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Pictet–Spengler condensations using 4-(2-aminoethyl)coumarins†

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Androgen-deprivation therapy (ADT) is only a palliative measure, and prostate cancer invariably recurs in a lethal, castration-resistant form (CRPC). Prostate cancer resists ADT by metabolizing weak, adrenal androgens to growth-promoting 5α-dihydrotestosterone (DHT), the preferred ligand for the androgen receptor (AR). Developing small-molecule inhibitors for the final steps in androgen metabolic pathways that utilize 17-oxidoreductases required probes that possess fluorescent groups at C-3 and intact, naturally occurring functionality at C-17. Application of the Pictet–Spengler condensation to substituted 4-(2-aminoethyl)coumarins and 5α-androstane-3-ones furnished spirocyclic, fluorescent androgens at the desired C-3 position. Condensations required the presence of activating C-7 amino or *N*,*N*-dialkylamino groups in the 4-(2-aminoethyl)coumarin component of these condensation reactions. Successful Pictet–Spengler condensation, for example, of DHT with 9-(2-aminoethyl)-2,3,6,7-tetrahydro-1*H*,5*H*,11*H*-pyrano[2,3-*f*]pyrido[3,2,1-*ij*]quinolin-11-one led to a spirocyclic androgen, (3*R*,5*S*,10*S*,13*S*,17*S*)-17-hydroxy-10,13-dimethyl-1,2,2′,3′,4,5,6,7,8,8′,9,9′,10,11,12,12′,13,13′,14,15,16,17-docosahydro-7′*H*,11′*H*-spiro-[cyclopenta[*a*]phenanthrene-3,4′-pyrido[3,2,1-*ij*]pyrido[4′,3′:4,5]pyrano[2,3-*f*]quinolin]-5′(1′*H*)-one. Computational modeling supported the surrogacy of the C-3 fluorescent DHT analog as a tool to study 17-oxidoreductases for intracrine, androgen metabolism.

Introduction

Prostate cancer growth and progression rely on the activation of the androgen receptor (AR) by the circulating, testicular androgen, testosterone (T) or its intracellular metabolite, 5α-dihydrotestosterone (DHT), the preferred ligand for AR transactivation.[1,2] Men, who present with advanced prostate cancer or who fail potentially curative therapy, undergo androgen-deprivation therapy (ADT) intended to lower circulating testosterone levels, to deprive the AR of activating ligands and to induce cancer regression.[3,4] Unfortunately, androgen-deprivation therapy is only a temporary, palliative measure, since prostate cancer produces intratumoral androgen levels during ADT that are low but sufficient to activate the AR[1,5] and promote cancer recurrence as lethal, castration-recurrent/resistant prostate cancer (CRPC). Current therapies for CRPC rely on inhibitors for enzymes that function well before the final steps in the biosynthetic pathways leading to DHT, and we sought to identify new, small-molecule inhibitors[6] for late-stage, NAD(P)H-dependent 17β-hydroxysteroid dehydrogenases.[7]

Prostate cancer cells utilize three, late-stage, androgen-metabolic pathways[8–12] driven by oxidoreductases to acquire DHT (**1**) (Fig. 1).

The "frontdoor" pathway converts the adrenal androgens, such as dehydroepiandrosterone and 4-androstene-3,17-dione (**2**), to testosterone (**3**) that subsequently undergoes Δ^4-reduction to DHT (**1**). The "primary backdoor" pathway converts the

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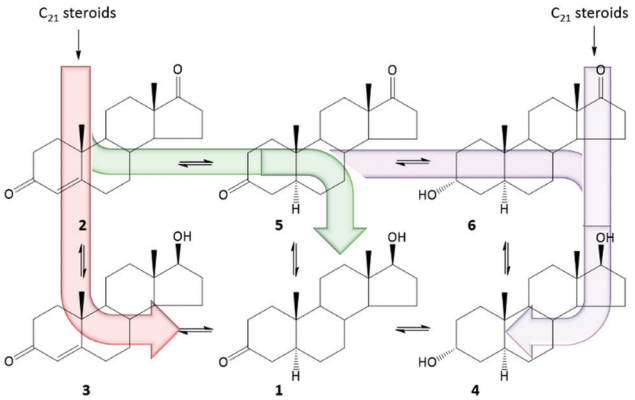
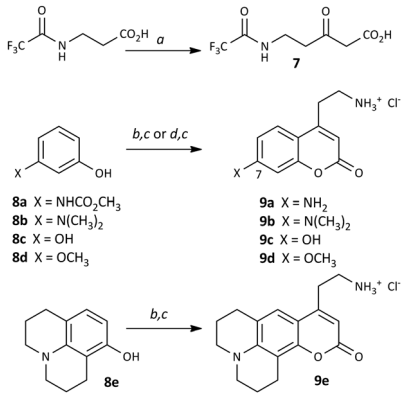


Fig. 1 Late-stage metabolic pathways converging on 5α-dihydrotestosterone (DHT). Frontdoor (orange), primary backdoor (green) and secondary backdoor (purple) pathways.



Scheme 1 Pechmann synthesis of substituted 4-(2-aminoethyl)-coumarins **9**. (a) $CH_3OC(=O)CH_2CO_2K$; (b) CH_3SO_3H, **7**; (c) conc. HCl; (d) $TiCl(OiPr)_3$, **7**.

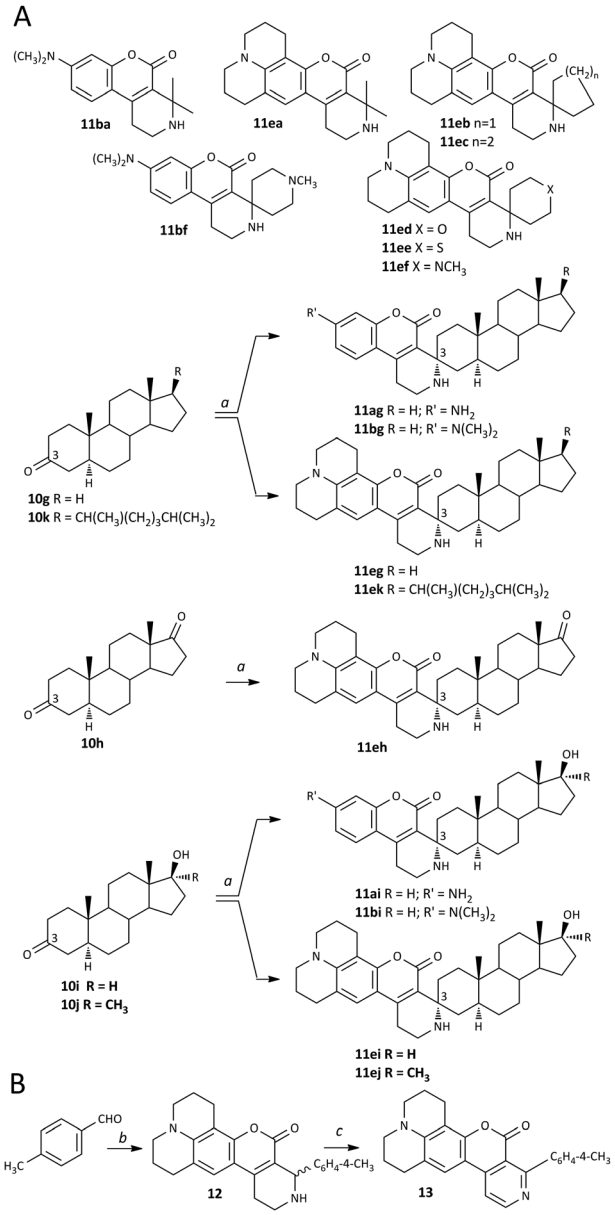
penultimate 5α-androstane-3α,17β-diol (**4**) directly to DHT[12–16] (**1**) without passing through testosterone (**3**) as an intermediate. The "secondary backdoor" pathway converts 4-androstene-3,17-dione (**2**) to 5α-androstane-3,17-dione (**5**) that undergoes reduction of the C-17-keto group to DHT[12,14,16–23] (**1**), also without passing through testosterone (**3**) as an intermediate. One commonality among these pathways involved C-17 redox reactions mediated by the aldo/ketoreductase[24–26] (AKR1C3; HSD17B5) and 17β-hydroxysteroid dehydrogenase[8] (HSD17B3) for the conversion of 4-androstene-3,17-dione (**2**) to testosterone (**3**); the conversion of 5α-androstane-3,17-dione (**5**) to DHT (**1**); and the conversion of 5α-androstan-3α-ol-17-one (**6**) to 5α-androstane-3α,17β-diol (**4**) (Fig. 1).

