Preparation and biological activity of tricyclic non-steroidal inhibitors of human steroid 5α-reductase

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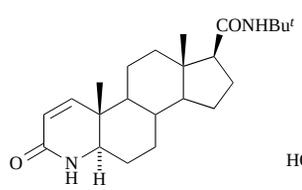
The synthesis and *in vitro* inhibitory studies against human types 1 and 2 steroid 5α-reductase of a series of 9,10-dihydrophenanthrene-2-carboxylic acids is reported. A (4-carboxy)phenyl substituent at C-7 provides a compound with activity against both isozymes. The structural features responsible for activity are discussed.

Introduction

In a preliminary communication we reported aryl acids of the type **5** as potent inhibitors of type 1 steroid 5α-reductase (SR).[1] SR is a membrane bound, NADPH-dependent enzyme which exists as two distinct isozymes (type 1 and type 2). These isozymes catalyse the stereospecific reduction of testosterone (T) to give dihydrotestosterone (DHT).[2] DHT is a potent androgen which has been implicated in a number of disorders of the skin (*e.g.* acne and hirsutism) and the prostate [*e.g.* benign prostatic hyperplasia (BPH) and prostatic cancer].[2,3] As such, the inhibition of SR has been identified as an important medicinal target.[2]



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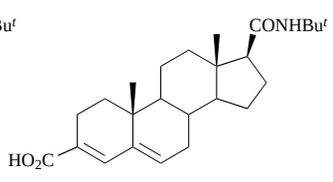

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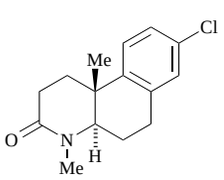

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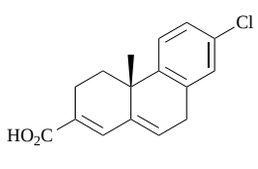

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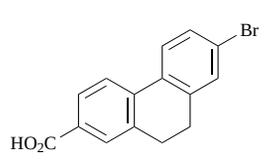

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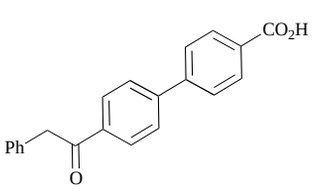

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The first generation of inhibitors of SR were steroid-based transition state analogues such as finasteride **1**[4] (type 1 IC_{50} 500 nM, type 2 IC_{50} 4.2 nM, currently marketed worldwide for the treatment of BPH) and epristeride **2** (type 1 $K_{i,app}$ 410 nM, type 2 $K_{i,app}$ 0.2 nM).[5] Many other steroid-based inhibitors of SR have subsequently been identified.[2] The recent identification[6] of two separate isozymes of SR (types 1 and 2) has now focused attention on developing isozyme-selective and dual isozyme inhibitors of SR.[2] Attention has also switched to developing non-steroidal, rather than steroidal, inhibitors of SR. Some of these efforts have identified[2] benzoquinolinones, *e.g.* **3** (type 1 $K_{i,app}$ 9–10 nM, type 2 $K_{i,app}$ >1000 nM),[7] aryl acids **5** (type 1 $K_{i,app}$ 26 nM, type 2 $K_{i,app}$ >10 000 nM),[1] phenanthridin-3-ones[8] and diazophenanthren-2-ones[9] as type 1 selective, non-steroidal inhibitors of SR. The diene acid **4** (type 1 $K_{i,app}$ 1200 nM, type 2 $K_{i,app}$ 260 nM),[10] biphenyl acids **6** (type 1 inactive, type 2 $K_{i,app}$ 60 nM),[11] indolecarboxylic acids[11] and benzophenone acids[11] have also been identified as type 2 selective inhibitors of SR.

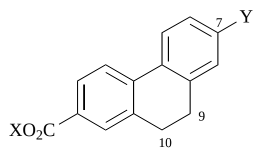
To date, dual isozyme, non-steroidal inhibitors of SR have not been reported. We now report the synthesis and biological evaluation of compound **21** as an initial step towards this goal. We also report the synthesis and inhibitory data on the analogues **18** and **20** which were designed (see below for details) to be selective inhibitors of the isozyme 1 and 2 of SR, respectively. The synthesis of these derivatives utilises a regioselective halogenation of 9,10-dihydroanthracene followed by a selective palladium-catalysed carbonylation to give **9**.[1] We also report the coupling of an arylboronic acid with **9** to give the biphenyl moiety of **17–21**. The synthesis and biological testing of **13**, **15** and **16** are also reported.

