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Synthesis of racemic and optically active forms of novel antimalarial agents, spirocyclopentanone–anthracene adducts, via tandem Michael addition–Dieckmann condensation reactions as the key steps

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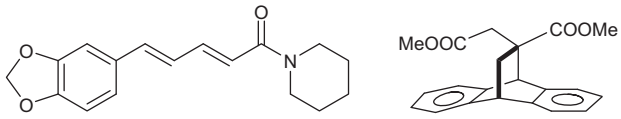
ABSTRACT

Spirocyclopentanone–anthracene adducts, novel antimalarial agents, have been synthesized by employing the racemic and the optically active dimethyl itaconate–anthracene adducts in a reaction with piperine via tandem Michael addition–Dieckmann condensation reactions as the key steps. All adducts exhibited antimalarial activity with IC_{50} values of 3.4–4.7 μg/mL, and importantly displayed non-cytotoxicity to *vero* cells.

1. Introduction

Malaria is a serious problem that is spreading in tropical and subtropical areas of the world.[1] Approximately 3.3 billion people were at risk of malaria in 2010.[2] Malaria is an infectious disease caused by the protozoa parasite of the genus *Plasmodium* and is carried from person to person by the anopheles mosquitoes. The parasites are of many types, but only five species of the genus *Plasmodium* cause malaria in humans.[2] The parasites that cause malaria in humans are *Plasmodium falciparum* (malaria tropica); *Plasmodium vivax* (malaria tertiana); *Plasmodium ovale* (malaria quartana); *Plasmodium malariae* (malaria tertiana) and *Plasmodium knowlesi*.[2] The first two species (*P. falciparum* and *P. vivax*) cause the most infections worldwide.[2] *P. falciparum* is the agent of severe and potentially fatal malaria because of antimalarial drug resistance.[3] Thus, the research and development of antimalarial drugs is especially important in order to eradicate this infectious disease.

Piperine **1** (Fig. 1) is a major alkaloid compound in *Piper nigrum*.[4] It displays a variety of pharmacological and other bioactivities such as antifungal,[5] antidiarrheal,[6] antiinflammatory,[7] insecticidal,[8] nematocidal activity,[4a] inhibition of life metabolism,[9] immunomodulatory[10], and antitumor activities.[10] Recently, piperine dimers such as chabamide[11] (antiplasmodial) and dipiperamide A, B, C[12] (CYP3A4 inhibitor) were discovered.



1 2

Figure 1. Structures of piperine **1** and dimethyl itaconate–anthracene adduct **2**.

Previously, the use of the dimethyl itaconate–anthracene adduct[13] (±)-**2** (Fig. 1) in racemic form has been reported as a building block in the syntheses of biologically active natural products, including methylenomycin B,[14] sarkomycin,[15] deepoxy-4,5-didehydromethylenomycin A,[16] methylenomycin A methyl esters,[16] diospyrol,[17] α-methylene-γ-butyrolactones[18a,b] such as methylenolactocin, nephrosterinic acid, and protolichesterinic acid, and α-methylene bis-γ-butyrolactones[18] such as cannadensolide, sporothriolide, and xylobovide. Moreover, optically active methylene lactones,[19] such as methylenolactocin, nephrosterinic acid and protolichesterinic acid, can be prepared by employing tandem aldol–lactonization reactions of the enantiomerically pure forms (+)-**2** and (−)-**2**.

Herein we report the synthesis of the racemic and optically active forms of a spirocyclopentanone–anthracene adduct via tandem Michael addition–Dieckmann condensation reactions as the key steps. The racemic and optically active forms of dimethyl itaconate–anthracene adducts **2** react with piperine **1** as a precursor

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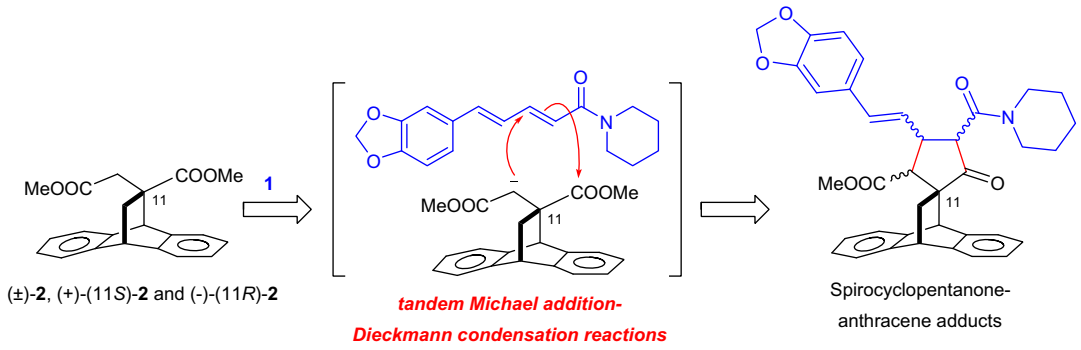


Scheme 1. Synthesis of spirocyclopentanone–anthracene adducts via tandem Michael addition–Dieckmann condensation reactions.