Our focus on developing small-molecule inhibitors for various 17-oxidoreductases, alone or in combination,[27] that perform these interconversions required fluorescent probes that had a C-3 fluorophore with excitation and emission patterns in the 550–650 nm range and that retained the intact, natural functionality at C-17. These combined challenges led us to select coumarins as fluorophores and to explore methodology for their attachment to the C-3 position of 5α-androstan-3-ones.[28–30] We now report the scope of the Pictet–Spengler condensation[31–34] of C-7 amino-substituted 4-(2-aminoethyl)-coumarins with aldehydes and ketones, NMR and X-ray crystallography studies that established the diastereoselectivity in adducts derived from 5α-androstan-3-ones, the mechanism of these reactions and computational modeling of adducts in the active site of 17β-hydroxysteroid dehydrogenase type 5[35,36] (17β-HSD5; AKR1C3). Our findings auger well for the application of these probes in drug discovery.

Results

Variants of the Pechmann condensation[37,38] provided access to C-7 substituted 4-(2-aminoethyl)coumarins **9a–9e** for this study (Scheme 1). Specifically, the condensation of 3-(trifluoroacetamido)propanoic acid with methyl potassium malonate furnished methyl 3-oxo-5-(trifluoroacetamido)-pentanoate (**7**); a second condensation with either methyl (3-hydroxyphenyl)carbamate (**8a**), 3-(*N*,*N*-dimethylamino)phenol (**8b**), resorcinol (**8c**), or 3-methoxyphenol (**8d**) furnished intermediate trifluoroacetamides; and the final, acid-catalyzed hydrolysis of these intermediates provided the 4-(2-aminoethyl)coumarins **9a–9d**, respectively, as their hydrochloride salts. A related procedure using a benzyloxy urethane in place of the trifluoroacetamide derivative (*i.e.*, methyl-5-benzyloxycarbonylamino-3-oxopentanoate) in a condensation with 8-hydroxy-2,3,6,7-tetrahydro-1*H*,5*H*-benzo[*ij*]quinolizine[38] (**8e**) furnished the 4-(2-aminoethyl)coumarin **9e** (Scheme 1). The Pictet–Spengler condensations of coumarins **9a**, **9b** or **9e** that possessed C-7 amino or C-7 *N*,*N*-dialkylamino groups with either acyclic and monocyclic ketones **10a–10f** or 3-keto-steroids **10g–10k** under acidic conditions afforded 1,2,3,4-tetrahydro-5*H*-chromeno[3,4-*c*]pyridin-5-ones **11** (Scheme 2A) in good yields (Table 1). For example, the condensation of coumarin **9e** with 5α-androstan-17β-ol-3-one (DHT) (**10i**) afforded a single diastereomer of the spirocyclic, fluorescent adduct **11ei** that was more compact than previously reported, bulky DHT analogs that possessed a linker between umbelliferone and 3β-amino-5α-androstan-17β-ol[28] or possessed a linker between fluorescein isothiocyanate and either a C-3 hydrazone or *O*-carboxymethyloxime derivative of DHT.[29,30] The hygroscopic nature of some of the hydrochloride and trifluoroacetate salts of the adducts **11** necessitated their isolation as *N*-acetyl derivatives, as noted in Table 1.

A range of steroidal and non-steroidal carbonyl compounds served to define the scope of these Pictet–Spengler reactions with coumarins **9** as illustrated by the condensation of 1-methylpiperidin-4-one (**10f**) with **9b** and the condensation of 5α-androstan-17β-ol-3-one (**10i**) with coumarins **9a** and **9e** (Scheme 2A). In general, condensations with unhindered ketones varying from acyclic to monocyclic ketones were successful in yields of 60–90% (Table 1). In contrast, the condensations of the coumarin **9e** either with hindered ketones such as 5α-androstan-17-one or with α,β-unsaturated ketones such as testosterone (**3**) were unsuccessful. This differential reactivity proved advantageous in the regiospecific modification of 5α-androstane-3,17-dione (**10h**) with coumarin **9e** that led



Scheme 2 Representative Pictet–Spengler reactions of C-7 amino-substituted 4-(2-aminoethyl)coumarins **9**. Panel A: (a) 1 : 10 (v/v) conc. HCl acid–abs. ethanol; panel B: (b) *p*-tolualdehyde; 1 : 10 (v/v) CH_2Cl_2–TFA, 25 °C; (c) $CuBr_2$, O_2, DBU, DMSO.

exclusively to the C-3 adduct **11eh** in 57% yield. Contrary to a prior report of a successful Pictet–Spengler reaction of an amino-substituted coumarin with formaldehyde,[39] condensations of aldehydes with coumarins **9** led to poor yields of isolated products, presumably because of adventitious air-oxidation. Deliberate efforts to oxidize the intermediate 1,2,3,6-tetrahydropyridines from condensations with aldehydes led to poor yields of pyridines. For example, a Pictet–Spengler reaction of coumarin **9e** with *p*-tolualdehyde followed by cupric bromide-catalyzed air oxidation[40] of the intermediate tetrahydropyridine **12** led to only a 20% yield of the 5*H*-chromeno[3,4-*c*]pyridin-5-one **13** (Scheme 2B).

Confirmation of the C-3*R* stereochemical assignments in the spirocyclic DHT adduct **11ei** (Scheme 2A) relied on two-dimensional ^1H–^{13}C heteronuclear single quantum coherence (HSQC), gradient-correlation spectroscopy (gCOSY) and 2D rotating frame NOESY (2D ROESY) experiments.[41,42] The resonance for the protonated amine in the spirocyclic ring system appeared at δ 9.24 ppm in the 2D ROESY spectrum and was the starting point for this stereochemical assignment at C-3. The ammonium group (NH_2^+) at C-3 was identified using a D_2O exchange experiment in DMSO-d_6. Correlations in the ROESY spectrum between this ammonium group and individually the C-1α C-2α, C-4α and C-5α protons (Fig. 2) confirmed the 3α-orientation of the ammonium group in **11ei**.

The Pictet–Spengler reactions of coumarins **9** and various ketones employed three different conditions that depended on the solubility of the ketone component and the desire, in the case of steroid condensations, to precipitate the products from acidic, ethanol solutions using water. A mixture of acetone–trifluoroacetic acid at reflux sufficed for reactions with acetone (condition A); trifluoracetic acid in dichloromethane at 25 °C; (condition B) effected the condensations of **9** with monocyclic ketones **10b–10f**, and 1 : 10 concentrated hydrochloric acid in ethanol at reflux (condition C) promoted successful reactions with steroidal ketones **10g–10k** to give the desired adducts **11** (Table 1). Depending on conditions, the acid-catalyzed condensation of 17α-methyl-5α-androstan-17β-ol-3-one (**10j**) with coumarin **9e** led not only to the expected product **11ej** but also to a Wagner–Meerwein rearrangement[43] product **14** (Fig. 3A). The rearrangement product **14** provided suitable crystals for an X-ray crystallographic structure determination (CSD deposition number CCDC 1888376†) that again confirmed the C-3α orientation of the ammonium group in accord with the aforementioned NMR-based stereochemical assignments (Fig. 3B).