Results and discussion

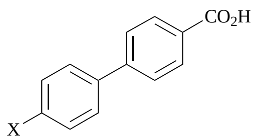
Compound **21** was designed to incorporate the structural features of the isozyme 1 selective inhibitors of type **5** and the isozyme 2 selective inhibitor **6** into the one molecule. The objective was to produce a molecule capable of inhibiting both the type 1 and type 2 isozymes of SR, *i.e.* a dual isozyme inhibitor. It was anticipated that the corresponding methyl ester **20** would display a reduced type 1 potency due to the masking of the A-ring acid, a group that is known to induce type 1 potency (see compound **5**, Table 1). Similarly, it was anticipated that **18** would have a reduced type 2 potency, relative to **21**, with the removal of the biphenyl acid which is known to induce type 2 potency as in compound **6** (Table 1).

The synthesis of compounds **5**, **11**, **13**, **15**, **16**, **18**, **20** and **21**, for evaluation against SR, is outlined in Schemes 1–4. 9,10-Dihydrophenanthrene **7** was treated with bromine in trimethyl

Table 1 Inhibition of types 1 and 2 human steroid 5α-reductase (SR)

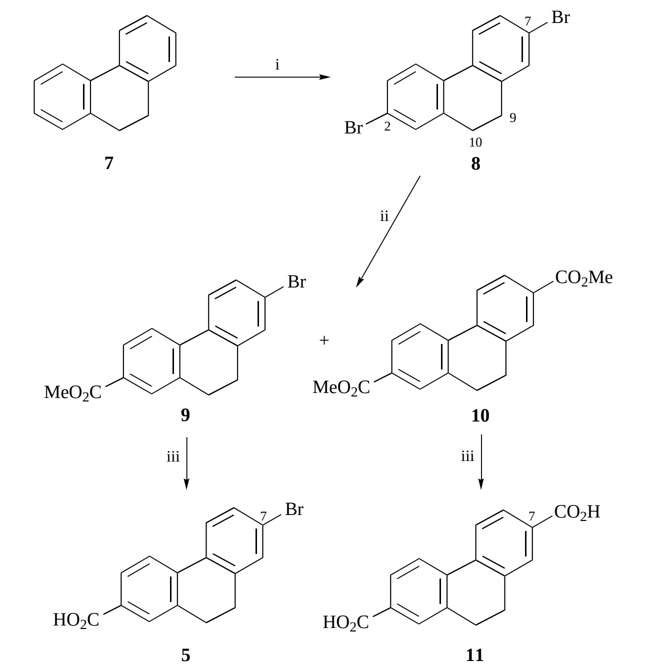


Compound	X	Y	Inhibition Type 1 SR	Inhibition Type 2 SR
5[a]	H	Br	100% @ 2.5 μM (0.026 μM)[b]	*c*
11	H	CO_2H	20% @ 2.5 μM	*c*
13	H	H (9,10-dehydro)	75% @ 2.5 μM (0.380 μM)[b]	*d*
15[a]	H	H	75% @ 2.5 μM (0.315 μM)[b]	*d*
16[a]	H	Cl	75% @ 2.5 μM (0.320 μM)[b]	50% @ 2.5 μM
18	H	Ph	45% @ 2.5 μM	2.200 μM[b]
20	Me	$4\text{-}HO_2CC_6H_4$	30% @ 2.5 μM	0.580 μM[b]
21	H	$4\text{-}HO_2CC_6H_4$	80% @ 2.5 μM	2.200 μM[b]



Compound	X	Y	Inhibition Type 1 SR	Inhibition Type 2 SR
6[e]	$COCH_2Ph$	—	*f*	0.060 μM[b]
22	H	—	10% @ 2.5 μM	20% @ 2.5 μM

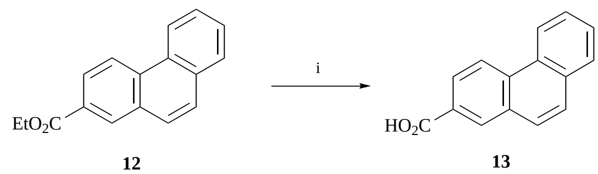
[a] Ref. 1. [b] $K_{i,app}$. [c] 50% @ 10 μM. [d] 20% @ 10 μM. [e] Ref. 11. [f] No inhibition @ 2.5μM.