(Scheme 1). All products were subsequently tested for antimalarial and cytotoxic activities.

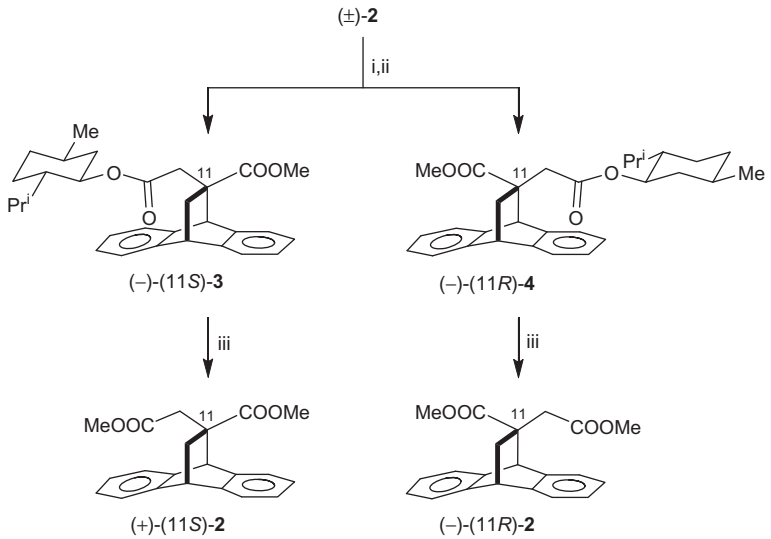
2. Results and discussion

Racemic dimethyl itaconate–anthracene adduct (±)-**2** was synthesized from dimethyl itaconate and anthracene via a [4+2] Diels–Alder reaction in high yield.[18a] It was then hydrolyzed and treated with (−)-menthol, followed by transmethylation to provide enantiomerically pure (+)-**2** and (−)-**2**, which have already been reported on,[19] as shown in Scheme 2.

Compound (±)-**2** was initially reacted with LDA to generate the corresponding ester enolate intermediate, which readily reacted with piperine **1** in THF via a tandem Michael addition followed by a Dieckmann condensation reaction. The reaction mixture was quenched with 30% hydrochloric acid solution at 0 °C, and then purified by flash column chromatography (silica gel, EtOAc/CH_2Cl_2/hexane = 2.0:0.5:7.5 as an eluent) to give two diastereomeric spirocyclopentanone–anthracene adducts (±)-**5** and (±)-**6**. The racemic and enantiomerically pure forms of **2** were applied to piperine **1** via tandem Michael addition–Dieckmann condensation reactions and the results are shown in Table 1.

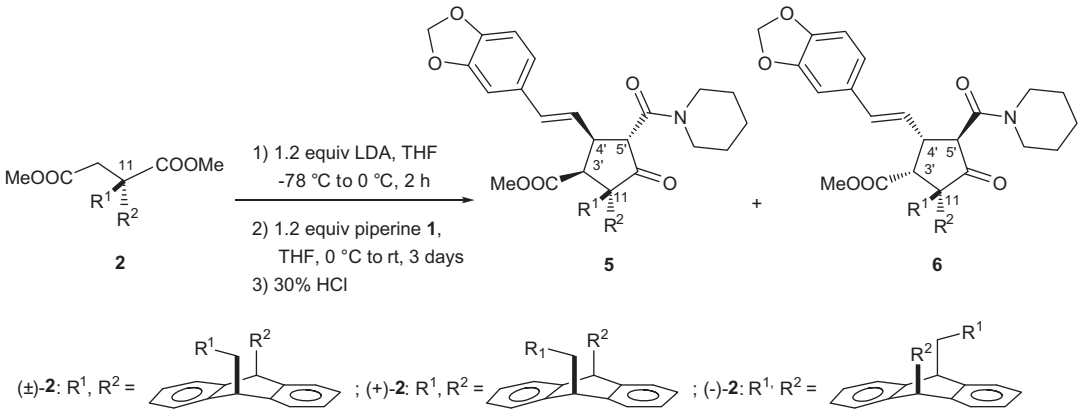
The relative configuration of the two spirocyclopentanone–anthracene adducts (±)-**5** and (±)-**6** was determined on the basis of their analytical and spectroscopic data. In particular, the ^{1}H NMR spectrum of adduct (±)-**5** showed three signals at the3′ [δ: 2.82 (H, d, J = 7.1 Hz)], 4′ [δ: 4.49 (H, m)], and 5′ [δ: 3.87 (H, d, J = 9.8 Hz)] positions, indicating that the configuration of the H-3′ between H-4′ and H-4′ between H-5′ was *cis*- and *trans*-, respectively. In a similar fashion, as for adduct (±)-**6**, the coupling constants of H-3′ between H-4′ and H-4′ between H-5′ were also established as *cis*- and *trans*-[3′ (δ: 2.35 (H, d, J = 7.1 Hz)), 4′ (δ: 3.93 (H, m)), and 5′ (δ: 4.00 (H, d, J = 11.3 Hz)]. The relative stereochemistry of the spirocyclopentanone–anthracene adducts (±)-**5** was confirmed by single-crystal X-ray diffraction analysis,[20] as shown in Figure 2. Therefore, the relative stereochemistries of the adduct (±)-**6** should be opposite to those of the adduct (±)-**5**. The relative stereochemistries at the 3′-, 4′- and 5′-position of the adduct (±)-**6** were confirmed by nOe difference experiments (Fig. 3), and the nOe results on (±)-**5** also showed stereochemistries corresponding to the X-ray results. Therefore, the relative stereochemistries of the adducts (−)-**5** and (−)-**6** from (+)-**2**, and the adducts (+)-**5** and (+)-**6** from (−)-**2** had similar configurations to the racemic adducts.

