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Synthesis of mono-, bis-spiro- and dispiro-β-lactams and evaluation of their antimalarial activities

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ABSTRACT

Some new mono-, bis-spiro- and dispiro-β-lactams have been synthesized from imines derived from 9*H*-fluoren-9-one and a ketene derived from 9*H*-xanthene-9-carboxylic acid or phenoxyacetic acid by a [2+2] cycloaddition reaction in good to excellent yields varying from 45 to 83%. The biological activity of these monocyclic β-lactams was successfully investigated against *Plasmodium falciparum* K14 resistant strain with excellent EC_{50} values up to 5 μM.

1. Introduction

The four-membered β-lactam ring is recognized as a key structural unit of the most widely employed class of β-lactam antibiotics.[1] The constant need for new drugs showing broader biological activity and the necessity for new β-lactam antibiotics to combat microorganisms that have built up resistance against traditional drugs,[2] have maintained the interest of organic chemists for almost half a century. A large number of methods are available for the syntheses of newer 2-azetidinones and the topic has been extensively reviewed.[3] The [2+2] cyclocondensation of ketenes with imines, a procedure commonly known as the Staudinger reaction is perhaps the most convenient procedure for the synthesis of the β-lactam ring skeleton.[4,5] Also, several syntheses of spiro-β-lactams have been reported in the literature,[6] and many researchers have accomplished the synthesis of spiro-β-lactams through cycloaddition reactions employing different ketenes and imines in recent years.[7] Spiro penicillins and cephalosporins have been reported[8] and only a few examples of simple spiro-β-lactams were known until 1990.[9] Spiro-β-lactams are interesting compounds, which can act as antiviral,[10] antibacterial agents.[7h] More recently, a cholesterol absorbtion inhibitor,[11] such as Sch 58053 **1** was discovered.[12] The proline derived β-lactams **2**[13] were designed as β-turn mimetics and used for synthetic applications. α,α-Disubstituted β-amino esters,[14] diazabicyclic compounds[15] and other densely functionalized nitrogenated five-membered-ring systems[16] have renewed the interest in these compounds.From a synthetic point of view spiro-β-lactam derivatives, such as dispiro-1,2,4-trioxolanes and dispiro-1,2,4,5-tetraoxanes have been recently obtained by using different methodologies and found to possess antimalarial activity.[8,9,13,17–21] In this article we report the synthesis in a single step of new mono-, bis-spiro- and dispiro-β-lactams by using a [2+2] cycloadition reaction (Staudinger reaction) and the evaluation of their potent antimalarial activity against *Plasmodium falciparum* K14 resistant strain.

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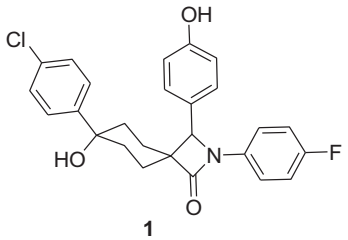
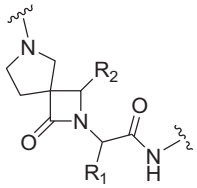


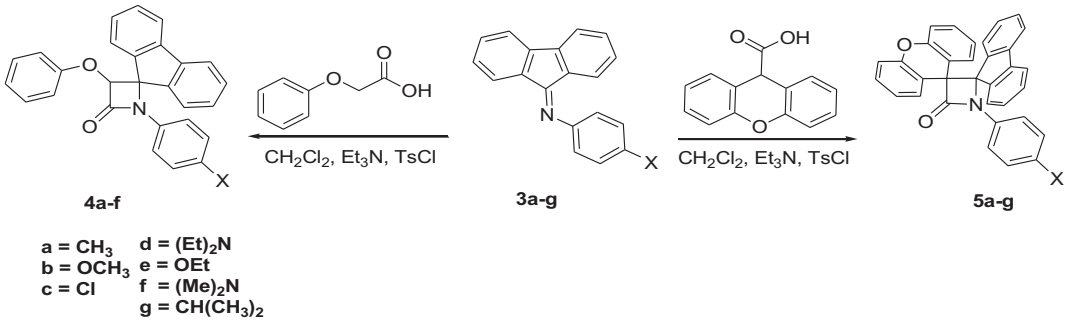
1
Sch 58053



2
Prolinederived β-turn mimetics

2. Results and discussion

The synthesis of spiro- and dispiro-β-lactams **4a**–**f** and **5a**–**g** was achieved according to the outlined procedure in Scheme 1. Typically, imines **3a**–**g** were prepared from 9*H*-fluoren-9-one and substituted anilines with catalytic acetic acid in refluxing ethanol. It is noteworthy that *ortho* and *meta* substituted amines do not react under these conditions. These Schiff bases were subsequently treated with 9*H*-xanthene-9-carboxylic acid or phenoxyacetic acid in the presence of triethylamine and tosyl chloride to afford the expected spiro- and dispiro-β-lactams **4a**–**f** and **5a**–**g** in good to excellent isolated yields varying from 45 to 83%.