Scheme 1 *Reagents and conditions:* i, Br_2, Me_3PO, 18 °C for 18 h, then −15 °C for 48 h; ii, $PdCl_2$, Ph_3P, Et_3N, MeOH–benzene (1 : 2.1), CO (575 psi), 150 °C for 4 h; iii, K_2CO_3, MeOH–H_2O (10 : 1), reflux, 18 h

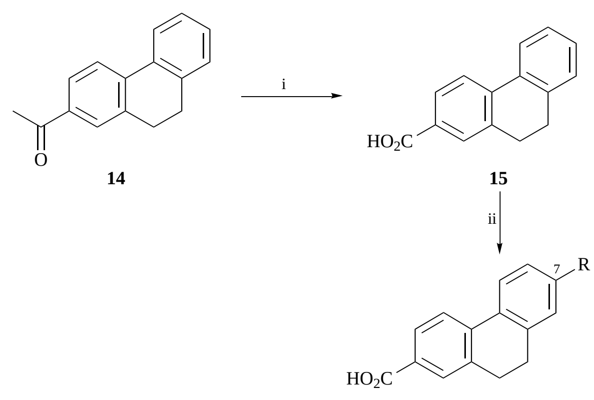
phosphate to give the dibromide **8** (Scheme 1). Trimethyl phosphate is a dipolar aprotic solvent that promotes[12] aromatic halogenation reactions by acting as an acid scavenger to generate a methyl halide, in this case methyl bromide. The observations of H(9) and H(10) as a four proton singlet in the ^1H NMR spectrum of **8** and also a positive NOE between H(9)/H(10) and H(1)/H(8) were consistent with the depicted strucure of **8**. The ^{13}C NMR spectrum of **8** showed seven resonances, which is also consistent with a structure possessing a C_2 axis of symmetry as in **8**.

A selective palladium(II)-catalysed carbonylation[13] of **8** at 575 psi and 150 °C gave a separable mixture of the methyl ester **9** (53%) and the dimethyl ester **10** (23%) (Scheme 1). Hydrolysis of **9** and **10** then gave the desired acids **5** and **11**, respectively. A similar hydrolysis of the ethyl ester **12** gave phenanthrene-2-carboxylic acid **13** (Scheme 2), which was also required for inhibitory studies.



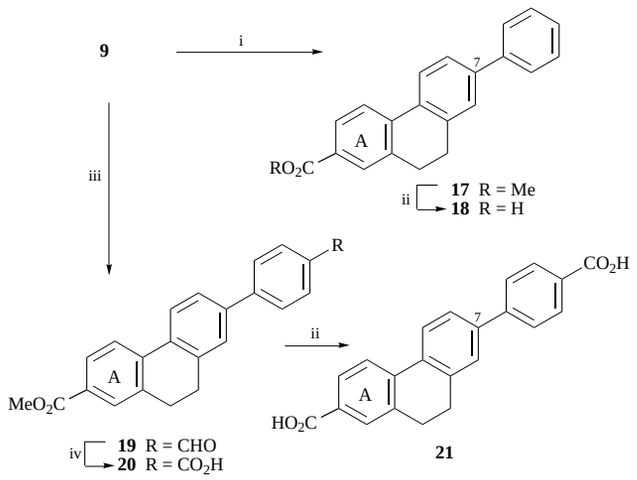
Scheme 2 *Reagents and conditions:* i, K_2CO_3, MeOH–H_2O (10 : 1), reflux, 18 h

Compound **5** was also prepared by bromination of **15** in trimethyl phosphate at room temperature (Scheme 3). Chlorination of **15** in trimethyl phosphate at 45–100 °C gave the chloro derivative **16**. Compounds **5** and **16** were obtained as the sole products from these halogenation reactions. The starting acid **15** was prepared by acetylating 9,10-dihydrophenanthrene to give **14** followed by oxidation with sodium hypobromite.[14]



5 R = Br
16 R = Cl

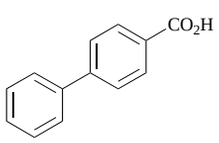
Scheme 3 *Reagents and conditions:* i, ref. 14; ii, Br_2, trimethyl phosphate (for **5**) or Cl_2, trimethyl phosphate (for **16**)



Scheme 4 *Reagents and conditions:* i, $Pd(PPh_3)_4$, K_2CO_3, phenylboronic acid, toluene; ii, K_2CO_3, $MeOH–H_2O$ (10:1), reflux, 18 h; iii, $Pd(PPh_3)_4$, K_2CO_3, 4-formylphenylboronic acid, toluene; iv, $NaClO_2$, NaH_2PO_4, 2-methylbut-2-ene, aq. *tert*-butyl alcohol

The biaryl acids **18** and **21** were prepared as shown in Scheme 4. The key biaryl intermediates **17** and **19** were prepared by palladium(0)-catalysed Suzuki coupling[15] of the aryl bromide **9** with either phenylboronic acid or 4-formylphenylboronic acid to give **17** and **19**, respectively (see steps i and iii in Scheme 4). A Lindgren oxidation[16] of the aldehyde **19** gave **20**. Finally, hydrolysis of the methyl esters of **17** and **20** gave the desired biaryls **18** and **21**, respectively.