Discussion

In the course developing small-molecule therapies for CRPC, we required fluorescent analogs for the androgens that appear in the penultimate, intratumoral pathways converging on 5α-dihydrotestosterone (DHT). Specifically, we focused our interest on inhibitors for the 17-oxidoreductases that appear in three places in the late-stage metabolism of androgens to DHT, and we required fluorescent androgen probes with fluorescent groups at C-3 and natural functionality at C-17. The acid-catalyzed Pictet–Spengler reaction of coumarins **9a**, **9b** and **9e** bearing C-7 amino or C-7 *N*,*N*-dimethylamino substituents with 3-ketosteroids, such as 5α-androstane-3,17-dione (**10h**) and 5α-androstan-17β-ol-3-one (**10i**), met these objectives and provided the adducts **11** with an intensely fluorescent, spirocyclic group attached at C-3 in good yield (Table 1). These reactions required either a 1 : 10 (v/v) mixture of trifluoroacetic acid and dichloromethane for ketones **10a–10f** or a 1 : 10 (v/v) mixture of concentrated hydrochloric acid and absolute ethanol for steroidal ketones **10g–10k**. A combination of sophisticated NMR experiments and an X-ray structure of a Wagner–Meerwein rearrangement[43]

Table 1 Synthesis conditions and yields of Pictet–Spengler adducts **11** formed from C-7 amino-substituted 4-(2-aminoethyl)coumarins **9** and ketones **10**

			Isolated yields of Pictet–Spengler adducts **11** from 4-(2-aminoethyl)coumarins		
Ketone		Conditions[a]	**9a**	**9b**	**9e**
Acetone	**10a**	A		**11ba** (88%)	**11ea** (94%)[d]
Cyclopentanone	**10b**	B			**11eb** (68%)[b]
Cyclohexanone	**10c**	B			**11ec** (63%)[b]
Tetrahydro-4*H*-pyran-4-one	**10d**	B			**11ed** (79%)
Tetrahydro-4*H*-thiopyran-4-one	**10e**	B			**11ee** (81%)[d]
1-Methylpiperidin-4-one	**10f**	B		**11bf** (73%)	**11ef** (84%)
5*a*-Androstan-3-one	**10g**	C	**11ag** (21%)[c]	**11bg** (70%)[c]	**11eg** (86%)[c]
5*a*-Androstane-3,17-dione	**10h**	C			**11eh** (57%)
5*a*-Androstan-17b-ol-3-one	**10i**	C	**11ai** (57%)[c]	**11bi** (85%)[c]	**11ei** (71%)[c]
17*a*-Methyl-5*a*-androstan-17*b*-ol-3-one	**10j**	C			**11ej** (20%)
5*a*-Cholestan-3-one	**10k**	C			**11ek** (78%)[c]

[a] Condition A: 1 : 10 (v/v) acetone–TFA, reflux; condition B: 1 : 10 (v/v) TFA–CH_2Cl_2, 25 °C; condition C: 1 : 10 (v/v) conc. HCl acid–abs. ethanol, reflux. [b] Isolated as *N*-acetyl derivative. [c] Isolated as hydrochloride salt. [d] Isolated as a trifluoroacetate salt.

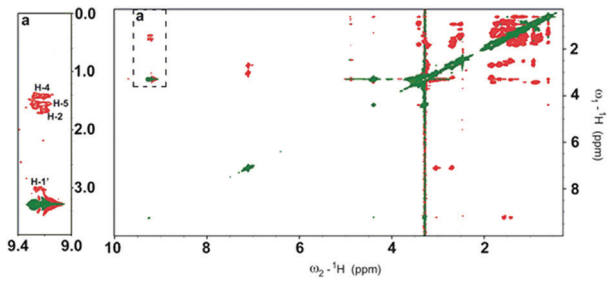


Fig. 2 2D ROESY spectrum of DHT analog **11ei**. Spectrum recorded using Agilent 400 MHz at 25 °C. Dotted lines show region expanded that displays the NOE connectivity between NH_2^+ and ring A protons (*i.e.*, H-5 (and H-1 overlapping), H-4, H-2, and CH_2 adjoining NH_2^+).

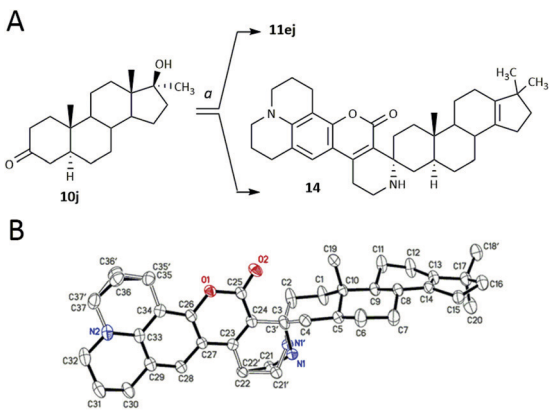


Fig. 3 Wagner–Meerwein Rearrangment. Panel A: Products of Pictet–Spengler condensation of coumarin **9e** and 17α-methyl-5α-androstan-17β-ol-3-one (**10j**). Panel B: An ellipsoid plot (50% probability) for the single-crystal structure of rearrangement product (**14**). Hydrogen atoms were omitted to enhance clarity.

product **14** (Fig. 3) established the C-3*R*-diastereoselectivity of these reactions with steroid substrates. Similar Pictet–Spengler reactions, however, of coumarins **9c** and **9d** bearing C-7 hydroxyl or methoxy substituents, respectively, in place of C-7 amino substituents were unsuccessful. Likewise, extension of this Pictet–Spengler reaction to the condensations of coumarins **9** with aldehydes was largely unsuccessful, even after attempts to effect the deliberate oxidation of the intermediate adduct to a pyridine (Scheme 2B).

The classic Pictet–Spengler reaction[31,32] involved an acid-catalyzed condensation of activated 2-(1*H*-indol-3-yl)ethan-1-amine with either an aldehyde or a ketone to give an intermediate imminium salt and a subsequent cyclization *via* a spirocyclic intermediate to provide a substituted 2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole. Superficially, the Pictet–Spengler reaction of coumarins **9** was a vinylogous extension of the classic reaction involving 3-(2-aminoethyl)-1*H*-indoles, as displayed in a skeletal format (Fig. 4), in which the iminium carbon linked to the α carbon in the indole case and to the ζ-carbon in the coumarin case.

A mechanism for these acid-catalyzed Pictet–Spengler reactions of coumarins **9a**, **9b** and **9e** with ketones **10** must take into account the following observations: [1] the requirement for highly acidic, aqueous conditions; [2] the rapid formation of the products from the intermediate, iminium salts derived from the condensation of the C-7 amino- or *N*,*N*-dialkylamino-substituted coumarins **9a**, **9b** and **9e** with ketones **10**; and [3] the failure of the 7-hydroxy- and 7-methoxy substituted 4-(2-aminoethyl)coumarins **9c** and **9d**, respectively, to participate in these reactions.

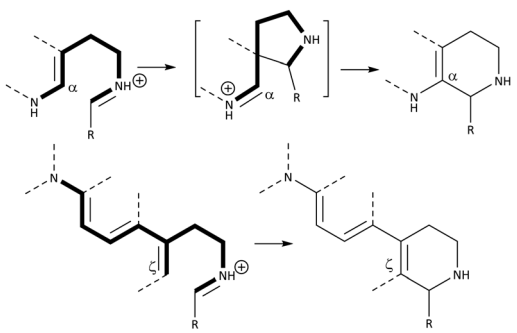
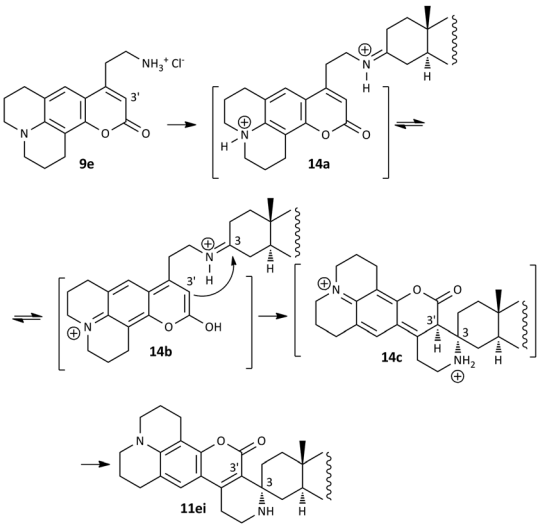


Fig. 4 Contrasting skeletons of key participants in Pictet–Spengler reactions.