Scheme 1. Synthesis of spiro- and dispiro-β-lactams.

The reaction progress was monitored by TLC and the presence of a new compound was easily confirmed. As an example, the IR spectrum for **5a** showed the characteristic absorption of a β-lactam carbonyl at 1755 cm^{-1}. The ^1H NMR spectrum exhibited the methyl protons as a singlet at 2.02 ppm, and aromatic protons as a multiplet at 6.53–8.32 ppm. The absence of doublets of H3 and H4 for the β-lactam ring confirms the spiro structure of **5a**. The ^{13}C NMR spectrum exhibited the following signals: methyl group at 20.8 ppm, C-3 at 64.2 ppm, C-4 at 69.1 ppm, and aromatic carbons at 116.3–164.3 ppm, the β-lactam carbonyl appearing at 169.1 ppm. The GC–MS analysis showed m/z=477 [M^+] as expected for this derivative. According to this methodology, we have been able to prepare numerous other spiro- and dispiro-β-lactams in good to moderate yield (Table 1), which have been unambiguously characterized by IR and NMR spectroscopy.

On the other hand, we envisioned, according to the same strategy, performing the synthesis of new bis-spiro- and bis-dispiro-β-lactams derivatives. Thus, treatment of imines **6a**,**b** with 9*H*-xanthene-9-carboxylic acid or phenoxyacetic acid in the presence of triethylamine and tosyl chloride afforded the expected bis-spiro and bis-dispiro-β-lactams **7** and **8a**,**b** in 45–48% isolated yields, respectively (Scheme 2).

All of these newly synthesized β-lactams derivatives were subsequently evaluated for their biological activities. First of all, it has been demonstrated that these compounds do not possess significant antimicrobial efficiency against Gram-positive *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli* or *Pseudomonas aeruginosa* and fungi, such as *Candidaalbicans* and *Candida glabrata* with MICs values greater than 125 μg/mL in all cases. On the other hand, none of the compounds exhibited specific antiviral activity, which means that they did not inhibit the replication (induction of viral cytopathogenicity) of any of the viruses tested (herpes simplex, vesicula stomatitis, vaccinia and coxsackie viruses) at a concentration that was ≥fivefold lower than the minimum cytotoxic concentration which has been determined against HEL, VERO or HeLa cells and which varied from 4 μM to >100 μM. Nevertheless, good to excellent antimalarial activities have been obtained against chloroquine-resistant *P. falciparum* K14 strain as outlined in Table 2 for spiro- and dispiro-β-lactams compounds with IC_{50} varying from 5 to 32.2 μM. It is also noteworthy that the best derivatives **4d** and **5e** present a low cytotoxicity up to 100 μM whatever the considered cells. On the other hand, no significant antimalarial activity was encountered for bis-spiro- and dispiro-β-lactams **7**, **8a**,**b**, all of this suggesting a quite strong influence of the structure of the considered lactam derivative on the mechanism of action.

