Me	rt, 4 h	**16a**, 31
3	R = CO_2Me	rt, 1 h	**16a**, 34
4	R = CO_2Me	0 ºC, 4 h	**16a**, 72
5	R = H	rt, 8 h	**16b**, 24
6	R = H	rt, 19 h	**16b**, 38
7	R = H	0 ºC, 4 h	**16b**, 28

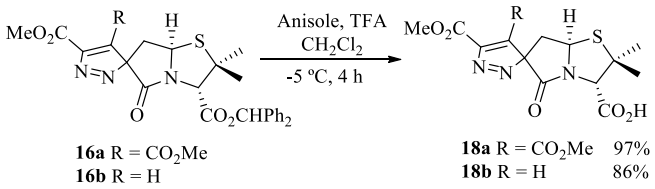
The work was also extended to the cycloaddition of diazo-γ-lactam **3** with *N*-substituted maleimides (Scheme 6). The reaction with an excess of *N*-methylmaleimide at room temperature gave spiro-γ-lactam **17a** in 12% yield. Carrying out the cycloaddition reaction with *N*-phenylmaleimide under the same reaction conditions afforded compound **17b** in 21% yield. The cycloaddition reactions with *N*-substituted maleimides were also studied at 0 ºC but the results obtained were unsatisfactory.



3 **17a** R = Me 12% **17b** R = Ph 21%

Scheme 6. 1,3-Dipolar cycloadditions of 6-diazo-γ-lactam **3** with *N*-substituted maleimides.

The conversion of the spiro-γ-lactams **16** bearing a benzhydryl ester moiety into the corresponding carboxylic acid derivative **18** was also studied (Scheme 7). Treatment of spiro-γ-lactams **16a** and **16b** with anisole and TFA in dichloromethane at -5 ºC gave spiro-γ-lactams **18a** and **18b** in 97% and 86% yield, respectively.



Scheme 7. Deprotection of the carboxylate group of spiro-γ-lactam **16**.

3.2. Biological evaluation

3.2.1. Anti-HIV activity

Initially, *in vitro* cytotoxicity assays in TZM-bl cells were performed for a wide range of γ-lactams, and some β-lactams previously synthesized (Figure 2) [23]. From a library of 19 compounds, 18 of them showed no cytotoxicity and their antiviral activity was evaluated (Table 2).

The antiviral activity of the selected 18 compounds was evaluated in a single-round viral infectivity assay against an HIV-1 isolate and resulted in the identification of three compounds (**4, BSS-975B** and **BSS-1028**) with moderate to good antiviral activity. Compound **4** was the only γ-lactam which exhibited activity, with a maximum percentage of inhibition (MPI) at 10 μg/mL of 64%. **BSS-1028** exhibited a MPI at 10 μg/mL of 79%. Although these compounds showed some degree of anti-HIV activity, they were not considered promising enough and their IC_{50} values were not calculated. Nevertheless, these molecules can still constitute a promising starting point for structural modulation on the search for additional compounds with anti-HIV activity.

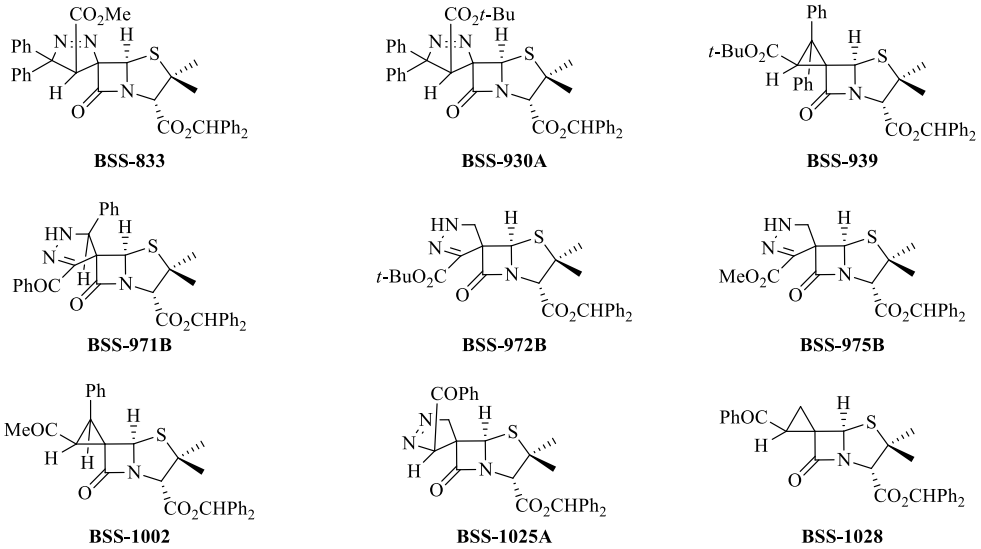


Figure 2. Library of tested spiro-β-lactams

Table 2. CC_{50} values and maximum percentage of inhibition of an HIV-1 isolate (SG3.1).