The stereochemical course of the reaction can be explained by considering the required transition state for the Michael addition reaction (1,4-addition) and the Dieckmann condensation reaction (ring closure). Dimethyl itaconate–anthracene adduct **2** was depro-



Scheme 2. Separation of the optically active dimethyl itaconate–anthracene adducts (+)-(11*S*)-**2** and (−)-(11*R*)-**2**. Reagents and conditions: (i) 1.3 equiv KOH, MeOH: H_2O (2:1), reflux 2 h, (97%); (ii) (a) 5.0 equiv $SOCl_2$, DMF (cat.), N_2, reflux 2 h, (b) 1.3 equiv (−)-(1*R*,2*S*,5*R*)-menthol, 1.3 equiv Et_3N, benzene, reflux 2 h [(−)-(11*S*)-**3**, 35%; (−)-(11*R*)-**4**, 35%]; (iii) excess anhydrous MeOH, H_2SO_4 (cat.), reflux 6 days [(+)-(11*S*)-**2**, 97%; (−)-(11*R*)-**2**, 89%].

Table 1
% Yield, % enantiomeric excess (ee) and the specific rotation [α] of the adducts **5** and **6**



Entry	Starting materials	Adducts	Yield[a] (%)	ee[b] (%)	[α][c]
1	(±)-**2**	(±)-**5**	23	—	—
2	(±)-**2**	(±)-**6**	15	—	—
3	(+)-**2**	(−)-**5**	33	>99	−18.5 (*c* 0.87, $CHCl_3$)
4	(+)-**2**	(−)-**6**	17	>99	−70.5 (*c* 0.58, $CHCl_3$)
5	(−)-**2**	(+)-**5**	14	>99	+17.5 (*c* 0.76, $CHCl_3$)
6	(−)-**2**	(+)-**6**	8	>99	+67.6 (*c* 0.60, $CHCl_3$)

[a] Isolated yield.
[b] Enantiomeric excesses (ee) of all products were determined by ^{1}H NMR using a chiral lanthanide shift reagent, tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato]praseodym(III), ($Pr(hfc)_3$).
[c] Specific rotation values of all products were measured at 24.4 °C and 589 nm.

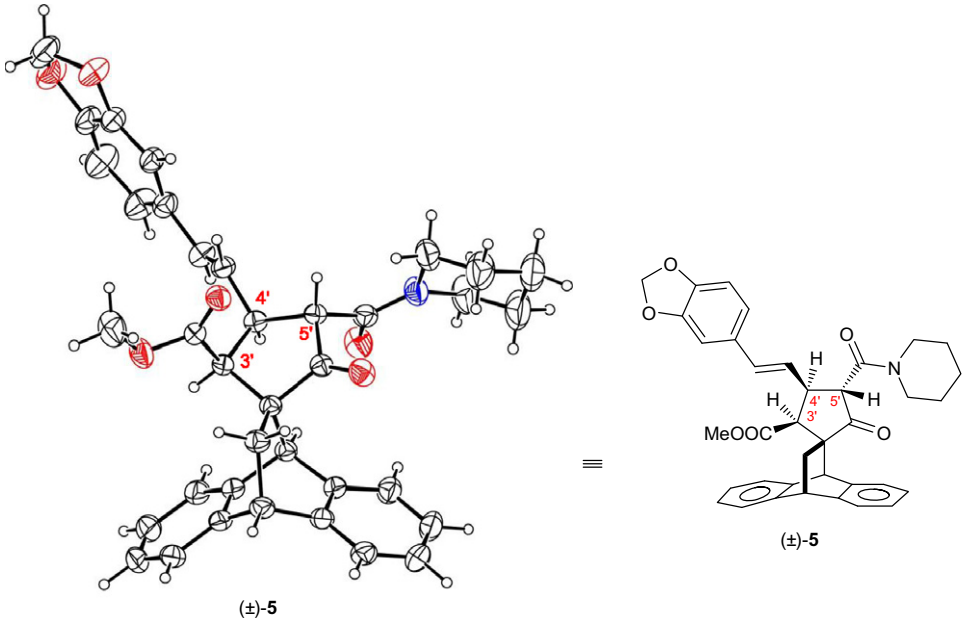


Figure 2. X-ray structure of the adduct (±)-**5** (ORTEP plot).