The compounds prepared, as detailed above, were assayed against types 1 and 2 SRs and the results obtained are summarised in Table 1. The first point to note is that the nature of the C(7) substituent (Y in Table 1) of the tricyclic aryl acids has a significant effect on the potency against the type 1 isozyme (*cf*. the type 1 activity of compounds **5**, **11**, **13**, **15**, **16**, **18** and **21**). This observation is consistant with reported structure–activity studies on other non-steroidal SR inhibitors of the type **3**.[7] It would also appear that the type 2 potency of the biaryl acids is decreased, relative to the reference compound **6**, by having the tricyclic moiety incorporated into the biaryl [*cf*. compounds **6** (type 2 $K_{i,app}$ 0.06 μM) and **20** (type 2 $K_{i,app}$ 0.58 μM)] and an ionizable group on the A-ring of the tricyclic group [*cf*. compounds **20** (type 2 $K_{i,app}$ 0.58 μM) and **21** (type 2 $K_{i,app}$ 2.2 μM)]. It should be noted that the unsubstituted biaryl acid **22** is a weak inhibitor of types 1 and 2 SRs, (Table 1).



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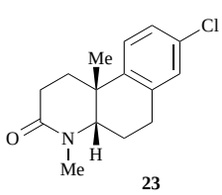

23

The results presented in Table 1 do, however, support our original proposition that the incorporation of a biaryl acid, as found in **6**, and a tricyclic aryl acid, as found in **5**, into one molecule would induce activity against types 1 and 2 SRs. The biaryl acid **20** inhibits type 2 SR ($K_{i,app}$ 0.58 μM) as would be expected based on the type 2 selective biaryl **6**. The related diacid **21** was expected to inhibit types 1 and 2 SRs and indeed it is an inhibitor of both isozymes (Table 1). It shows significantly greater activity towards types 1 and 2 SRs than the diacid **11**, which lacks both a biaryl acid group, which is responsible for type 2 activity, and also an optimum[1] C(7) substituent for type 1 activity. It should be noted that compound **21** also shows significantly greater type 1 potency than **18** and **20**, compounds that do not possess the A-ring aryl acid group known to promote type 1 activity.

Previous studies have noted that an extended planar structure is required to give potent non-steroidal inhibitors of type 1 SR.[7b] In particular, the *cis*-fused and, as a consequence, bowl-shaped **23** shows a significantly reduced potency as compared to the *trans*-fused analogue **3**. The fact that the planar aromatic derivative **13** was found to be a potent inhibitor of type 1 SR (Table 1) adds support to this observation. A loss of planarity in the biaryl derivatives **18** and **21** may also contribute to a lower activity against type 1 SR.

An enormous amount of structure–activity work has gone into optimising the inhibitor activity of steroid-based inhibitors of SR.[2] However, with a few exceptions, little such work has been carried out on non-steroidal compounds.[2] The work presented here demonstrates the feasibility of incorporating the structural features of type 1 and type 2 selective, non-steroidal inhibitors of SR into the one molecule. Further work is needed to find the optimum combination of functional groups to give a potent dual inhibitor of SR.

Experimental

Melting points are uncorrected. ^{1}H NMR Spectra were recorded with a Bruker AM-250 spectrometer and are reported as δ units (in ppm) in the solvent specified; *J* values are given in Hz. Mass spectra were obtained with a Finnigan-MAT quadrupole instrument. Chromatography refers to flash chromatography using Kieselgel 60 (230–400 mesh) silica gel. Recombinant type 1 and type 2 human steroid 5α-reductases were prepared as previously described.[17]

Enzymatic assay for SR activity[17]

SR activity was determined by following the conversion of T to 5α-reduced steroids, represented by the sum of DHT and androstanediol (ADIOL). [^{14}C]-T in ethanol was deposited in test tubes and concentrated to dryness in a SAVANT Speed-Vac evaporator. Buffer and NADPH were added and each tube was equilibrated to assay temperature. A cofactor regenerating system ($NADP^+$ to NADPH) consisting of 1 mM glucose-6-phosphate and 0.5 units cm^{-3} glucose-6-phosphate dehydrogenase was included in each assay. The reaction was initiated by the addition of an aliquot of enzyme preparation to give a final volume of 0.5 cm^3. All assays were carried out in 50 mM sodium citrate, pH 5.0, at 37 °C. The reaction was stopped after a maximum of 40 min by addition of 4 cm^3 ethyl acetate containing 0.2 μmol of T, DHT, ADIOL and androstenedione each as carriers and markers. After separation from the aqueous layer, the organic solvent was removed under reduced pressure and the thus isolated steroids were separated by TLC, developing twice with chloroform–acetone (9:1) and the relative content of radiolabel in the substrate (T) and the products (DHT plus ADIOL) was determined for each lane using a BIOSCAN imaging scanner.