Compound	CC_{50} (µg/mL)	MPI (10 µg/mL)
4	44.53	64%
8	51.07	40%
12	60.87	48%
14	53.37	47%
15	48.95	46%
16[a]	30.34	18%
17[a]	46.61	0%
17b	52.94	0%
18[a]	52.34	30%
18b	108.6	27%
BSS-833	48.7	0%
BSS-930A	48.1	0%
BSS-939	42.34	0%
BSS-971B	51.14	4%
BSS-972B	9.28	----
BSS-975B	50.1	56%
BSS-1002	50.25	0%
BSS-1025A	48.91	0%
BSS-1028	48.5	79%

3.2.2. Anti-Plasmodium activity

In order to evaluate the antiplasmodial potential of some spiro-γ-lactams and spiro-β-lactams, *in vitro* assays against the hepatic stage of *P. berghei* infection were performed (Table 3, Figures S31-S34). Compounds were tested at 1 and 10 μM, and DMSO was used as a negative control. Our results showed that none of the tested compounds displayed cytotoxicity against the Huh-7 host cells. Among the 17 compounds evaluated, 9 had an IC_{50} against *P. berghei* under 10 μM.

Table 3. Compound activity against *P. Berghei* hepatic stages.

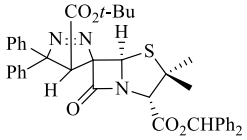
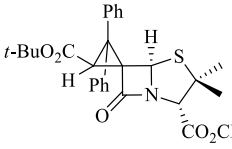
Compound	**IC_{50} against *P. berghei hepatic stages* (μM)**
8	>10
12	>10
13	>10
14	>10
17[a]	Between 1 and 10
17b	>10
18[a]	>10
18b	>10
BSS-833	Between 1 and 10
BSS-930A	Between 1 and 10
BSS-939	Between 1 and 10
BSS-971B	Between 1 and 10
BSS-972B	Between 1 and 10
BSS-975B	>10
BSS-1002	Between 1 and 10
BSS-1025A	Between 1 and 10
BSS-1028	Between 1 and 10

Since **BSS-930A** and **BSS-939** appeared as the most potent of the 9 molecules showing anti-*Plasmodium* activity below 10 μM, their IC_{50} values were determined and found to be 3.32 ± 0.23 and 2.67 ± 0.22 μM, respectively (Table 4).

These two compounds share a high structural similarity. In fact, **BSS-930A** is a precursor of **BSS-939**, the latter being obtained from the N_2 extrusion and subsequent ring contraction of the former's pyrazole ring. The fact that all **BSS-939**'s pharmacophoric features are present on **BSS-930A** is a likely explanation for the similar activities observed for both compounds. The high structural similarity observed between these two

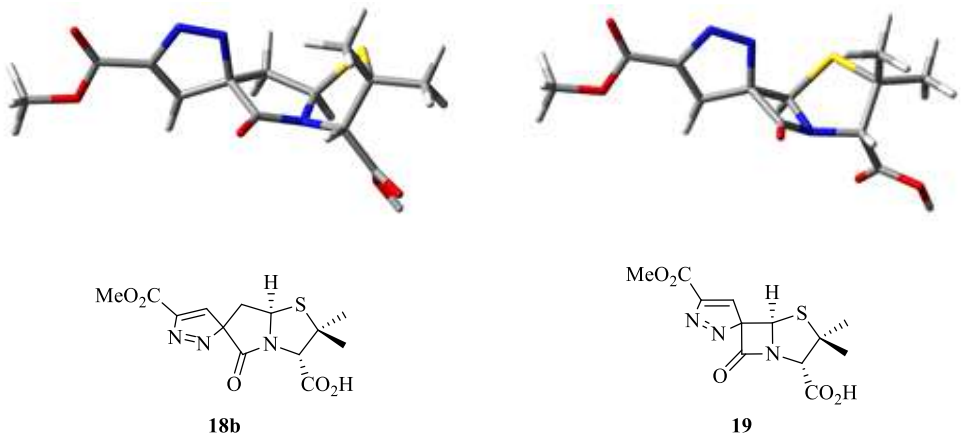
active molecules suggests that their common substructure may constitute a scaffold for structural modulation and optimization on the search for new molecules with potential activity against *Plasmodium* species.

Table 4. IC_{50} values against *P. berghei* hepatic stages. Values are reported as the mean of independent determinations ± the standard deviation.