tonated at the α-proton of the ester by LDA to give the enolate **7**. Next, piperine **1** was added and attacked with the enolate anion **7** on the *Si*-face to form the eight-membered ring chelated transition state **8**. After the Michael addition reaction, transition state **9** was attained to give the favorable envelope-chair-like form, whereupon all of the large substituents occupied the less sterically demanding equatorial orientations, and the following Dieckmann condensation reaction via a 5-*exo*-trig cyclization, led to the major product **5**. Conversely, piperine **1** was attacked with enolate-anion **7** on the *Re*-face through the chelated transition states **10** and **11**. This led to the formation of minor product **6** because of the large steric repulsion between the vinylbenzo[1,3]dioxole being eclipsed with the anthracene groups present in the chelated transition state, as depicted in Scheme 3.

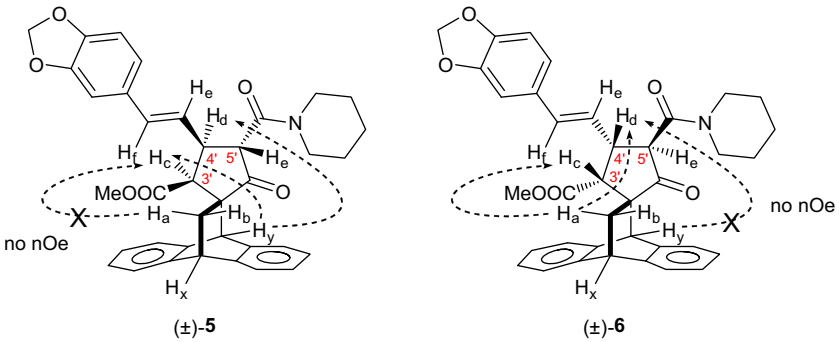
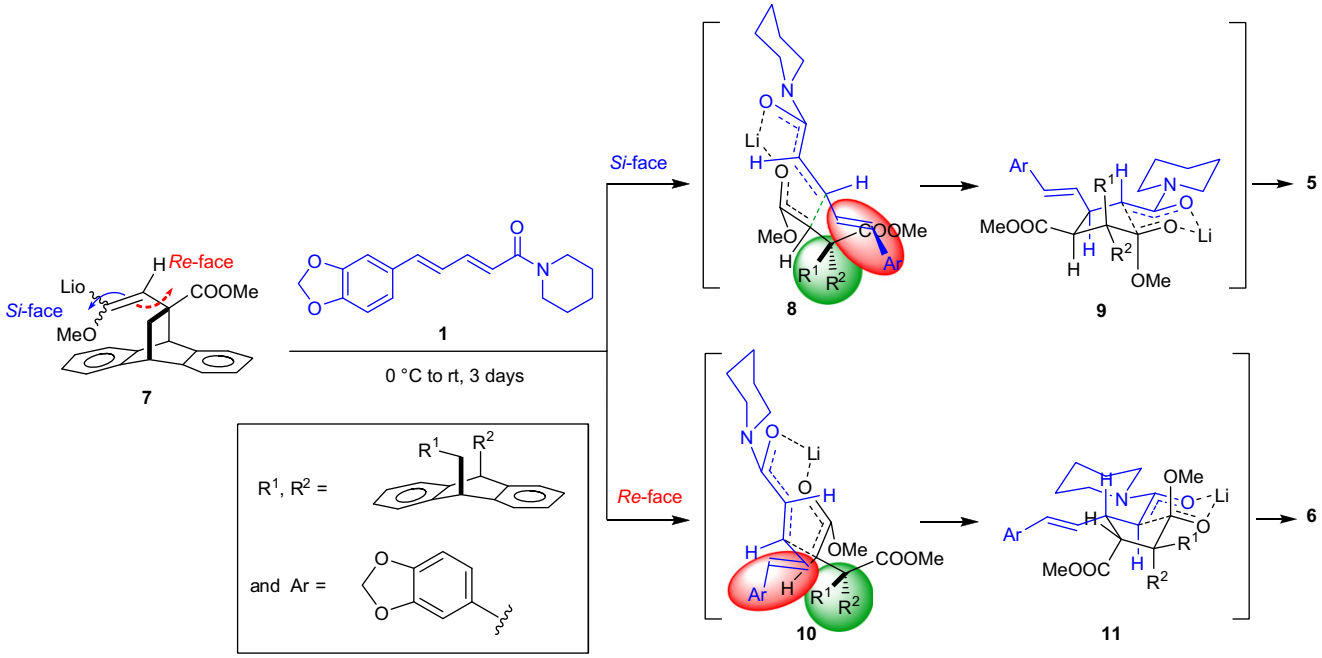


Figure 3. The nOe difference results of the adducts (±)-**5** and (±)-**6**.



Scheme 3. The proposed mechanism of Michael addition–Dieckmann condensation reactions of the enolate anion **7** with **1**.