3. Conclusion

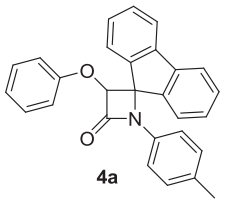
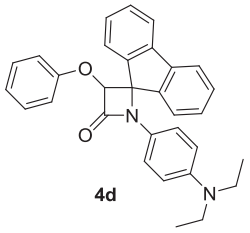
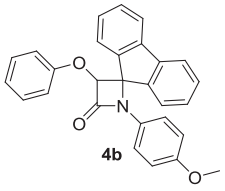
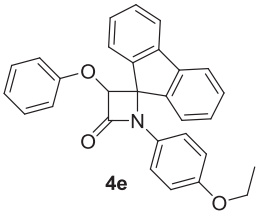
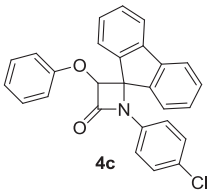
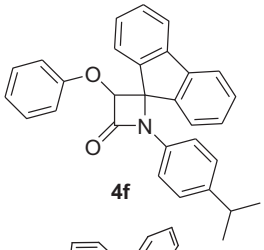
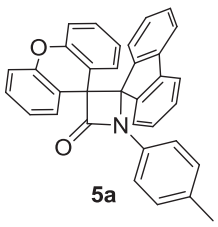
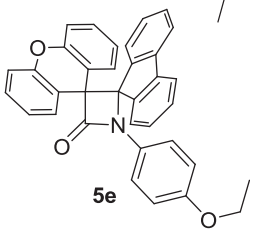
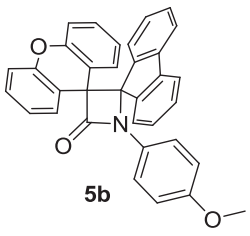
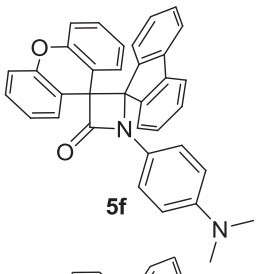
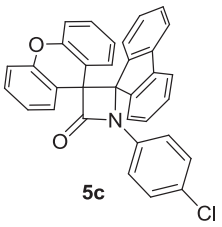
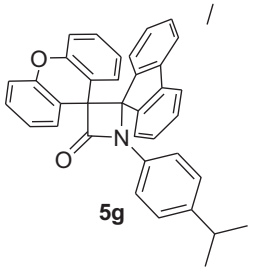
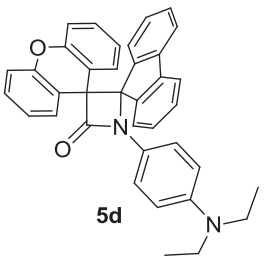
In conclusion, we have been able to prepare some new mono-, bis-spiro- and dispiro-β-lactams from imines derived from 9*H*-fluoren-9-one and ketenes derived from 9*H*-xanthene-9-carboxylic acid or phenoxyacetic acid and presenting good to excellent antimalarial activities against a *P. falciparum* K14 resistant strain. Further work is now under current investigation in order to improve the structure of potent more active compounds and elucidate the mechanism of action of these derivatives since this latter remains unclear to date.

4. Experimental section

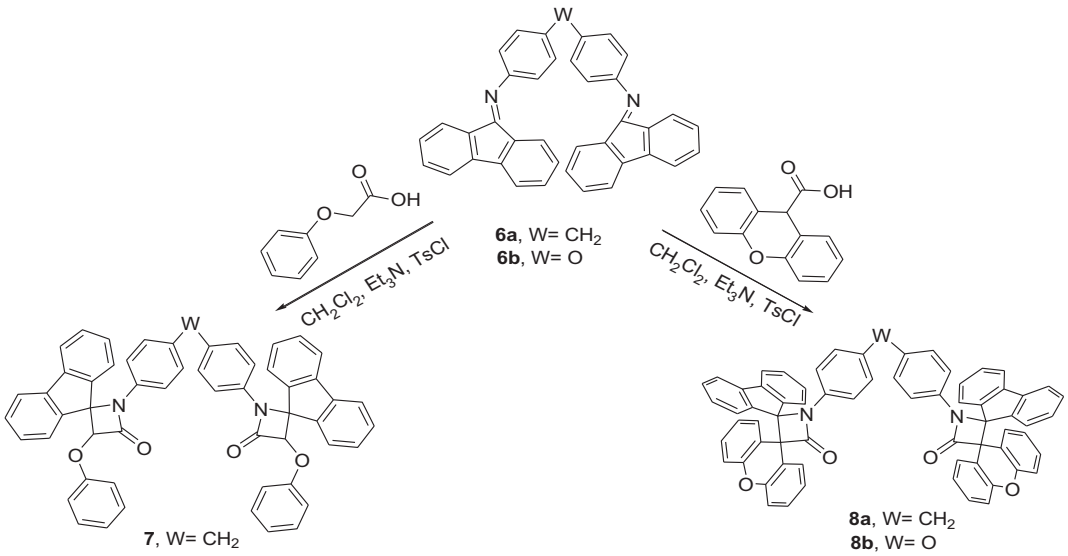
4.1. General

All needed chemicals were purchased from Merck, Fluka and Acros chemical companies. All reagents and solvents were dried prior to use according to standard methods.[22] IR spectra were run on a Shimadzu FT-IR 8300 spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 and $CDCl_3$ using a Bruker Avance DPX instrument (^1H NMR 250 MHz, ^{13}C NMR 62.9 MHz). Chemical shifts were reported in parts per million (δ) downfield from TMS. All of the coupling constants (J) are in hertz. The mass spectra were recorded on a Shimadzu GC–MS QP 1000 EX instrument. Elemental analyses were run on a Thermo Finnigan Flash EA-1112 series. Melting points were determined in open capillaries with Buchi 510 melting point apparatus. Thin-layer chromatography was carried out on silica gel F_{254} analytical sheets obtained from Fluka. Column chromatography was performed on Merck Kieselgel (230–270 mesh).