Potential inhibitors in ethanol were added to the assay tubes with substrate (T) and the contents were evaporated to dryness. All other procedures were the same as outlined above for the SR activity assay. Inhibition data are quoted as a percentage at an inhibitor concentration of 2.5 μM (10 μM for less active compounds) and inhibition constants ($K_{i,app}$) were estimated for the most potent inhibitors by Dixon analysis[17] with initial substrate concentrations of 1.0 μM T and 200 to 400 μM NADPH.

7-Bromo-9,10-dihydrophenanthrene-2-carboxylic acid 5

Method A. A mixture of **9**, prepared as detailed below (30 mg, 0.10 mmol) and potassium carbonate (26 mg, 0.19 mmol) was refluxed for 18 h in methanol (2.5 cm^3) and water (0.25 cm^3). The methanol was removed under reduced pressure and water (1 cm^3) and 10% aqueous HC1 (5 cm^3) were added. After stirring for 30 min, the white solid was isolated by filtration to give the title compound (27 mg, 89%), mp 321–322 °C (Found: C, 59.2; H, 3.5. $C_{15}H_{11}BrO_2$ requires C, 59.4; H, 3.7%);

ν_{max}(Nujol)/cm^{-1} 3200–2500 (br) and 1690; δ_H([$^2H_6$]DMSO) 2.87 (4 H, br s), 7.55 (1 H, dd, *J* 1.5 and 8.3), 7.56 (1 H, d, *J* 1.5), 7.84–7.87 (3 H, m) and 7.94 (1 H, d, *J* 8.2); *m/z* (ES +ion) 305 ([M + H]$^+$).

Method B. A solution of bromine (31 mg, 10 μl, 0.19 mmol) in trimethyl phosphate (0.1 cm^3) was added dropwise to a solution of 9,10-dihydrophenanthrene-2-carboxylic acid **15**[14] (20 mg, 0.09 mmol) in trimethyl phosphate (0.1 cm^3). The solution was stirred at room temp. with protection from the light for 18 h and then left in a refrigerator for 6 h. Ice-water (5 cm^3) was added and the resulting solid was collected by vacuum filtration to give **5** (12 mg, 44%); δ_H as recorded above.

Methyl 7-bromo-9,10-dihydrophenanthrene-2-carboxylate 9

Bromine (4.75 g, 1.53 cm^3, 29.7 mmol) in trimethyl phosphate (10 cm^3) was added dropwise to a solution of 9,10-dihydrophenanthrene **7** (2.5 g, 13.9 mmole) in trimethyl phosphate (15 cm^3) with protection from light. The solution was stirred for 18 h at room temp. and then left in a freezer for 2 days. The white solid was collected and recrystallised from chloroform to give 2,7-dibromo-9,10-dihydrophenanthrene **8**, mp 162–164 °C (lit.,[12] 163–165 °C); δ_H($CDCl_3$) 2.82 (4 H, s), 7.37 (2 H, d, *J* 2), 7.41 (2 H, dd, *J* 2.1 and 8.3) and 7.54 (2 H, d, *J* 8.3); δ_C($CDCl_3$) 28.6, 121.5, 125.1, 130.2, 131.1, 132.6 and 139.1.

A high pressure bomb was charged with **8** (1.50 g, 4.44 mmol), palladium(II) chloride (12 mg), triphenylphosphine (35 mg), triethylamine (750 μl), methanol (3.5 cm^3), benzene (7.5 cm^3) and finally carbon monoxide at 450 psi.[13] The temperature was raised to 150 °C (pressure 575 psi) and the reaction mixture was stirred for 4 h. Unreacted carbon monoxide was purged and the solution was evaporated to dryness. Purification by flash silica chromatography using diethyl ether–hexane (1:4) gave recovered starting material **8** (356 mg, 24%). Further elution gave **9** (745 mg, 53%), mp 119–120 °C (Found: C, 60.9; H, 4.0. $C_{16}H_{13}BrO_2$ requires C, 60.6; H, 4.1%); δ_H($CDCl_3$) 2.85–2.95 (4 H, m), 3.93 (3 H, s), 7.42–7.4 (2 H, m), 7.64 (1 H, d, *J* 8.2), 7.75 (1 H, d, *J* 8.1), 7.91 (1 H, d, *J* 1.5) and 7.96 (1 H, dd, *J* 1.9, 8.2); *m/z* (ES +ion) 317/318 ([M + H]$^+$). The final fraction contained dimethyl 9,10-dihydrophenanthrene-2,7-dicarboxylate **10**[18] (300 mg, 23%) which was used in subsequent steps without further purification.