Table 2
Antimalarial activity and cytotoxicity of adducts **5** and **6**

Compounds	IC_{50} (μg/mL)	
	Antimalarial activity[a,b]	Cytotoxicity[a,c] (*vero* cells)
Piperine **1**	Inactive	Non-cytotoxic
(±)-**5**	4.7	Non-cytotoxic
(±)-**6**	3.4	Non-cytotoxic
(−)-**5**	4.3	Non-cytotoxic
(−)-**6**	3.6	Non-cytotoxic
(+)-**5**	4.0	Non-cytotoxic
(+)-**6**	4.5	Non-cytotoxic

[a] All biological activities resulted from the average of multiple (three) determinations.
[b] The IC_{50} values of the standard antimalarial compounds chloroquine diphosphate,[23] mefloquine and dihydroartemisinin (DHA) were 0.16, 0.012 and 0.001 μg/mL, respectively.
[c] The IC_{50} value of the standard compound ellipticine was 0.976 μg/mL for the *vero* cells.

The antimalarial activity of the synthetic compounds was determined by means of a microculture radioisotope technique based on the method described by Desjardins et al.[21] The adducts were screened in vitro for activity against the parasite *P. falciparum* (K1, multi-drug resistance strain). Mefloquine and dihydroartemisinin (DHA) were used as positive standard controls. The results of the antimalarial activity and cytotoxicity against *vero* cells of the adducts **5** and **6** are summarized in Table 2.

Adducts (±)-**5** and (±)-**6** exhibited antimalarial activity against the parasite *P. falciparum* (K1, multi-drug resistance strain) with IC_{50} values of 4.7 and 3.4 μg/mL, respectively (Table 2). Interestingly, adducts (±)-**5** and (±)-**6** displayed non-cytotoxicity against *vero* cells, as shown in Table 2. The differences in pharmacological activity between the drug and the receptor site generally depend on their ability to react selectively at an asymmetric center in the biological system which is due to the influence of the steric factors, such as optical and geometric isomerism, conformational isomerism, isosterism, and bioisosterism.[22] We were therefore interested in investigating the antimalarial activity of adducts **5** and **6** in enantiomerically pure forms, as outlined in Table 2. Adducts (−)-**5**, (−)-**6**, (+)-**5**, and (+)-**6** displayed antimalarial activity against the parasite *P. falciparum* (K1, multi-drug resistance strain) with IC_{50} values of 4.3, 3.6, 4.0, and 4.5 μg/mL respectively, as shown in Table 2. From the above results, it can be seen that all optically active products showed non-specific antimalarial activity against the parasite *P. falciparum*.

3. Conclusions

In conclusion, we have developed an enantioselective synthesis for the racemic and enantiomerically pure forms of spirocyclopentanone–anthracene adducts **5** and **6** using tandem Michael addition–Dieckmann condensation reactions as the key steps. These compounds exhibited in vitro antimalarial activity, and importantly exhibited non-cytotoxicity against *vero* cells. Further studies are underway in order to improve the activity of our novel class of antimalarial agents.

4. Experimental

4.1. General methods

All reactions were carried out under a nitrogen or argon atmosphere. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Melting points were determined by using a Gallenkamp Electrothermal apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX 500 MHz spectrometers and chemical shifts were given in ppm downfield from tetramethylsilane (TMS). All NMR spectra were measured in $CDCl_3$ and chemical shifts are reported as δ-values in parts per million (ppm) relative to residue $CHCl_3$ as the internal reference (^{1}H: δ 7.26, ^{13}C: δ 77.00) and coupling constants (J values) are reported in Hertz (Hz). Peak multiplicities are indicated as follows: s (singlet), d (doublet), dd (doublet of doublets), and m (multiplet). Infrared spectra were taken with a FT-IR model TENSER 27 (Bruker) spectrometer and absorption frequencies were reported in reciprocal centimeters (cm^{-1}). Mass spectra (electrospray ionization mode, ESI-MS) were measured on a micromass Q-TOF-2™ (Waters) spectrometer. Optical rotations were measured in $CHCl_3$ and MeOH with the Sodium D-line (589 nm) on a Jasco P-1030 digital polarimeter. Flash column chromatography was performed employing Merck silica gel 60 and Merck silica gel 60H. Preparative thin layer chromatography (PLC) plates were carried out using Merck silica gel 60 PF_{254}. Analytical thin layer chromatography was performed with Merck silica gel 60 F_{254} aluminum plates. Solvents were dried over CaH_2 and distilled before use. Tetrahydrofuran (THF) was freshly distilled from sodium and benzophenone ketyl under nitrogen. Diisopropylamine was distilled over CaH_2 and stored under nitrogen. *n*-Butyllithium was purchased from Fluka and Across as solution in hexane and titrated periodically according to the 2,5-dimethoxybenzyl alcohol method. Enantiomeric excess was determined by ^{1}H NMR spectroscopy using the chiral lanthanide shift reagent, tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato]praseodym(III), Pr(hfc)$_3$.