Table 1
Structures and isolated yields of synthesized spiro- and dispiro-β-lactams

Entry	Product	Yield[a] (%)	Entry	Product	Yield[a] (%)
1	 **4a**	73	4	 **4d**	76
2	 **4b**	70	5	 **4e**	75
3	 **4c**	72	6	 **4f**	48
7	 **5a**	55	11	 **5e**	83
8	 **5b**	55	12	 **5f**	65
9	 **5c**	77	13	 **5g**	63
10	 **5d**	80			

[a] Isolated yield of pure products.



Scheme 2. Synthesis of bis-spiro- and dispiro-β-lactams **7**, **8a**,**b**.

Table 2
Antimalarial activity and cytotoxicity of the new mono-, bis-spiro- and dispiro-β-lactams

Compound	IC_{50} (μM) *P. falciparum* K14	Minimum cytotoxic concentration (μM)		
		HEL	VERO	HeLa
4a	10.7	100	100	100
4c	28.5	>100	≥4	20
4d	6.4	≥100	100	>100
4f	11.1	>100	100	>100
5a	18.0	>100	≥20	20
5b	32.2	>100	100	>100
5c	28.1	>100	4	>4
5d	15.8	≥100	100	>100
5e	5.0	>100	100	>100
5f	29.8	≥100	>100	>100
5g	17.3	≥100	20	>100
7	>50	≥100	>20	>100
8a	>50	≥100	100	>100
8b	>50	≥100	100	>100

4.2. General procedure for the preparation of Schiff bases 3a–g

A mixture of an aromatic amine (1.00 mmol) and 9*H*-fluoren-9-one (1.50 mmol) with catalytic acetic acid (100 μL) was refluxed in ethanol (20 mL) for 12 h. Evaporation of the solvent afforded the crude imines either as oil or solid, which were used for the next step without further purification.

4.3. General procedure for the synthesis of spiro- and dispiro-β-lactams 4a–f and 5a–g

A mixture of the previous crude Schiff base, triethylamine (5.00 mmol), 9*H*-xanthen-9-carboxylic acid or phenoxyacetic acid (1.50 mmol) and tosyl chloride (1.50 mmol) in dry CH_2Cl_2 (15 mL) was stirred at room temperature for 15 h. Then it was washed with HCl 1 N (20 mL), saturated $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was dried (Na_2SO_4), filtered and the solvent was evaporated to give the product as a crystal, which was then purified by recrystallization from ethanol.

*4.3.1. 3-Phenoxy-1-p-tolylspiro[azetidine-2,9′-fluoren]-4-one (**4a**).* White solid (yield 73%). Mp: 140–142 °C. IR (KBr, cm^{-1}): 1742 (CO β-lactam). ^1H NMR δ (ppm): 2.08 (Me, s, 3H), 5.57 (H-3, s, 1H), 6.34–7.95 (ArH, m, 17H). ^{13}C NMR δ (ppm): 20.7 (Me), 72.4 (C-4), 86.9 (C-3), 114.8, 117.4, 120.3, 120.8, 121.9, 123.1, 126.2, 127.6, 127.7, 128.4, 129.1, 129.4, 129.7, 130.2, 133.8, 134.2, 138.1, 140.3, 140.6, 141.1, 156.4 (aromatic carbons), 162.2 (CO β-lactam). GC–MS *m*/*z*=477 [M^+]. Analysis calculated for $C_{28}H_{21}NO_2$: C, 83.35; H, 5.25; N, 3.47%. Found: C, 83.30; H, 5.23; N, 3.53%.

*4.3.2. 1-(4-Methoxyphenyl)-3-phenoxyspiro[azetidine-2,9′-fluoren]-4-one (**4b**).* White crystal (yield 70%). Mp: 196–198 °C. IR (KBr, cm^{-1}): 1758 (CO β-lactam). ^1H NMR δ (ppm): 3.60 (OMe, s, 3H), 5.56 (H-3, s, 1H), 6.05–8.18 (ArH, m, 17H). ^{13}C NMR δ (ppm): 55.2 (OMe), 72.6 (C-4), 87.0 (C-3), 114.2, 114.9, 119.0, 120.3, 121.1, 123.2, 126.4, 127.8, 128.4, 129.2, 129.7, 130.2, 138.2, 140.4, 140.7, 141.1, 156.5, 156.6 (aromatic carbons), 162.0 (CO β-lactam). GC–MS *m*/*z*=419 [M^+]. Analysis calculated for $C_{28}H_{21}NO_3$: C, 80.17; H, 5.05; N, 3.34%. Found: C, 80.20; H, 5.11; N, 3.38%.