Scheme 3 Mechanism of Pictet–Spengler reactions of 4-(2-aminoethyl)coumarins **9** with ketones (**10**).

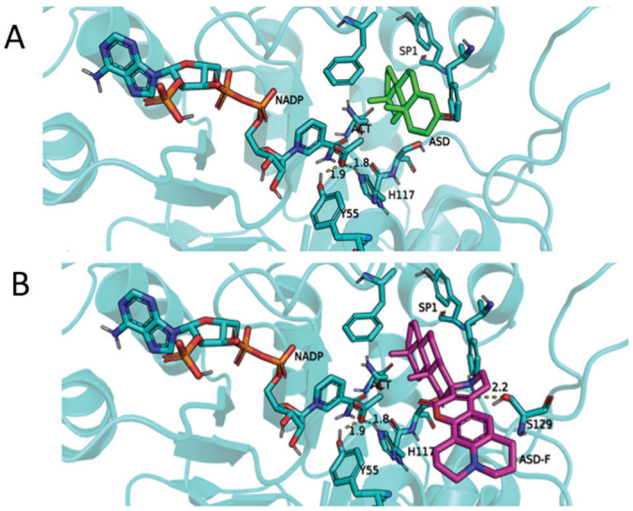


Fig. 5 Computational modeling. Panel A: 5α-Androstane-3,17-dione (**5**) in the SP1 binding site of AKR1C3 (PDB: 1XF0) containing proximal $NADP^+$.[35] Panel B: Pictet–Spengler adduct (**11eh**) derived from 5α-androstane-3,17-dione (**5**) in the SP1 binding site of AKR1C3 (PDB: 1XF0) containing proximal $NADP^+$.[35]

We propose a mechanism for the successful Pictet–Spengler condensations of C-7 amino-substituted 4-(2-aminoethyl)-coumarins **9a**, **9b** and **9e** with ketones **10** that involves an initial, rapid condensation leading to an intermediate imine and a rate-determining cyclization to a tetrahydropyridine product **11**. For example, the acid-catalyzed condensation of coumarin **9e** with 5α-androstan-17β-ol-3-one (**10i**) furnishes an intermediate, biscationic iminium ion **14a** (Scheme 3). The acidic conditions (est. pH 1) used for these condensations and the calculated pK_a values for 7-(*N*,*N*-dimethylamino)coumarin (pK_a 3.48) and *N*-methylcyclohexanimine (pK_a 10.14), using the ChemAxon software (version 19.18; ChemAxon, Inc., Cambridge, MA), support a biscationic structure involving protonation of both the imine and aniline moieties in **14a**. The agreement between calculated pK_a values for *N*,*N*-dimethylaniline (pK_a 5.02) and experimental values (pK_a 5.06[44]–5.07[45]) supported the veracity of these calculated pK_a values. This suggestion for a biscationic species finds precedent a similar species generated from *p*-aminostyrene with strong acids having Hammett acidities H_0 greater than one.[46] In our case, protonation followed by tautomerization leads to the biscationic, enolic form of the coumarin **14b** that undergoes rate-limiting condensation to furnish the iminoquinone methide **14c**. Addition of the *Si*-face of the enol to the β-face of the iminium ion in **14b** (*i.e.*, equatorial addition) produces an iminoquinone methide **14c** with C-3*R* and C-3′*R* stereochemistry. The alternative, *Re*-face addition provides the C-3′*S* epimer but proceeds through a transition state that is more sterically hindered than that from the *Re*-face based on MM2 calculations for the relative stability of the 3*R*,3′*R* and the 3*R*,3′*S* products. Final, irreversible deprotonation of **14c** at C-3′ affords the observed product **11ei** (Scheme 3).

Heating the adduct **11ef** with tetrahydro-4*H*-thiopyran-4-one (**10e**) in dichloromethane-trifluoroacetic acid (condition B) for 3 days failed to provide any of the spirocyclic ketone-exchange product **11ee**. In summary, an electron-donating partner in the coumarin-based Pictet–Spengler reaction is a vinylogous analog of the indole partner in the classic Pictet–Spengler reaction. The activating C-7 amino substituent in the coumarin moiety **9e** counterbalances the deactivating carbonyl group and leads to a biscationic enol intermediate, such as **14b** (Scheme 3) that then leads to successful condensations. Analogous reactions of 4-(2-aminoethyl)coumarins **9c** and **9d** (Scheme 1) that possess either a C-7 hydroxy or C-7 methoxy group fail to generate a bicationic enol intermediate and hence, fail to produce adducts **11** just as benzofuran cases fail in the classic Pictet–Spengler reactions.

Computational modeling of the binding of the fluorescent, spirocyclic adduct **11eh** in the ligand-binding domain of human 17β-hydroxysteroid dehydrogenase-5 (17β-HSD5) indicated that the compact nature of this fluorescent androgen **11eh** did not interfere with binding to the active site. The adduct **11eh** adopted the same pose as the naturally occurring ligand, 5α-androstane-3,17-dione (**5**; **ASD**)[35] (Fig. 5).

The BCD rings of **11eh** were inserted into the SP1 binding pocket in an identical fashion as seen for **5**, and the C-18 and C-19 angular methyl groups of **5** and **11ei** projected into the oxyanion hole of 17β-HSD5 bounded by Y55, H117 and $NADP^+$. Hydrogen bonding with S129 further stabilizes the observed binding mode with **11eh**. These binding features indicated that the fluorescent ASD-based adduct **11eh** possessed a binding mode that matched that of ASD itself. In a similar fashion, the ASD adduct **11eh** occupied the same binding pocket seen for a previously described inhibitor, 3-carboxamido-1,3,5-(10)-estratrien-17*R*-spiro-2-(5,5-dimethyl-6-oxo)tetrahydropyran (EM1404) that bound to17β-HSD5 (PDB: 1ZQ5).[36]

Conclusions

In summary, the Pictet–Spengler condensation of substituted 4-(2-aminoethyl)coumarins and ketones furnished fluorescent

(4*aS*,14*bS*)-1,2,3,4,4*a*,8,9,12,13,14*b*-decahydro-5*H*,7*H*,11*H*-pyrido-[3,2,1-*ij*]pyrido[4′,3′:4,5]pyrano[2,3-*f*]quinolin-5-ones. This work describes the scope of this variant of the Pictet–Spengler reaction with various coumarins, proposes a mechanism consistent with the substituents in the coumarin moiety, and defines the stereochemistry at C-3 in spirocyclic products derived from 3-ketosteroids by a combination of detailed NMR studies and an X-ray structure. Computational modeling supported the surrogacy of a C-3 fluorescent derivative of 5α-androstan-17β-ol-3-one as a tool to study 17-oxidoreductases for intracrine, androgen metabolism in prostate cancer. Future studies will describe applications of these fluorescent androgens for image flow cytometry and will elucidate the effects of these fluorescent androgens on the prevention of prostate cancer growth promotion during ADT and on the stimulation of androgen receptor-regulated gene expression.