9,10-Dihydrophenanthrene-2,7-dicarboxylic acid 11

Compound **10** (50 mg, 0.17 mmol), prepared as detailed above, and potassium carbonate (47 mg, 0.34 mmol) were refluxed for 18 h in methanol (5 cm^3) and water (0.5 cm^3) as previously described to give **11**[18] (43 mg, 94%); δ_H([$^2H_6$]DMSO) 2.92 (4 H, br s), 7.85–7.90 (4 H, m) and 8.00–8.03 (2 H, m).

Phenanthrene-2-carboxylic acid 13

A mixture of ethyl phenanthrene-2-carboxylate[19] **12** (500 mg, 0.20 mmol) and potassium carbonate (47 mg, 0.34 mmol) was refluxed for 18 h in methanol (5 cm^3) and water (0.5 cm^3) as described for **5** to give the title compound **13** (quant.), mp 262–263 °C (lit.,[19] 258.5–260 °C) (Found: C, 81.1; H, 4.3. $C_{15}H_{10}O_2$ requires C, 81.1; H, 4.5%); ν_{max}(Nujol)/cm^{-1} 3100–2500 (br), 1685, 1620 and 1600; δ_H([$^2H_6$]DMSO) 7.70–7.80 (2 H, m), 7.92–8.10 (3 H, m), 8.17 (1 H, dd, *J* 1.9, 8.7), 8.60 (1 H, d, *J* 1.6) and 8.88–8.99 (2 H, m).

7-Chloro-9,10-dihydrophenanthrene-2-carboxylic acid 16

Chlorine (240 μl of 133 mg cm^{-3} solution of Cl_2 in trimethyl phosphate) was added slowly to a stirred suspension of **15**[14] in trimethyl phosphate (0.5 cm^3) at 45 °C. After the addition, the temperature was raised slowly to 100 °C and the mixture was held at this temperature for 1.5 h. The solution was cooled to room temperature and ice-water (5 cm^3) was added. The resulting precipitate was isolated by filtration and recrystallised from chloroform to give **16** (26 mg, 49%) (Found: C, 69.4; H, 4.1. $C_{15}H_{11}ClO_2$ requires C, 69.6; H, 4.3%); ν_{max}(Nujol)/cm^{-1} 3100–2500 (br), 1690 and 1610; δ_H([$^2H_6$]DMSO) 2.87 (4 H, br s), 7.4 (1 H, dd, *J* 2 and 6.6), 7.42 (1 H, d, *J* 2) and 7.85–7.98 (4 H, m); *m/z* (DEI) 258 ([M]$^+$), 213 and 178.

7-Phenyl-9,10-dihydrophenanthrene-2-carboxylic acid 18

To a stirred mixture **9** (50 mg, 0.16 mmol) and tetrakis(triphenylphosphine)palladium(0) (6 mg, 0.01 mmol) in toluene (0.5 cm^3), under argon, was added an aqueous 2 M solution of potassium carbonate (160 μl) followed by phenylboronic acid (20 mg, 0.16 mmol). The reaction mixture was heated at 85 °C for 18 h and then diluted with dichloromethane (5 cm^3). The solution was washed with saturated aqueous sodium hydrogen carbonate (2×) and dried, and the solvent was evaporated under reduced pressure. Purification by preparative silica chromatography eluting with diethyl ether–hexane (1:4) gave **17** (39 mg, 78%) which was used without further purification; mp 149–150 °C; δ_H($CDCl_3$) 2.96 (4 H, s), 3.93 (3 H, s), 7.34–7.38 (1 H, m), 7.46 (2 H, t, *J* 7.8), 7.49 (1 H, d, *J* 1.8), 7.56 (1 H, dd, *J* 1.9 and 8.0), 7.64 (2 H, d, *J* 7.0), 7.81–7.86 (2 H, m), 7.93 (1 H, d, *J* 1.7) and 7.97 (1 H, dd, *J* 1.8 and 8.2); *m/z* (ES, +ion) *m/z* 315 ([M+H]$^+$).