*4.3.3. 1-(4-Chlorophenyl)-3-phenoxyspiro[azetidine-2,9′-fluoren]-4-one (**4c**).* White solid (yield 72%). Mp: 126–128 °C. IR (KBr, cm^{-1}): 1758 (CO β-lactam). ^1H NMR δ (ppm): 5.91 (H-3, s, 1H,), 6.27–8.36 (ArH, m, 17H). ^{13}C NMR δ (ppm): 72.7 (C-4), 87.1 (C-3), 114.9, 118.8, 120.5, 121.0, 122.1, 123.1, 126.3, 127.9, 128.6, 129.1, 129.2, 129.8, 130.1, 130.4, 134.8, 137.7, 140.4, 140.7, 140.8, 156.5 (aromatic carbons), 162.5 (CO β-lactam). GC–MS *m*/*z*=405 [M^+, ^{37}Cl], 403 [M^+, ^{35}Cl]. Analysis calculated for $C_{27}H_{18}ClNO_2$: C, 76.50; H, 4.28; N, 3.30%. Found: C, 76.54; H, 4.31; N, 3.32%.

*4.3.4. 1-(4-(Diethylamino)phenyl)-3-phenoxyspiro[azetidine-2,9′-fluoren]-4-one (**4d**).* Green crystal (yield 76%). Mp: 152–154 °C. IR (KBr, cm^{-1}): 1789 (CO β-lactam). ^1H NMR δ (ppm): 0.98 (Me, t, 6H, *J*=7), 3.13 (CH_2, q, 4H, *J*=7), 5.54 (H-3, s, 1H,), 6.30–7.78 (ArH, m, 17H). ^{13}C NMR δ (ppm): 12.5 (Me), 44.3 (CH_2), 72.6 (C-4), 87.0 (C-3), 111.7, 114.1, 119.4, 120.3, 120.7, 121.8, 123.3, 125.3, 126.5, 127.7, 128.4, 129.1, 130.1, 138.7, 140.4, 140.7, 141.6, 145.1, 156.7 (aromatic carbons), 161.6 (CO β-lactam). GC–MS *m*/*z*=475 [M^+]. Analysis calculated for $C_{31}H_{28}N_2O_2$: C, 80.84; H, 6.13; N, 6.08%. Found: C, 80.81; H, 6.18; N, 6.05%.

*4.3.5. 1-(4-Ethoxyphenyl)-3-phenoxyspiro[azetidine-2,9′-fluoren]-4-one (**4e**).* Yellow crystal (yield 75%). Mp: 140–142 °C. IR (KBr, cm^{-1}): 1755 (CO β-lactam). ^1H NMR δ (ppm): 1.26 (Me, t, 3H, *J*=7), 3.79 (CH_2, q, 2H, *J*=7), 5.56 (H-3, s, 1H), 6.60–8.32 (ArH, m, 17H). ^{13}C

NMR δ (ppm): 14.7 (Me), 63.4 (CH_2), 72.6 (C-4), 87.0 (C-3), 114.3, 114.8, 114.9, 119.0, 120.4, 120.9, 121.9, 123.2, 126.4, 127.8, 128.5, 129.2, 129.6, 129.9, 130.2, 138.2, 140.4, 140.7, 141.2, 155.9, 156.6 (aromatic carbons), 162.0 (CO β-lactam). GC–MS *m*/*z*=433 [M^+]. Analysis calculated for $C_{29}H_{23}NO_3$: C, 80.35; H, 5.35; N, 3.23%. Found: C, 80.33; H, 5.32; N, 3.25%.

*4.3.6. 1-(4-Isopropylphenyl)-3-phenoxyspiro[azetidine-2,9′-fluoren]-4-one (**4f**).* White solid (yield 48%). Mp: 144–146 °C. IR (KBr, cm^{-1}): 1758 (CO β-lactam). ^1H NMR δ (ppm): 1.13 (Me, d, 6H, *J*=3.8), 2.69(CH, m, 1H), 5.57 (H-3, s, 1H), 6.34–8.30 (ArH, m, 17H). ^{13}C NMR δ (ppm): 23.8 (Me), 33.5 (CH), 72.5 (C-4), 87.1 (C-3), 114.9, 117.6, 120.4, 120.9, 122.0, 123.2, 126.4, 127.0, 127.8, 128.5, 129.2, 129.9, 130.2, 134.2, 138.2, 140.4, 140.7, 141.2, 145.2, 156.6 (aromatic carbons), 162.4 (CO β-lactam). GC–MS *m*/*z*=431 [M^+]. Analysis calculated for $C_{30}H_{25}NO_2$: C, 83.50; H, 5.84; N, 3.25%. Found: C, 83.52; H, 5.81; N, 3.30%.