Experimental

Chemicals were purchased from Millipore Sigma (St. Louis, MO, USA) or Fisher Scientific (Hampton, NH, USA) or were synthesized according to literature procedures. Solvents were used from commercial vendors without further purification unless otherwise noted. Nuclear magnetic resonance spectra were acquired on a Varian (^{1}H at 400 MHz and ^{13}C at 100 MHz or ^{1}H at 500 MHz and ^{13}C at 125 MHz) instruments. High resolution electrospray ionization (ESI) mass spectra were recorded on an LTQ-Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The FT resolution was set at 100 000 (at 400 *m*/*z*). Samples were introduced through direct infusion using a syringe pump with a flow rate of 5 μL min^{-1}. Purity of compounds was established using combustion analyses (Atlantic Microlabs, Inc. Norcross, GA, USA). Compounds were chromatographed on preparative layer Merck silica gel F254 (Fisher Scientific) plates unless otherwise indicated.

Methyl 3-oxo-5-((2,2,2-trifluoroacetyl)amino)pentanoate (7)

To a solution of 22.3 g (250 mmol) of β-alanine in 125 mL of methanol at 25 °C was added 35 mL (250 mmol) of triethylamine. After 5 min, 37 mL (312 mmol, 1.25 eq.) of ethyl trifluoroacetate was added, and the mixture was allowed to stir for 24 h at 25 °C. The solvent was evaporated under reduced pressure, and the residue was diluted with 50 mL of H_2O and acidified with concentrated hydrochloric acid. The mixture was extracted with ethyl acetate, and the combined organic layers were washed with brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was evaporated at reduced pressure to give 42.1 g (91%) of 3-(2,2,2-trifluoroacetamido)propanoic acid[47] as a white solid: mp 114–116 °C (lit.[47] mp 114–116 °C). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 12.3 (s, 1H), 9.47 (br s, 1H), 3.42–3.3 (m, 2H), and 2.54–2.46 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 172.5, 156.4 (q, $^2J_{CF}$ = 35.7 Hz), 116 (q, $^1J_{CF}$ = 286.9 Hz), 35.6, 32.8. A suspension of 3.08 g (32.3 mmol) of magnesium chloride and 7.2 g (46.2 mmol, 1.4 eq.) of monomethyl monopotassium malonate in 45 mL of anhydrous THF was stirred for 4 h at 50 °C under a nitrogen atmosphere. In a second flask, 6 g (37 mmol, 1.2 eq.) of 1.1′-carbonyldiimidazole was added portionwise to a solution of 5.7 g (30.8 mmol) of 3-[(2,2,2-trifluoroacetyl)amino]propanoic acid in 30 mL of anhydrous THF at 0–5 °C. The mixture in the second flask was stirred for 1 h at 25 °C and was added dropwise to the methylmagnesium malonate suspension at 25 °C. The mixture was stirred for 16 h, concentrated, and diluted with ethyl acetate. The ethyl acetate solution was washed with saturated, aqueous $NaHCO_3$ solution and brine. After drying over anhydrous $MgSO_4$, the mixture was filtered and concentrated. The product was chromatographed on silica gel using 2% methanol-dichloromethane to give 6.1 g of 7 as a colorless oil (82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (br s, 1H), 3.27 (s, 3H), 3.64–3.56 (m, 2H), 3.48 (s, 2H), 2.87 (t, J = 5.7 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 202.1, 167.4, 157.4 (q, $^2J_{CF}$ = 37 Hz), 115.8 (q, $^1J_{CF}$ = 287.6 Hz), 52.7, 48.8, 41.5, 34.5. HRMS (ESI) calcd for $C_8H_{11}O_4NF_3$ [MH$^+$]: 242.0635. Found: 242.0636.

Methyl *N*-(3-hydroxyphenyl)carbamate (8a)

A solution of 10 g (91.6 mmol) of 3-aminophenol in 35 mL of ethyl acetate was refluxed for 30 min. To the clear solution was added 4 mL (45.8 mmol, 0.5 eq.) of methyl chloroformate dropwise over a period of 30 min. The mixture was cooled to 25 °C. The white solid was collected by vacuum filtration and washed with 1 : 1 ethyl acetate–hexanes to give 7.67 g (50%) of **8a**: ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.5 (s, 1H, NH), 9.34 (s, 1H, OH), 6.98–7.05 (m, 2H), 6.82–6.84 (m, 1H), 6.36–6.39 (m, 1H), 3.63 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 158.1, 154.3, 140.6, 129.8, 109.9, 109.4, 105.7, 51.9.

7-Amino-4-(2-aminoethyl)-2*H*-chromen-2-one dihydrochloride (9a)

To a stirred solution of 4.94 g (20.5 mmol) of methyl 3-oxo-5-[(2,2,2-trifluoroacetyl)amino]-pentanoate (7) in 60 mL of methanesulfonic acid was added 3.42 g (20.5 mmol, 1 eq.) of methyl *N*-(3-hydroxyphenyl)carbamate (**8a**) portion-wise at 0 °C. The mixture was stirred at 25 °C for 16 h and quenched by pouring into cold water. The precipitate was collected by filtration and recrystallized from methanol to give 5.59 g (76%) of methyl (2-oxo-4-(2-(2,2,2-trifluoroacetamido)ethyl)-2*H*-chromen-7-yl)-carbamate: mp 215–216 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.19 (s, 1H), 9.57 (t, J = 5.2 Hz, 1H), 7.77 (d, J = 8.4 Hz, 1H), 7.57 (d, J = 1.6 Hz, 1H), 7.4 (dd, J = 8.4 Hz, 1.6 Hz, 1H), 6.19 (s, 1H), 3.71 (s, 3H), 3.51 (q, J = 6.8 Hz, 2H), 2.99 (t, J = 6.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 159.9, 156.4 (q, $^2J_{CF}$ = 35.7 Hz), 154.2, 153.8, 153.1, 142.8, 125.6, 115.9 (q, $^1J_{CF}$ = 287 Hz), 114.3, 113.4, 112.2, 104.6, 52.1 (CH_3), 38 (CH_2), 30.1 (CH_2). HRMS (ESI) calcd for $C_{15}H_{14}F_3N_2O_5$ [MH$^+$]: 359.085. Found: 359.085. Anal. calcd for $C_{15}H_{13}F_3N_2O_5$: C, 50.29; H, 3.66; N, 7.82. Found: C, 50.53; H, 3.75; N, 7.80. A solution of 5 g (14 mmol) of methyl (2-oxo-4-(2-(2,2,2-trifluoroacetamido)ethyl)-2*H*-chromen-7-yl)carbamate was refluxed in 60 mL of concentrated hydrochloric acid for 42 h. The solvent

was removed *in vacuo*. The residue was recrystallized from methanol to give 3.63 g (94%) of **9a** as a bishydrochloride salt: ^{1}H NMR (400 MHz, D_2O) δ 7.61 (d, J = 9.2 Hz, 1H), 6.83–6.91 (m, 2H), 6.2 (s, 1H), 3.37 (t, J = 7.2 Hz, 2H), 3.19 (t, J = 7.2 Hz, 2H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (s, 1H), 7.52 (d, J = 8.4 Hz, 1H), 6.6 (d, J = 8.4 Hz, 1H), 6.46 (s, 1H), 5.98 (s, 1H), 3.2–3 (m, 4H). ^{13}C NMR (100 MHz, D_2O) δ 162.9, 153.6, 152.5, 138.5, 126.2, 117.9, 116.1, 113.2, 109, 37.4, 28.6. HRMS (ESI) calcd for $C_{11}H_{13}N_2O_2$ [MH^+]: 205.0972. Found: 205.0977. Anal. calcd for $C_{11}H_{14}Cl_2N_2O_2$: C, 47.67; H, 5.09; N, 10.11. Found: C, 47.41; H, 4.94; N, 9.95.