4.2. General procedure for the synthesis of 4′-((*E*)-2-(benzo[*c*]-[1,3]dioxol-1-yl)vinyl)-3′-methoxycarbonyl-5′-(piperidine-1-carbonyl)cyclopentanone-2′-spiro-11-9,10-dihydro-9,10-ethanoanthracenes (±)-5 and (±)-6

To a 250 mL round-bottomed flask equipped with a magnetic stirrer fitted with a three-way stopcock with a septum cap and nitrogen inlet were added THF (50 mL) and dry diisopropylamine (7.9 mL, 55.97 mmol) via syringes. The mixture was cooled down to −78 °C, after which *n*-butyllithium (1.4 M in hexane, 33.0 mL, 46.64 mmol) was added and the mixture left to stir at 0 °C for 1 h. A solution of (±)-dimethyl itaconate–anthracene adduct (±)-**2** (13.1 g, 38.9 mmol) in THF (50 mL) was introduced to the LDA solution at −78 °C, then the mixture was stirred at 0 °C for 2 h. A solution of piperine **1** (13.3094 g, 46.6 mmol) in THF (100 mL) was added to the anion solution at −78 °C after which the reaction mixture was left to stir at room temperature for 3 days. The reaction mixture was quenched with a saturated aqueous ammonium chloride solution at 0 °C and the crude mixture was extracted several times with CH_2Cl_2. The dichloromethane solution was washed with H_2O, a saturated NaCl solution, then dried over $MgSO_4$, filtered and evaporated to dryness. The crude product was purified by flash column chromatography (silica gel) using EtOAc/CH_2Cl_2/hexane = 2:0.5:7.5 as eluent to give the diastereoisomeric spirocyclopentanone–anthracene adducts, 4′-((*E*)-2-(benzo[*c*][1,3]dioxol-1-yl)vinyl)-3′-methoxycarbonyl-5′-(piperidine-1-carbonyl)cyclopentanone-2′-spiro-11-9,10-dihydro-9,10-ethanoanthracenes (±)-**5** and (±)-**6** in 23% (2.37 g), and 15% (1.55 g) respectively.

4.2.1. Compound (±)-5

White solid; mp: 205.9–207.6 °C (from CH_2Cl_2/hexane); R_f (15% EtOAc/5%CH_2Cl_2/hexane) 0.50; IR (thin film): ν_{max} 2850, 2930, 1730, 1438, 1370, 1250, 1100 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.49–1.69 (m, 6H, CH_2), 1.31, 2.24, 4.32 (ABX system, J = 12.7, 2.7, 2.5 Hz, 3H, CH_2, ArCH), 2.82 (d, J = 7.1 Hz, 1H, CH_3OOCCH), 3.44, 3.80, 3.92 (m, 4H, CH_2NCH_2), 3.55 (s, 3H, COOMe), 3.87 (d, J = 9.8 Hz, 1H, COCHCON), 4.49 (m, 1H, C═CHCH), 4.86 (s, 1H, ArCH), 5.80 (dd, J = 15.7, 8.1 Hz, 1H, C═CHCH), 5.96 (s, 2H, OCH_2O), 6.54 (d, J = 15.7 Hz, 1H, ArCH═C), 6.73 (s, 2H, ArH-piperine), 6.84 (s, 1H, ArH-piperine), 7.02–7.45 (m, 8H, ArH-anthracene); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.6, 25.9, 26.6, 35.8, 42.0, 44.1, 44.3, 46.8, 47.6, 51.6, 53.3, 55.8, 60.6, 101.1, 105.5, 108.3, 121.8, 122.9, 123.7, 125.2, 125.4, 125.7, 125.8, 126.0, 126.6, 126.8, 132.8, 131.2, 138.7, 139.4, 143.5, 144.2, 147.4, 148.1, 165.4, 173.8, 207.4; HRMS (ESI) calcd for $C_{37}H_{35}NO_6Na$ (M+Na)$^+$: m/z 612.2361, found 612.2362.

4.2.2. Compound (±)-6

White solid; mp: 223.4–225.9 °C (from CH_2Cl_2/hexane); R_f (15% EtOAc/5%CH_2Cl_2/hexane) 0.36; IR (KBr-pellet): ν_{max} 2850, 2930, 1730, 1460, 1370, 1250, 1100 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.40–1.71 (m, 6H, CH_2), 1.90, 2.07, 4.37 (ABX system, J = 12.9, 2.9, 2.2 Hz, 3H, CH_2, ArCH), 2.35 (d, J = 6.6 Hz, 1H, CH_3OOCCH), 3.32, 3.54, 3.66 (m, 4H, CH_2NCH_2), 3.78 (s, 3H, COOMe), 3.93 (m, 1H, C═CHCH), 4.00 (d, J = 11.3 Hz, 1H, COCHCON), 4.43 (s, 1H, ArCH), 5.72 (dd, J = 15.7, 7.4 Hz, 1H, C═CHCH), 5.94 (s, 2H, OCH_2O), 6.33 (d, J = 15.7 Hz, 1H, ArCH═C), 6.65–6.82 (m, 3H, ArH-piperine), 6.90–7.44 (m, 8H, ArH-anthracene); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.6, 25.6, 26.7, 41.1, 41.7, 43.6, 44.2, 47.5, 47.8, 51.5, 53.9, 57.9, 58.0, 101.1, 105.5, 108.3, 121.8, 122.6, 123.9, 124.2, 124.4, 125.5, 125.6, 125.9, 126.5, 126.9, 131.2, 132.4, 140.6, 141.4, 143.0, 143.5, 147.3, 148.0, 165.2, 174.0, 210.4; HRMS (ESI) calcd for $C_{37}H_{35}NO_6Na$ (M+Na)$^+$: m/z 612.2361, found 612.2362.