*4.3.7. 1-(4-Methylphenyl)-dispiro[azetidine((2,9′-fluoren)(3,9′-xanthen))]-4-one (**5a**).* Yellow crystal (yield 55%). Dec: 222–224 °C. IR (KBr, cm^{-1}): 1755 (CO β-lactam). ^1H NMR δ (ppm): 2.02 (Me, s, 3H), 6.30–8.99 (ArH, m, 20H). ^{13}C NMR δ (ppm): 20.8 (Me), 61.7 (C-3), 78.5 (C-4), 116.3, 116.7, 117.2, 117.4, 120.0, 122.2, 122.8, 123.1, 125.3, 126.0, 126.9, 127.3, 128.6, 128.9, 129.5, 134.1, 134.6, 139.7, 140.0, 152.0, 153.5, 164.3 (aromatic carbons), 169.1 (CO β-lactam). GC–MS *m*/*z*=477 [M^+]. Analysis calculated for $C_{34}H_{23}NO_2$: C, 85.51; H, 4.85; N, 2.93%. Found: C, 85.30; H, 4.73; N, 3.13%.

*4.3.8. 1-(4-Methoxyphenyl)-dispiro[azetidine((2,9′-fluoren)(3,9′-xanthen))]-4-one (**5b**).* White crystal (yield 77%). Dec: 238–240 °C. IR (KBr, cm^{-1}): 1751 (CO β-lactam). ^1H NMR δ (ppm): 3.64 (OMe, s, 3H), 6.30–8.36 (ArH, m, 20H). ^{13}C NMR δ (ppm): 55.3 (OMe), 69.2 (C-3), 78.6 (C-4), 114.2, 116.4, 118.9, 120.0, 122.3, 122.8, 125.4, 126.1, 127.3, 128.6, 128.9, 130.0, 139.7, 140.1, 153.5, 156.3 (aromatic carbons), 164.0 (CO β-lactam). GC–MS *m*/*z*=493 [M^+]. Analysis calculated for $C_{34}H_{23}NO_3$: C, 82.74; H, 4.70; N, 2.84%. Found: C, 82.65; H, 4.71; N, 2.81%.

*4.3.9. 1-(4-Chlorophenyl)-dispiro[azetidine((2,9′-fluoren)(3,9′-xanthen))]-4-one (**5c**).* White solid (yield 61%). Dec: 226–228 °C. IR (KBr, cm^{-1}): 1758 (CO β-lactam). ^1H NMR δ (ppm): 6.40–8.05 (ArH, m, 20H). ^{13}C NMR δ (ppm): 69.5 (C-3), 78.7 (C-4), 116.5, 117.2, 118.4, 120.2, 121.0, 122.0, 122.9, 124.0, 125.3, 126.2, 127.2, 128.8, 129.1, 129.2, 129.4, 129.6, 135.0, 139.2, 140.1, 153.5 (aromatic carbons), 164.7 (CO β-lactam). GC–MS *m*/*z*=499 [M^+, ^{37}Cl], 497 [M^+, ^{35}Cl]. Analysis calculated for $C_{33}H_{20}ClNO_2$: C, 79.59; H, 4.05; N, 2.81%. Found: C, 79.54; H, 4.12; N, 2.85%.

*4.3.10. 1-(4-(Diethylamino)phenyl)-dispiro[azetidine((2,9′-fluoren)(3,9′-xanthen))]-4-one (**5d**).* White solid (yield 80%). Dec: 220–222 °C. IR (KBr, cm^{-1}): 1739 (CO β-lactam). ^1H NMR δ (ppm): 1.01 (Me, t, 6H, *J*=7), 3.15 (CH_2, q, 4H, *J*=7), 6.35–8.39 (m, ArH, 20H). ^{13}C NMR δ (ppm): 12.6 (Me), 44.3 (CH_2), 69.1 (C-3), 78.7 (C-4), 111.8, 116.4, 116.8, 119.3, 120.0, 120.4, 122.6, 122.8, 123.2, 125.6, 125.7, 126.0, 127.0, 128.6, 128.9, 129.8, 140.2, 140.3, 145.0, 153.6 (aromatic carbons), 163.4 (CO β-lactam). GC–MS *m*/*z*=534 [M^+]. Analysis calculated for $C_{37}H_{30}N_2O_2$: C, 83.12; H, 5.66; N, 5.24%. Found: C, 83.20; H, 5.62; N, 5.21%.