Compound	**IC_{50} against *P. berghei* hepatic stages (μM)**
BSS-930A 	3.32 ± 0.23
BSS-939 	2.67 ± 0.22

3.3. Computational Studies

Quantum chemical calculations, at the DFT level of theory, were carried out in order to explore the structure and the preferred conformations of selected molecules. Conformational studies of compounds **18b** and **19** showed that both γ- and β-lactam compounds presented similar minimum energy conformations, despite the difference on the lactam ring size (Figure 3).



18b **19**

Figure 3. Optimized geometries of γ-lactam **18b** and its β-lactam analogue **19**.

Similar results were obtained for the superposition studies between γ- and β-lactam minimum energy conformations which presented an almost perfect overlap between the pharmacophoric features of both molecules. These results indicate that the γ-lactam molecule should be capable of reproducing the active β-lactam analogue pharmacophoric three-dimensional disposition and, consequently, it should also be able to reproduce its interactions with molecular targets. The molecular superposition and the pharmacophoric features shared between the two molecules are represented on Figure 4.

Notwithstanding such structural, conformational and pharmacophoric similarities, the γ-lactam molecule showed no relevant anti-HIV and anti-*Plasmodium* activity, contrary to its β-lactam counterpart. Such result suggests that the presence of the 4-membered β-lactam ring appears to play a crucial role in the both anti-HIV and anti-*Plasmodium* activity of spiro-lactams.

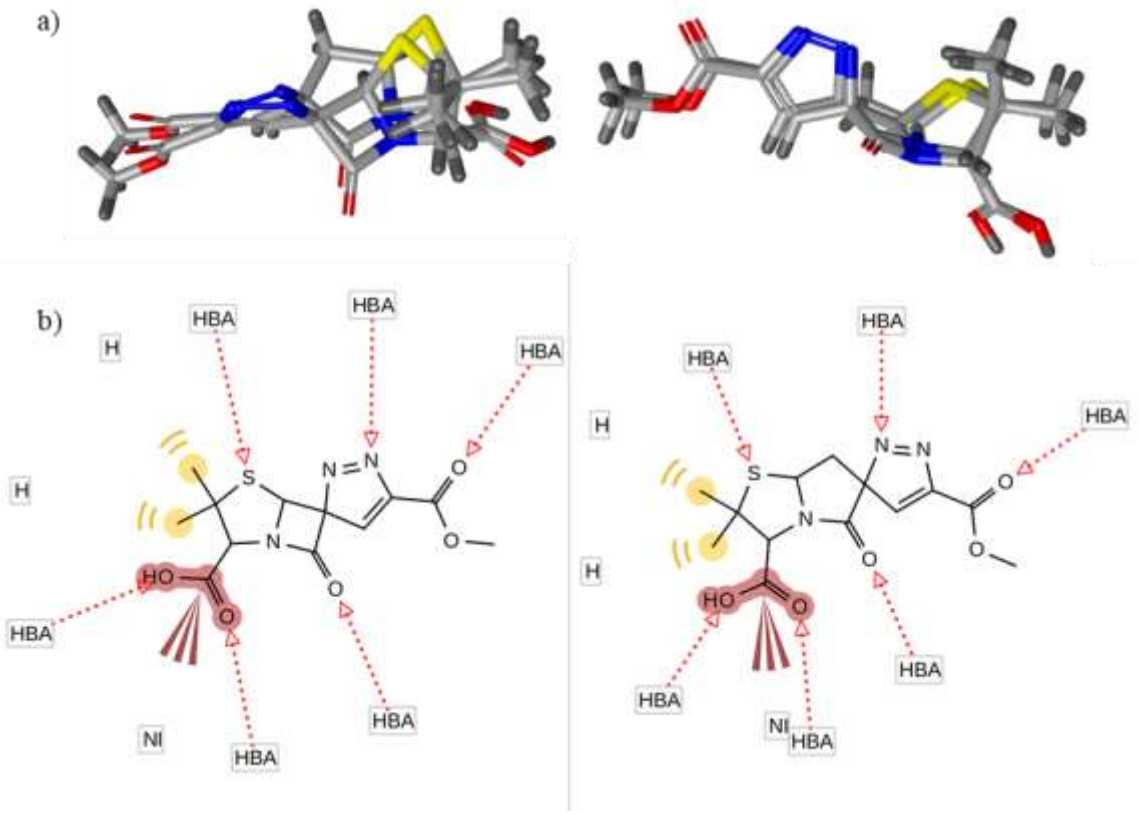


Figure 4. a) Three-dimensional superposition between γ-lactam **18b** and its β-lactam analogue **19** minimum energy conformations; **b)** Depiction of the shared pharmacophoric features between the lactams **18b** and **19** minimum energy conformations. The represented features are Hydrogen Bond Acceptors (HBA), hydrophobic features (H) and Negative Ionizable (NI).