4.3. Resolution to prepare optically active dimethyl itaconate–anthracene adducts (+)-2 and (−)-2

4.3.1. (−)-11-Carbomethoxy-11-((−)-menthoxyacetyl)-9,10-dihydro-9,10-ethanoanthracenes (11*S*)-3 and (11*R*)-4

A solution of KOH (1.08 g, 19.4 mmol) in H_2O (140 mL) was added to a solution of (±)-dimethyl itaconate–anthracene adduct, **2** (5.01 g, 14.9 mmol), in MeOH (270 mL), and heated at reflux for 2 h. The cooled reaction mixture was diluted with water (130 mL) and acidified to pH 2–3 by 10% HCl, then extracted with CH_2Cl_2, dried over $MgSO_4$, filtered, and evaporated to dryness. The crude product was crystallized from CH_2Cl_2–hexane to give the corresponding (±)-monoacid (4.65 g, 97%) as a white solid; mp: 207–209 °C. A mixture of the monoacid adduct (4.65 g, 14.4 mmol), thionyl chloride (5.3 mL, 72.1 mmol), and DMF (3 drops, as catalyst) was heated at reflux for 2 h after which the solvent was removed under reduced pressure. A mixture of the acid chloride obtained, benzene (160 mL), triethylamine (2.6 mL,

18.8 mmol), and (−)-menthol (2.93 g, 18.8 mmol) was heated at reflux for 2 h, filtered through Celite 545, diluted with H_2O, and extracted with CH_2Cl_2. The solution was washed with H_2O, saturated NaCl solution, dried over $MgSO_4$, filtered and evaporated to dryness. The crude product was purified by flash column chromatography (silica gel) using EtOAc/acetone/hexane = 0.5:0.3:9.2 as eluent to give two diastereoisomers, (−)-**3** (2.33 g, 35%, >99% de) as a white solid; mp: 185–186 °C (from EtOAc/hexane); (lit.[19] mp: 185–187 °C (from EtOAc/hexane)); $[\alpha]_{589}^{30} = -108.8$ (*c* 1.10, $CHCl_3$); {lit.[19] $[\alpha]_D^{30} = -109.0$ (*c* 1.29, $CHCl_3$)}; and (−)-**4** (2.32 g, 35%, >99% de) as a white solid; mp: 101–103 °C (from MeOH); (lit.[19] mp: 101–103 °C (from MeOH)); $[\alpha]_{589}^{30} = -51.2$ (*c* 1.10, $CHCl_3$);}lit.[19] $[\alpha]_D^{30} = -51.4$ (*c* 1.20, $CHCl_3$)} whose IR, ^1H, ^{13}C NMR, and mass spectroscopic data were identical to those previously published.[19]

4.3.2. (11*S*)-11-Carbomethoxy-11-methoxyacetyl-9,10-dihydro-9,10-ethanoanthracene (+)-2

To a solution of (−)-**3** (2.01 g, 4.4 mmol) in excess anhydrous MeOH (600 mL) was added concd H_2SO_4 (10 mL) and the mixture was heated under reflux for 7 days. Next, H_2O was added, and the mixture neutralized with aqueous $NaHCO_3$ solution, then extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with H_2O, dried over $MgSO_4$, filtered, and evaporated to dryness. The crude product was recrystallized from EtOAc/hexane to give optically active adduct (+)-**2** (1.43 g, 97% yield, >99% ee) as a white solid; mp: 153–155 °C (from EtOAc/hexane); $[\alpha]_{589}^{29} = +38.2$ (*c* 1.01, $CHCl_3$); {lit.[19] $[\alpha]_D^{29} = +38.7$ (*c* 1.06, $CHCl_3$)} whose IR, ^1H, ^{13}C NMR, and mass spectroscopic data were identical to those previously published.[19]

4.3.3. (11*R*)-11-Carbomethoxy-11-methoxyacetyl-9,10-dihydro-9,10-ethanoanthracene (−)-2