4-(2-Aminoethyl)-7-*N*,*N*-dimethylamino-2*H*-chromen-2-one hydrochloride (9b)

To a stirred suspension of 1.37 g (10 mmol) of 3-(*N*,*N*-dimethyamino)phenol and 2.41 g (10 mmol) of methyl 3-oxo-5-[(2,2,2-trifluoroacetyl)amino]-pentanoate (7) in 30 mL of toluene was added 20 mL of 1 M (20 mmol) chlorotriisopropyloxytitanium(IV) in hexanes. The mixture was refluxed for 10 h under a nitrogen atmosphere, cooled and diluted with 40 mL of hexane. The precipitate was collected by filtration and washed with 40 mL of hexane. The product was purified by recrystallization from methanol to give 2.2 g (67%) of a yellow solid: mp 195–196 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.57 (t, J = 5.6 Hz, 1H, N*H*), 7.58 (d, J = 9.2 Hz, 1H), 6.71 (dd, J = 9.2, 2.4 Hz, 1H), 6.52 (d, J = 2.4 Hz, 1H), 5.91 (s, 1H), 3.49 (dt, J = 6.8, 5.6 Hz, 2H), 3.01 (s, 6H), 2.93 (t, J = 6.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.6, 156.2 (q, $^2J_{CF}$ = 35.7 Hz), 155.5, 153.5, 152.8, 125.4, 115.9 (q, $^1J_{CF}$ = 286.5 Hz), 109.1, 108.1, 107.8, 97.6, 39.7, 38.2, 30.1. HRMS (ESI) calcd for $C_{15}H_{16}F_3N_2O_3$ [MH^+]: 329.1108. Found: 329.1108. Anal. calcd for $C_{15}H_{15}F_3N_2O_3$: C, 54.88; H, 4.61; N, 8.53. Found: C, 55.01; H, 4.43; N, 8.58. A mixture of 2.2 g (6.7 mmol) of *N*-(2-(7-(*N*,*N*-dimethylamino)-2-oxo-chromen-4-yl)ethyl)-2,2,2-trifluoroacet-amide in 4.5 mL of concentrated hydrochloric acid was refluxed for 4 h. After cooling, the product was concentrated *in vacuo*. The residue was recrystallized from methanol to give 1.14 g (63%) of **9b**: ^{1}H NMR (400 MHz, D_2O) δ 7.54 (d, J = 8.8 Hz, 1H), 6.83 (dd, J = 8.8, 2.8 Hz, 1H), 6.53 (d, J = 2.8 Hz, 1H), 6.03 (s, 1H), 3.37 (t, J = 7.2 Hz, 2H), 3.14 (t, J = 7.2 Hz, 2H), 3.04 (s, 6H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (s, 3H), 7.64 (d, J = 9 Hz, 1H), 6.75 (dd, J = 8.8, 2.4 Hz, 1H), 6.58 (d, J = 2.4 Hz, 1H), 6.03 (s, 1H), 3.2–2.98 (m, 10H). ^{13}C NMR (100 MHz, D_2O) δ 163.8, 154.4, 153.1, 150.1, 125.7, 112.6, 112, 110.1, 102.1, 41.8, 37.7, 28.7. ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.6, 155.5, 152.4, 152.3, 125.7, 109.8, 109.4, 108.4, 98.6, 40.2, 37.8, 28.8. HRMS (ESI) calcd for $C_{13}H_{17}N_2O_2$ [MH^+]: 233.1285. Found: 233.1283. Anal. calcd for $C_{13}H_{17}ClN_2O_2$: C, 58.10; H, 6.38; N, 10.42; Cl, 13.19. Found: C, 58.08; H, 6.45; N, 10.45; Cl, 13.26.

4-(2-Aminoethyl)-7-hydroxy-2*H*-chromen-2-one hydrochloride (9c)

To a stirred solution of 3.3 g, (13.7 mmol) of methyl 3-oxo-5-((2,2,2-trifluoroacetyl)amino)pentanoate (7) in 25 mL of methanesulfonic acid was added 1.37 g (12.4 mmol) of resorcinol portionwise at 0–5 °C. The mixture was stirred for 3 h at this temperature and was quenched by pouring into 100 mL of ice water. The precipitate was collected by filtration and recrystallized from methanol to give 2.32 g (62%) of the 2,2,2-trifluoro-*N*-(2-(7-hydroxy-2-oxo-2*H*-chromen-4-yl)ethyl)acet-amide: mp 219–220 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (s, 1H, OH), 9.57 (t, J = 5.4 Hz, 1H, NH), 7.68 (d, J = 8.8 Hz, 1H), 6.81 (dd, J = 8.8, 2.4 Hz, 1H), 6.72 (d, J = 2.4 Hz, 1H), 6.1 (s, 1H), 3.5 (dt, J = 7, 5.4 Hz, 2H), 2.97 (t, J = 7 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.2, 160.2, 156.4 (q, $^2J_{CF}$ = 36.1 Hz), 155.2, 153.5, 126.2, 115.9 (q, $^1J_{CF}$ = 287.8 Hz), 113, 111.2, 110.5, 102.5, 38.1, 30.2. HRMS (ESI) calcd for $C_{13}H_{11}F_3NO_4$ [MH^+]: 302.0635. Found: 302.0635. Anal. calcd for $C_{13}H_{10}F_3NO_4$: C, 51.84; H, 3.35; N, 4.65. Found: C, 51.98; H, 3.50; N, 4.62. A mixture of 1.51 g (5 mmol) of 2,2,2-trifluoro-*N*-(2-(7-hydroxy-2-oxo-chromen-4-yl)ethyl)acetamide in 20 mL of concentrated hydrochloric acid was refluxed for 2 h. After cooling, the product was concentrated *in vacuo*. The residue was recrystallized from methanol to give 945 mg (78%) of **9c**: ^{1}H NMR (400 MHz, D_2O) δ 7.67 (d, J = 8.8 Hz, 1H), 6.95 (dd, J = 8.8, 2 Hz, 1H), 6.81 (d, J = 2 Hz, 1H), 6.26 (s, 1H), 3.43 (t, J = 7.6 Hz, 2H), 3.23 (t, J = 7.6 Hz, 2H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.7 (s, 1H), 8.11 (s, 3H), 7.7 (d, J = 8.8 Hz, 1H), 6.84 (dd, J = 8.8, 1.6 Hz, 1H), 6.76 (d, J = 1.6 Hz, 1H), 6.2 (s, 1H), 3.2–3 (m, 4H). ^{13}C NMR (100 MHz, D_2O) δ 163.4, 159.9, 153.9, 153.2, 125.6 (CH), 113.4 (CH), 111, 109.7 (CH), 102.5 (CH), 37.4 (CH_2), 28.5 (CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.4, 160.2, 155.2. 152, 126.2 (CH), 113.1 (CH), 111.2 (CH), 110.8, 102.6 (CH), 37.5 (CH_2), 28.8 (CH_2). HRMS (ESI) calcd for $C_{11}H_{12}O_3N$ [MH^+]: 206.0812. Found: 206.0813. Anal. calcd for $C_{11}H_{12}ClNO_3$: C, 54.67; H, 5.01; N, 5.80. Found: C, 54.81; H, 4.97; N, 5.80.