The structural similarity of compounds **BSS-930A** and **BSS-939** suggests that the two molecules' minimum energy conformations are also similar (Figure 5). This was confirmed by the conformational study where both molecules showed a very similar conformation at their minimum energies, except for the orientation of the two phenyl substituents at spiro-rings. Such positional difference is related to the different rings sharing one carbon atom with the penicillanic bicyclic system, a 4,5-dihydro-3*H*-pyrazole (**BSS-930A**) *vs.* a cyclopropane (**BSS-939**) ring and may explain the slight difference observed between **BSS-930A** and **BSS-939** anti-*Plasmodium* activities.

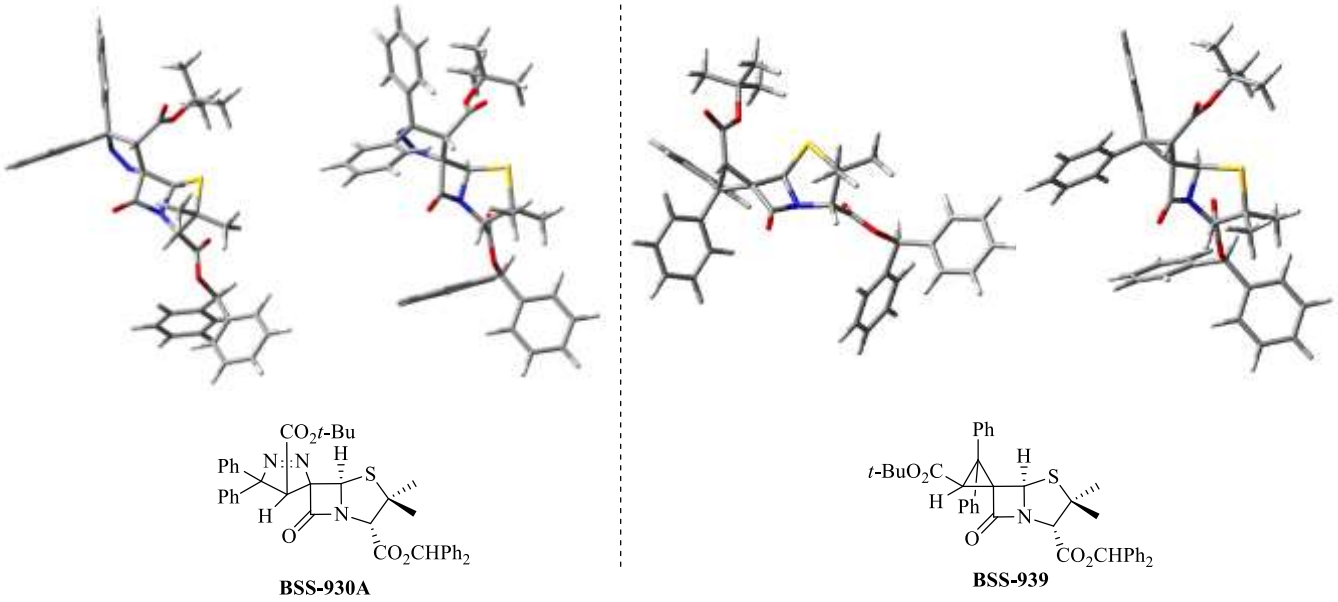


Figure 5. Optimized geometries of **BSS-930A** and **BSS-939**.

CONCLUSION

In summary, a structural modulation of previous lead compounds with antimicrobial activity was carried out, leading to the synthesis of new chiral spiro-γ-lactams. Assessment of the *in vitro* activity of a wide range of spiro-β-lactams and spiro-γ-lactams against HIV and *Plasmodium* led to the identification of one derivative with good anti-HIV activity, and two with promising anti-*Plasmodium* activity. The results suggest that the β-lactamic core is an important structural feature to ensure activity against both HIV and *Plasmodium*. This information will be a good starting point for other structural modulations aiming at the development of new and more potent drugs against HIV and *Plasmodium*.

SUPPORTING INFORMATION

Supporting Information is available at …

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

A.J.S.A., N.G.A., C.C.C., M.I.M.E., M.I.L.S., S.M.M.L. and T.M.V.D.P.M., thanks the Coimbra Chemistry Centre (CQC) supported by the Portuguese Agency for Scientific Research, "Fundação para a Ciência e a Tecnologia" (FCT), through Project UID/QUI/00313/2019. A.J.S.A. and N.G.A. thank FCT for fellowships SFRH/BD/128910/2017 and PD/BD/135287/2017, respectively. We also acknowledge the UC-NMR facility for obtaining the NMR data (www.nmrccc.uc.pt). Ana Filipa Teixeira is gratefully acknowledged for *Plasmodium* parasite production. M.P. acknowledges FCT for grant PTDC-SAU-INF-29550-2017 and for contract CEEC-2017.

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