Under the same conditions, adduct (−)-**4** provided (−)-**2** (89% yield, >99% ee) as white solid; mp: 154–155 °C (from EtOAc/hexane); $[\alpha]_{589}^{29} = -38.8$ (*c* 1.02, $CHCl_3$); {lit.[19] $[\alpha]_D^{29} = -39.0$ (*c* 1.18, $CHCl_3$)} whose IR, ^1H, ^{13}C NMR, and mass spectroscopic data were identical to those previously published.[19]

4.4. Synthesis of enantiomerically pure 4′-((*E*)-2-(benzo[*c*][1,3]-dioxol-1-yl)vinyl)-3′-methoxycarbonyl-5′-(piperidine-1-carbonyl) cyclopentanone-2′-spiro-11-9,10-dihydro-9,10-ethanoanthracenes (−)-5 and (−)-6 from (+)-2

Compounds (−)-5 and (−)-6 were obtained when (+)-2 was employed in the above typical procedure. Compound (−)-(3′*S*,4′*S*,5′*S*,11*R*)-5 (33%): white solid; mp: 205.9–207.6 °C (from CH_2Cl_2/hexane); $[\alpha]_{589}^{24.4} = -18.5$ (c 0.87, $CHCl_3$) whose IR, 1H NMR, ^{13}C NMR, and mass spectroscopic data were identical to those previously reported.

Compound (−)-(4′*R*,4′*R*,5′*R*,11*R*)-**6** (17%): white solid, mp: 223.4–225.9 °C (from CH_2Cl_2/hexane); $[\alpha]_{589}^{24.4} = -70.5$ (*c* 0.58, $CHCl_3$) whose IR, ^1H NMR, ^{13}C NMR, and mass spectroscopic data were identical to those previously reported.

4.5. Synthesis of enantiomerically pure 4′-((*E*)-2-(benzo[*c*][1,3]-dioxol-1-yl)vinyl)-3′-methoxycarbonyl-5′-(piperidine-1-carbonyl)cyclopentanone-2′-spiro-11-9,10-dihydro-9,10-ethanoanthracenes (+)-5 and (+)-6 from (−)-2

Compounds (+)-**5** and (+)-**6** were obtained when (−)-**2** was employed in the above typical procedure. Compound (+)-(3′*R*,4′*R*,5′*R*,11*S*)-**5** (14%): white solid, mp: 205.9–207.6 °C (from CH_2Cl_2/hexane); $[\alpha]_{589}^{24.4} = +17.5$ (*c* 0.76, $CHCl_3$) whose IR, ^1H NMR, ^{13}C NMR, and mass spectroscopic data were identical to those previously reported.

Compound (+)-(3′*S*,4′*S*,5′*S*,11*S*)-**6** (8%): white solid, mp: 223.4–225.9 °C (from CH_2Cl_2/hexane); $[\alpha]_{589}^{24.4} = +67.6$ (*c* 0.60, $CHCl_3$) whose IR, ^1H NMR, ^{13}C NMR, and mass spectroscopic data were identical to those previously reported.

4.6. X-ray crystallographic analysis of adducts (±)-5

White crystals of (±)-**5** were obtained in the mixture of CH_2Cl_2/hexane by slow evaporation. X-ray diffraction data were measured on a Bruker-Nonius kappaCCD diffractometer with graphite monochromated Mo Kα radiation (λ = 0.71073 Å) at 298(2) K. The structure was solved by direct methods by SIR97,[24] and refined with full-matrix least-squares calculations on F^2 using SHELXL-97.[25]

4.6.1. Crystal data of adduct 5

$C_{37}H_{35}NO_6{\cdot}H_2O$, MW = 607.68, monoclinic, dimensions: 0.25 × 0.15 × 0.10 mm^3, D = 1.255 g/cm^3, space group $P2_1/c$, Z = 4, a = 18.7483(9), b = 9.9445(2), c = 19.2932(9) Å, β = 116.5848(14)°, V = 3216.8(2) $Å^3$, reflections collected/unique: 9565/4844 (R_{int} = 0.025), number of observation [$>2\sigma(I)$] 3722, final R indices [$I > 2\sigma(I)$]: R_1 = 0.0785, wR_2 = 0.2569.

4.7. Bioassay procedures

The in vitro antimalarial activity was evaluated against the parasite *P. falciparum* (K1, multi-drug resistance strain), which was cultured continuously according to the method of Trager and Jensen.[26] Quantitative assessment of the antimalarial activity in vitro was determined by means of the microculture radioisotope technique based on the method described by Desjardins et al.[21] The inhibitory concentration (IC_{50}) represents the concentration that causes 50% reduction in the parasite growth as indicated by the in vitro uptake of [3*H*]-hypoxanthine by *P. falciparum*. The standard compounds, mefloquine and dihydroartemisinin (DHA), exhibited IC_{50} values of 0.012 and 0.001 μg/mL, respectively, in this test system. The cytotoxicity of the purified compounds against *vero* cells (African green monkey kidney) was evaluated employing the colorimetric method as described by Hunt et al.[27] Ellipticine was used as the reference substance, exhibiting the activity toward *vero* cells, with IC_{50} of 0.976 /mL.

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