4-(2-Aminoethyl)-7-methoxy-chromen-2-one hydrochloride (9d)

To a stirred solution of 2.65 g (11 mmol) of methyl 3-oxo-5-[(2,2,2-trifluoroacetyl)amino]pentanoate (7) in 20 mL of methanesulfonic acid was added 1.24 g (10 mmol) of 3-methoxyphenol portionwise at 0–5 °C. The mixture was stirred for 30 min at this temperature and diluted with ice water. The precipitate was collected by filtration and recrystallized from methanol to give 2.28 g (72%) of 2,2,2-trifluoro-*N*-(2-(7-methoxy-2-oxo-2*H*-chromen-4-yl)ethyl)acetamide: mp 156–158 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (t, J = 4.4 Hz, 1H, N*H*), 7.78 (d, J = 8.8 Hz, 1H), 7.02 (d, J = 2.4 Hz, 1H), 6.98 (dd, J = 8.8, 2.4 Hz, 1H), 6.17 (s, 1H), 3.86 (s, 3H), 3.51 (dt, J = 7, 4.4 Hz, 2H), 3 (t, J = 7 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.4, 160, 156.4 (q, $^2J_{CF}$ = 35.7 Hz), 155.1, 153.3, 126 (CH), 115.9 (q, $^1J_{CF}$ = 286.9 Hz), 112.2 (quaternary C and CH), 111.4 (CH), 101 (CH), 55.9 (CH_3), 38 (CH_2), 30.2 (CH_2). HRMS (ESI) calcd for $C_{14}H_{13}F_3NO_4$ [MH^+]: 316.0791. Found: 316.0793. Anal. calcd for $C_{14}H_{12}F_3NO_4$: C, 53.34; H, 3.84; N, 4.44. Found: C, 53.44; H, 3.97; N, 4.29. A mixture of 2.69 g (8.54 mmol) of 2,2,2-trifluoro-*N*-(2-(7-methoxy-2-oxo-chromen-4-yl)-ethyl)acetamide was refluxed in 25 mL of concentrated hydrochloric acid for 3 h. After cooling, the product was concentrated *in vacuo*. The residue was recrystallized from methanol to give 1.67 g (76%) of **9d**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (s, 3H), 7.79 (d, J = 8.4 Hz, 1H), 7.08–6.96 (m, 2H), 6.28 (s, 1H), 3.87 (s, 3H), 3.2–3 (m, 4H). ^{1}H NMR (400 MHz, D_2O) δ 7.72 (d, J = 8.8 Hz, 1H), 7.05 (dd, J = 8.8, 2.4 Hz, 1H), 7.02 (d, J = 2.4 Hz, 1H), 6.29 (s, 1H), 3.91 (s, 3H), 3.39 (t, J = 7.4 Hz, 2H), 3.22 (t, J = 7.4 Hz, 2H). ^{13}C NMR

(100 MHz, DMSO-d_6) δ 162.4, 160, 155.2, 151.9, 126.1, 112.2 (two C), 112, 101.1, 56, 37.5, 28.8. HRMS (ESI) calcd for $C_{12}H_{14}NO_3$ [MH^+]: 220.0968. Found 220.0976. Anal. calcd for $C_{12}H_{14}ClNO_3$: C, 56.37; H, 5.52; N, 5.48. Found: C, 56.32; H, 5.59; N, 5.54.

8-(2-Aminoethyl)-2,3,4,5-tetrahydro-1*H*,4*H*-11-oxa-3*a*-aza-benzo-[*de*]anthracen-10-one hydrochloride (9e)

The procedure of Wirtz and Kazmaier[38] was repeated using 8.33 g (44 mmol) of 8-hydroxy-2,3,6,7-tetrahydro-1*H*,5*H*-benzo[*ij*]quinolizine, 10.8 g (44 mmol) of methyl-5-(benzyloxy-carbonylamino)-3-oxopentanoate, and 88 mL (88 mmol, 2 eq.) of a 1 M solution of chlorotriisopropyloxy-titanium(IV) in hexanes to afford 13 g (77%) of a 1 : 9 mixture of the benzyl and isopropyl [2-(10-oxo-2,3,5,6-tetrahydro-1*H*,4*H*,10*H*-11-oxa-3*a*-aza-benzo[*de*]anthracen-8-yl)ethyl]-carbamates as a yellow solid. To 5.05 g (13.6 mmol) of this mixture of esters was added 12 mL of concentrated HCl. The solution was heated at 95 °C for 9 h, cooled, and concentrated *in vacuo*. The residue was suspended in a mixture of methanol–acetone and was filtered to afford 4.3 g (98%) of **9e** as a yellow hydrochloride salt: mp 238–239 °C (lit.[38] mp 114–118 °C for free base). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8 (s, 3H), 7.22 (s, 1H), 5.93 (s, 1H), 3.3–3.16 (m, 4H), 3.12–2.92 (m, 4H), 2.8–2.7 (m, 4H), 1.94–1.84 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.7, 152.2, 150.9, 145.6, 121.7, 118, 107.5, 106.8, 105.7, 49.2, 48.7, 37.9, 28.9, 27, 21, 20.1, 20. HRMS (ESI) calcd for $C_{17}H_{21}N_2O_2$ [MH^+]: 285.1598. Found 285.1597. Anal. calcd for $C_{17}H_{21}ClN_2O_2{\cdot}H_2O$: C, 60.26; H, 6.84; N, 8.27. Found: C, 60.46; H, 6.57; N, 8.19.

Condition A for the Pictet–Spengler reaction of 4-(2-aminoethyl)-coumarins 9 with acetone. 8-(*N*,*N*-Dimethylamino)-4,4-dimethyl-1,2,3,4-tetrahydro-5*H*-chromeno[3,4-*c*]pyridin-5-one (11ba)

To a stirred solution of 50 mg (0.19 mmol) of **9b** in 0.5 mL of trifluoroacetic acid were added 0.5 mL of acetone. The mixture was stirred under reflux for 30 min. After cooling, the mixture was neutralized with a saturated, aqueous solution of $NaHCO_3$ and stirred for 2 h at 25 °C. A precipitate was collected by filtration and purified by chromatography using 1 : 25 methanol–dichloromethane (R_f 0.29) to provide 45 mg (88%) of **11ba**. mp 179–181 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.47 (d, J = 9 Hz, 1H), 6.71 (dd, J = 9, 2.6 Hz, 1H), 6.5 (d, J = 2.6 Hz, 1H), 2.99 (s, 6H), 2.96 (t, J = 5.8 Hz, 2H), 2.67 (t, J = 5.7 Hz, 2H), 1.38 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.27, 153.73, 152.04, 148.14, 124.98, 123.54, 109.02, 108.6, 96.88, 51.42, 36.69, 26.68, 26.03. HRMS (ESI) calcd for $C_{16}H_{21}N_2O_2$ [MH^+]: 273.1598. Found: 273.1600.

Condition B for the Pictet–Spengler reaction of 4-(2-aminoethyl)-coumarins 9 with monocyclic ketones. 2,2′,3,3′,5,6,8′,9′,12′,13′-Decahydro-7′*H*,11′*H*-spiro[pyran-4,4′-pyrido[3,2,1-*ij*]pyrido-[4′,3′:4,5]pyrano[2,3-*f*]quinolin]-5′(1′*H*)-one (11ed)

To a stirred suspension of 100 mg (0.31 mmol, 1 eq.) of **9e** in 2 mL dichloromethane was added 0.2 mL of trifluoroacetic acid followed by 125 mg (1.25 mmol, 4 eq.) of tetrahydro-4*H*-pyran-4-one. The mixture was stirred at 25 °C for 2–5 days with monitoring by TLC for the disappearance of starting material. Diethyl ether (*ca.* 5 mL) was added to the mixture. A precipitate was collected by filtration to provide 2,2′,3,3′,5,6,8′,9′,12′,13′-decahydro-7′*H*,11′*H*-spiro[pyran-4,4′-pyrido[3,2,1-*ij*]pyrido-[4′,3′:4,5]pyrano[2,3-*f*]quinolin]-5′(1′*H*)-one 2,2,2-trifluoroacetate that was treated with saturated, aqueous solution of $NaHCO_3$ to give a free base that was purified by chromatography using 1 : 10 methanol–dichloromethane (R_f 0.51) to furnish 90 mg (79%) of **11ed**. mp 204–206 °C (decomp). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.06 (s, 1H), 3.87–3.71 (m, 2H), 3.57 (dd, J = 10.8, 4.9 Hz, 2H), 3.21 (q, J = 5.6 Hz, 4H), 2.89 (t, J = 5.6 Hz, 2H), 2.76–2.68 (m, 4H), 2.68–2.59 (m, 4H), 1.95–1.8 (m, 4H), 1.2 (d, J = 13.1 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.36, 149.97, 149.19, 144.75, 121.2, 117.78, 107.99, 104.85, 62.46, 51.34, 49.15, 48.66, 36.09, 31.91, 27.12, 26.06, 21.11, 20.24, 19.85. HRMS (ESI) calcd for $C_{22}H_{27}N_2O_3$ [MH^+]: 367.2016. Found: 367.2020.