Compound **17** (30 mg, 0.10 mmol) and potassium carbonate (27 mg, 0.20 mmol) were refluxed for 18 h in methanol (2 cm^3) and water (0.2 cm^3) as previously described. Recrystallisation from acetic acid gave the title compound **18** (quant.), mp >340 °C (decomp.) (HRMS: found, 300.1143. $C_{21}H_{16}O_2$ requires 300.1150); ν_{max}(Nujol)/cm^{-1} 3100–2500 (br) and 1680; δ_H([$^2H_6$]DMSO) 2.90 (4 H, br s), 7.37 (1 H, t, *J* 7.2), 7.48 (2 H, t, *J* 7.6), 7.61–7.63 (2 H, m), 7.72 (2 H, d, *J* 7.5), 7.79–7.83 (3 H, m) and 7.93 (1 H, d, *J* 8.5); *m/z* (ES, +ion) 301 ([M+H]$^+$).

Ethyl 7-(4-formylphenyl)-9,10-dihydrophenanthrene-2-carboxylate 19

To a stirred solution of **9** (110 mg, 0.35 mmol) and tetrakis(triphenylphosphine)palladium(0) (15 mg, 0.01 mmol) in toluene (1 cm^3) under argon, was added aqueous potassium carbonate (320 μl of 2 M solution) followed by a solution of 4-formylphenylboronic acid (50 mg, 0.33 mmol) in methanol (0.5 cm^3). The reaction mixture was stirred at 85 °C for 3.5 h and then left at room temp. for 18 h. The solution was washed with 5% aqueous HCl and saturated aqueous sodium hydrogen carbonate and dried, and the solvent was evaporated under reduced pressure to give a brown solid. Recrystallisation from chloroform–hexane gave **19** (58 mg, 49%), mp 198–199 °C (Found: C, 80.7; H, 5.7. $C_{23}H_{18}O_3$ requires C, 80.7; H, 5.3%); δ_H($CDCl_3$) 2.99 (4 H, s), 3.95 (3 H, s), 7.55 (1 H, d, *J* 1.8), 7.62 (1 H, dd, *J* 1.9 and 8.1), 7.80–8.00 (8 H, m) and 10.07 (1 H, s); *m/z* (ES, +ion) 343 ([M+H]$^+$).

4-(7-Methoxycarbonyl-9,10-dihydrophenanthren-2-yl)benzoic acid 20

To a stirred mixture of **19** (50 mg, 0.15 mmol), *tert*-butyl alcohol (2.5 cm^3) and 2-methylbut-2-ene, at 0 °C, was added a solution of sodium phosphate (205 mg, 1.48 mmol) and sodium chlorite (165 mg, 1.46 mmol) in water (0.8 cm^3). The mixture was stirred under argon and allowed to warm to room temp. over 18 h. Acetic acid (1.5 cm^3) and water were added and the resulting precipitate was collected by filtration and washed with ethyl acetate (5 cm^3) and dichloromethane (5 cm^3) to give **20** (39 mg, 73%), mp 298–300 °C (Found: C, 76.8; H, 4.7. $C_{23}H_{18}O_4$ requires C, 77.1; H, 5.1%); ν_{max}(Nujol)/cm^{-1} 3100–2500 (br), 1715, 1680 and 1605; δ_H([$^2H_6$]DMSO) 2.95 (4 H, s), 3.87 (3 H, s), 7.73 (2 H, m), 7.85–7.92 (4 H, m) and 8.00–8.05 (4 H, m); *m/z* (ES, −ion) 357 ([M − H]$^-$).

7-(4-Carboxyphenyl)-9,10-dihydrophenanthrene-2-carboxylic acid 21

A mixture of **20** (20 mg, 0.06 mmol) and potassium carbonate (30 mg, 0.22 mmol) was refluxed for 18 h in methanol (2 cm^3) and water (0.2 cm^3), as previously described, to give **21** (quant.),

mp >400 °C (Found: C, 76.9; H, 4.8. $C_{22}H_{16}O_4$ requires C, 76.7; H, 4.7%) (HRMS: found 344.1056. $C_{22}H_{16}O_4$ requires 344.1049); ν_{max}(Nujol)/cm^{-1} 3100–2500 (br), 1680 and 1605; δ_H([$^2H_6$]DMSO) 2.95 (4 H, s), 7.75 (2 H, m), 7.85–7.91 (4 H, m) and 8.00–8.05 (4 H, m); *m/z* (EI) 344 (30%), 207 (68) and 91 (100).