*4.3.11. 1-(4-Ethoxyphenyl)-dispiro[azetidine((2,9′-fluoren)(3,9′-xanthen))]-4-one (**5e**).* White solid (yield 83%). Mp: 228–230 °C. IR (KBr, cm^{-1}): 1751 (CO β-lactam). ^1H NMR δ (ppm): 1.30 (Me, t, 3H, *J*=7), 3.83 (CH_2, q, 2H, *J*=7), 6.60–8.32 (ArH, m, 20H). ^{13}C NMR δ (ppm): 14.7 (Me), 63.5 (CH_2), 69.2 (C-3), 78.7 (C-4), 114.8, 116.4, 116.8, 118.9, 120.1, 122.3, 122.8, 123.2, 125.4, 126.1, 127.4, 128.7, 129.0, 129.7, 130.0, 139.8, 140.1, 153.5, 155.7 (aromatic carbons), 164.0 (CO β-lactam). GC–MS *m*/*z*=507 [M^+]. Analysis calculated for $C_{35}H_{25}NO_3$: C, 82.82; H, 4.96; N, 2.76%. Found: C, 82.85; H, 4.93; N, 2.75%.

*4.3.12. 1-(4-(Dimethylamino)phenyl)-dispiro[azetidine((2,9′-fluoren)(3,9′-xanthen))]-4-one (**5f**).* White solid (yield 65%). Dec: 230–232 °C. IR (KBr, cm^{-1}): 1735 (CO β-lactam). ^1H NMR δ (ppm): 2.78 (Me, s, 6H), 6.44–8.32 (ArH, m, 20H). ^{13}C NMR δ (ppm): 40.6 (Me), 69.2 (C-3), 78.6 (C-4), 112.7, 116.3, 117.1, 118.9, 120.0, 120.3, 121.5, 122.5, 122.8, 123.8, 125.5, 126.0, 126.8, 126.9, 127.5, 128.5, 128.8, 129.3, 129.5, 140.1, 140.2, 147.7, 153.6 (aromatic carbons), 163.5 (CO β-lactam). GC–MS *m*/*z*=507 [M^+]. Analysis calculated for $C_{35}H_{26}N_2O_2$: C, 82.98; H, 5.17; N, 5.53%. Found: C, 82.93; H, 5.21; N, 5.51%.

*4.3.13. 1-(4-Isopropylphenyl)-dispiro[azetidine((2,9′-fluoren)(3,9′-xanthen))]-4-one (**5g**).* White solid (yield 63%). Mp: 238–240 °C. IR (KBr, cm^{-1}): 1751 (CO β-lactam). ^1H NMR δ (ppm): 1.25 (Me, d, 6H, *J*=3.8), 2.74 (CH, m, 1H), 6.42–8.05 (ArH, m, 20H). ^{13}C NMR δ (ppm): 23.8 (Me), 33.5 (CH), 69.1 (C-3), 78.5 (C-4), 116.3, 117.0, 117.5, 119.8, 120.0, 122.2, 122.8, 123.8, 125.4, 126.0, 126.6, 126.9, 127.3, 128.6, 128.9, 129.3, 129.4, 134.3, 139.7, 140.0, 145.0, 151.0, 153.5 (aromatic carbons), 164.3 (CO β-lactam). GC–MS *m*/*z*=506 [M^+]. Analysis calculated for $C_{36}H_{27}NO_2$: C, 85.52; H, 5.38; N, 2.77%. Found: C, 85.50; H, 5.34; N, 2.72%.

4.4. General procedure for the preparation of bis-Schiff bases 6a,b

A mixture of 9*H*-fluoren-9-one (3.00 mmol) and bisamine (1.00 mmol) with catalytic amount of acetic acid was refluxed in ethanol (20 mL) for 12 h. Evaporation of the solvent afforded the crude imines as oil, which were used for the next step without further purification.

4.5. General procedure for the synthesis of bis-spiro- and dispiro-β-lactams 7 and 8a,b

A mixture of previous crude Schiff base **6a**,**b**, triethylamine (10.00 mmol), 9*H*-xanthen-9-carboxylic acid or phenoxyacetic acid (3.00 mmol) and tosyl chloride (3.00 mmol) in dry CH_2Cl_2 (15 mL) was stirred at room temperature for 24 h. Then it was washed with HCl 1 N (20 mL), saturated $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and the solvent was evaporated to give the product as a solid, which was then purified by column chromatography, EtOAc/petroleum ether (8/2) as the eluent solvent.