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References

1 A. D. Abell, M. Brandt, M. A. Levy and D. A. Holt, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 1365.
2 D. A. Holt, M. A. Levy and B. W. Metcalf, *Advances in Medicinal Chemistry*, ed. B. E. Maryanoff and C. A. Maryanoff, JAI Press, London, 1993, vol. 2, pp. 1–29; A. D. Abell and B. R. Henderson, *Curr. Med. Chem.*, 1995, **2**, 583.
3 J. C. Lamb, M. A. Levy, R. K. Johnson and J. T. Isaacs, *Prostate* (*NY*), 1992, **21**, 15; A. R. Diani, M. J. Mulholland, K. L. Shull, M. F. Kubicek, G. A. Johnson, H. J. Schostarez, M. N. Brunden and A. E. Buhl, *J. Clin. Endocrinol. Metab.*, 1992, **74**, 505; F. Kuttenn, I. Mowszowicz, G. Shaison and P. Mauvais-Jarvis, *J. Endocrinol.*, 1977, **75**, 83; G. L. Sansone and R. M. Reisner, *J. Invest. Dermatol.*, 1971, **56**, 366.
4 G. H. Ramusson, G. F. Reynolds, N. G. Steinberg, E. Walton, G. F. Patel, T. Liang, M. A. Cascieri, A. H. Cheung, J. R. Brooks and C. Berman, *J. Med. Chem.*, 1986, **29**, 2298.
5 D. A. Holt, M. A. Levy, H.-J. Oh, J. M. Erb, J. I. Heaslip, M. Brandt, H.-Y. Lan-Hargest and B. W. Metcalf, *J. Med. Chem.*, 1990, **33**, 943; D. A. Holt, M. A. Levy, D. L. Ladd, H.-J. Oh, J. M. Erb, J. I. Heaslip, M. Brandt and B. W. Metcalf, *J. Med. Chem.*, 1990, **33**, 937.
6 E. P. Jenkins, S. Andersson, J. Imperato-McGinley, J. D. Wilson and D. W. Russell, *J. Clin. Invest.*, 1992, **89**, 293.
7 C. D Jones, J. E. Audia, D. E. Lawhorn, L. A. McQuaid, B. L. Neubauer, A. J. Pike, P. A. Pennington, N. B. Stamm, R. E. Toomey and K. S. Hirsch, *J. Med. Chem.*, 1993, **36**, 421; K. S. Hirsch, C. D. Jones, J. E. Audia, S. Andersson, L. A. McQuaid, N. B. Stamm, B. L. Neubauer, P. Pennington, R. E. Toomey and D. W. Russell, *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 5277; A. D. Abell, K. F. Erhard, H.-K. Yen, D. S. Yamashita, M. Brandt, H. Mohammed, M. A. Levy and D. A. Holt, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 1365.
8 R. A. Mook Jr, K. Lackey and C. Bennett, *Tetrahedron Lett.*, 1995, **36**, 3969.
9 A. D. Abell, M. Brandt, M. A. Levy and D. A. Holt, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 883.
10 A. D. Abell, M. Brandt, M. A. Levy and D. A. Holt, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 2327.
11 D. A. Holt, D. S. Yamashita, A. L. Konialian-Beck, Y. I. Luengo, A. D. Abell, D. J. Bergsma and M. A. Levy, *J. Med. Chem.*, 1995, **38**, 13.
12 D. E. Pearson, USP 3 988 369, 1976; D. E. Pearson, M. G. Frazer, V. S. Frazer and L. C. Washburn, *Synthesis*, 1976, 621.
13 Y. Sugi, Y. Iwai, K. Takeuchi, S. Takagi and Y. Doi, EP 0 397 418 A1, 1990.
14 J. W. Brown, D. J. Byron, D. J. Harwood, N. M. Smith and R. C. Wilson, *J. Chem. Soc., Perkin Trans. 2*, 1984, 271.
15 N. Miyaura and A. Suzuki, *Chem. Rev.*, 1995, **95**, 2457.
16 B. O. Lindgren and T. Nilsson, *Acta Chem. Scand.*, 1973, **27**, 888; B. S. Ba, W. E. Childeis and H. W. Pinnick, *Tetrahedron*, 1981, **37**, 2091.
17 M. A. Levy, M. Brandt, K. M. Sheedy, J. T. Dinh, D. A. Holt, L. M. Garrison, D. J. Bergsma and B. W. Metcalf, *J. Steroid Biochem. Mol. Biol.*, 1994, **48**, 197.
18 R. Paioni and W. Jenny, *Helv. Chim. Acta*, 1970, **53**, 141.
19 E. Mosettig and J. Van de Kamp, *J. Am. Chem. Soc.*, 1930, **52**, 3704.

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