*4.5.1. (4-(4(4-Oxo-3-phenoxyspiro[azetidine-2,9′-fluoren]1-yl)benzyl)phenyl)3-phenoxyspiro[azetidine-2,9′-fluoren]-4-one (**7**).* White solid (yield 45%). Mp: 188–190 °C. IR (KBr, cm^{-1}): 1735 (CO β-lactam). ^1H NMR δ (ppm): 3.56 (CH_2, s, 2H), 5.53 (H-3, s, 2H), 6.32–8.21 (ArH, m, 34H). ^{13}C NMR δ (ppm): 40.6 (CH_2) 72.4 (C-4), 86.9 (C-3), 114.8, 117.5, 120.3, 120.8, 121.9, 122.3, 123.1, 126.3, 127.7, 128.4, 129.1, 129.2, 129.8, 130.1, 134.4, 136.9, 137.9, 140.2, 140.5, 140.9, 156.4 (aromatic carbons), 162.3 (CO β-lactam). Analysis calculated for $C_{55}H_{38}N_2O_4$: C, 83.52; H, 4.84; N, 3.54%. Found: C, 83.55; H, 4.87; N, 3.51%.

*4.5.2. (4(4(4-Oxo-dispiro[azetidine((2,9′-fluoren)(3,9′-xanthen))]1-yl)benzyl)phenyl)dispiro[azetidine((2,9′-fluoren)(3,9′-xanthen))]4-one (**8a**).* Yellow solid (yield 45%). Dec: 210–212 °C. IR (KBr, cm^{-1}): 1735 (CO β-lactam). ^1H NMR δ (ppm): 3.74 (CH_2, d, 2H, *J*=10.8), 6.80–7.80 (ArH, m, 40H). ^{13}C NMR δ (ppm): 40.6 (CH_2), 69.1 (C-3), 78.5 (C-4), 116.4, 117.1, 117.5, 119.8, 120.0, 122.1, 122.8, 123.8, 125.4, 126.0, 127.2, 128.6, 128.9, 129.1, 129.3, 129.4, 134.7, 136.9, 139.6, 140.0, 153.4 (aromatic carbons), 164.4 (CO β-lactam). Analysis

calculated for $C_{67}H_{42}N_2O_4$: C, 85.69; H, 4.51; N, 2.98%. Found: C, 85.71; H, 4.56; N, 2.93%.

*4.5.3. (4(4(4-Oxo-dispiro[azetidine((2,9′-fluoren)(3,9′-xanthen))]1-yl)phenoxy)phenyl)dispiro[azetidine((2,9′-fluoren)(3,9′-xant hen))] 4-one (**8b**).* White solid (yield 48%). Mp: 188–190 °C. IR (KBr, cm^{-1}): 1735 (CO β-lactam). ^1H NMR δ (ppm): 6.63–8.33 (ArH, m, 40H). ^{13}C NMR δ (ppm): 69.3 (C-3), 78.6 (C-4), 116.4, 117.1, 118.5, 118.9, 119.2, 120.0, 121.4, 122.1, 122.8, 123.9, 125.4, 126.1, 127.2, 128.7, 129.0, 129.4, 132.1, 139.4, 140.1, 153.4, 153.5 (aromatic carbons), 164.2 (CO β-lactam). Analysis calculated for $C_{66}H_{40}N_2O_5$: C, 84.24; H, 4.28; N, 2.98%. Found: C, 84.25; H, 4.31; N, 2.95%.

4.6. General procedure for antimalarial activity measurements

The chloroquine-resistant *P. falciparum* strain K14 (Southeast Asia) was cultured in vitro in complete medium consisting of RPMI 1640 (In Vitrogen) supplemented with 27.5 mM $NaHCO_3$, 20 mg/L gentamycin, and 10% human serum.[23] Parasites were grown at 37 °C in human O+ red blood cells at a 6% haematocrit under a 5% CO_2, 10% O_2 and 85% N_2 atmosphere. Cultures were synchronized by sorbitol treatments.[24] Stock solutions of lactam derivatives were prepared in sterile DMSO (10 mM) and later dilutions were with complete culture medium. Increasing concentrations of lactam derivatives (100 μL/well, top concentration=50 μM) were distributed in a 96-well plate; DMSO (0.5% vol/vol, top concentration) was distributed for control. Then, 100 μL from a culture containing >95% ring (0–20 h postinvasion) at a 0.8% parasitaemia and 3% haematocrit in complete medium was added per well along with 1.0 μCi of 3*H*-hypoxanthine with a specific activity of 14.1 Ci/mmol (Perkin–Elmer, Courtaboeuf, France). Parasites were grrown for 42 h at 37 °C. Plates were then freeze-thawed and harvested on filters. Dried filters were moistened in scintillation liquid mixture (Microscint O; Perkin–Elmer) and counted in a Top Count Microbeta counter (Perkin–Elmer). Percentage growth inhibition was calculated from the parasite-associtaed radioactivity. 100% 3*H*-hypoxanthine incorporation was determined from a control grown in the absence of lactam derivatives. The concentration of drug giving 50% inhibition of label incorporation (IC_{50}) was determined by nonlinear regression analysis of log-based dose–response curve (Riasmart; Packard). Each concentration was estimated from independent experiments in triplicate.

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