Condition C for the Pictet–Spengler reaction of 4-(2-aminoethyl)coumarins 9 with steroidal ketones. (3*R*,5*S*,10*S*,13*S*,17*S*)-17-Hydroxy-10,13-dimethyl-1,2,2′,3′,4,5,6,7,8,8′,9,9′,10,11,12,12′,13,13′,14,15,16,17-docosahydro-7′*H*,11′*H*-spiro[cyclopenta[*a*]-phenanthrene-3,4′-pyrido[3,2,1-*ij*]pyrido[4′,3′:4,5]pyrano[2,3-*f*]-quinolin]-5′(1′*H*)-one hydrochloride (11ei)

To a suspension of 66 mg (0.21 mmol, 1.2 eq.) of **9e** hydrochloride in 2 mL of absolute ethanol was added 50 mg (0.17 mmol, 1 eq.) of 5α-androstan-17β-ol-3-one (**10i**). To this suspension in a sealed tube was added 0.2 mL of concentrated HCl, and the mixture was stirred under reflux for 24 h. The suspension became a clear solution within the first hour of heating, and a precipitate of the desired product then appeared. The reaction was quenched by the addition of *ca.* 3 mL of water, and the precipitate was collected by filtration to provide 73 mg (71%) of **11ei**. Additional purification was achieved by recrystallization from methanol: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.41–9.13 (m, 2H), 7.14 (s, 1H), 4.43 (br s, 1H), 3.45 (t, 2H), 3.29–3.22 (m, 4H), 3.12–3 (m, 2H), 2.82–2.64 (m, 4H), 2.57 (t, J = 14.2 Hz, 1H), 1.96–1.8 (m, 4H), 1.8–1.7 (m, 2H), 1.7–1.55 (m, 4H), 1.56–1.41 (m, 4H), 1.42–1.3 (m, 3H), 1.28–1.06 (m, 5H), 1.05–0.77 (m, 7H), 0.65 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.61, 149.23, 147.42, 145.69, 121.59, 118.47, 114.52, 106.22, 104.7, 80.04, 58.69, 52.91, 50.82, 49.16, 48.65, 42.59 (two C), 36.7, 35.26, 35.05, 34.83, 32.41, 31.64, 31.17, 29.84, 27.47, 27.09, 25.4, 23.05, 22.54, 20.88, 20.12, 19.96, 19.69, 11.42, 11.37. HRMS (ESI) calcd for $C_{36}H_{49}N_2O_3$ [MH^+]: 557.3738. Found: 557.3744. Anal. calcd for $C_{36}H_{49}ClN_2O_3{\cdot}H_2O$: C, 70.74; H, 8.41; N, 4.58; Cl, 5.80. Found: C, 70.51; H, 8.43, N, 4.66; Cl, 5.72. The hydrochloride salt of the desired product was suspended in dichloromethane and washed with an aqueous, saturated solution of $NaHCO_3$. The dichloromethane layer was dried over anhydrous Na_2SO_4, filtered, concentrated and purified by chromatography on silica gel using 1 : 10 methanol–dichloromethane (R_f 0.55) to afford **11ei** as a free base: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.03 (s, 1H), 4.4 (d, J = 4.8 Hz, 1H), 3.48–3.37 (m, 1H), 3.2 (q, J = 5.6 Hz, 4H), 2.84 (t, J = 5.7 Hz, 2H), 2.7 (q, J = 6 Hz, 4H), 2.58 (t, J = 5.6 Hz, 2H), 2.52–2.43 (m, 1H), 2.32 (t, J = 13 Hz, 1H), 1.95–1.76 (m, 6H), 1.75–1.67 (m, 1H), 1.66–1.54 (m, 2H), 1.54–1.43 (m, 2H), 1.39–1.26 (m, 4H), 1.25–1.03 (m, 5H), 1–0.75 (m, 7H), 0.74–0.64 (m, 1H), 0.62 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.5, 149.38, 149.08, 144.58, 122.52, 121.11, 117.68,

108.18, 104.87, 80.11, 53.89, 53.82, 50.79, 49.16, 48.67, 42.59, 39.94, 36.76, 36.13, 35.69, 35.28, 35.25, 33.32, 31.48, 29.88, 28.18, 27.84, 27.13, 26.3, 23.11, 21.16, 20.28, 20.22, 19.88, 11.61, 11.39. HRMS (ESI) calcd for $C_{36}H_{49}N_2O_3$ [MH^+]: 557.3738. Found: 557.3738. Anal. calcd for $C_{36}H_{48}N_2O_3$: C, 77.66; H, 8.69; N, 5.03. Found: C, 77.41; H, 8.95, N, 4.99.

4,4-Dimethyl-1,2,3,4,8,9,12,13-octahydro-5*H*,7*H*,11*H*-pyrido-[3,2,1-*ij*]pyrido[4′,3′:4,5]pyrano[2,3-*f*]quinolin-5-one 2,2,2-trifluoroacetate (11ea)

The procedure described under Condition A was repeated using 60 mg (0.19 mmol) of 9-(2-aminoethyl)-2,3,6,7-tetrahydro-1*H*,5*H*,11*H*-pyrano[2,3-*f*]pyrido[3,2,1-*ij*]quinolin-11-one hydrochloride (**9e**) in 0.5 mL of trifluoroacetic acid and 0.5 mL of acetone. The mixture was cooled and diluted with 3 mL of diethyl ether. A precipitate was collected by filtration to provide 77 mg (94%) of analytically pure **11ea** as trifluoroacetate salt: mp 230–231 °C (decomp). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (s, 2H), 7.15 (s, 1H), 3.43 (t, J = 6.4 Hz, 2H), 3.25 (q, J = 6.4 Hz, 4H), 3.02 (t, J = 6.1 Hz, 2H), 2.79–2.64 (m, 4H), 1.95–1.8 (m, 4H), 1.68 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.26, 149.27, 146.13, 145.65, 121.61, 118.42, 115.21, 106.08, 104.88, 55.14, 49.17, 48.65, 34.93, 27.07, 23.8, 22.28, 20.9, 20, 19.74. HRMS (ESI) calcd for $C_{20}H_{25}N_2O_2$ [MH^+]: 325.1911. Found: 325.1911. Anal. calcd for $C_{22}H_{25}F_3N_2O_4$: C, 60.27; H, 5.75; N, 6.39. Found: C, 59.99; H, 5.63, N, 6.29.

3′-Acetyl-2′,3′,8′,9′,12′,13′-hexahydro-7′*H*,11′*H*-spiro[cyclo-pentane-1,4′-pyrido[3,2,1-*ij*]pyrido[4′,3′:4,5]pyrano[2,3-*f*]quinolin]-5′(1′*H*)-one (11eb)

To 123 mg (0.26 mmol) of 2′,3′,8′,9′,12′,13′-hexahydro-7′*H*,11′*H*-spiro[cyclopentane-1,4′-pyrido[3,2,1-*ij*]pyrido[4′,3′:4,5]pyrano[2,3-*f*]-quinolin]-5′(1′*H*)-one 2,2,2-trifluoroacetate obtained under Condition B in 2 mL of dichloromethane at 0 °C was added 59 mg (0.58 mmol, 2.2 eq.) of triethylamine followed by 25 mg (0.32 mmol, 1.2 eq.) of acetyl chloride. The mixture was stirred at 25 °C for 2 h, poured into water, and extracted with dichloromethane. The organic layers were dried over anhydrous $MgSO_4$ filtered, and concentrated. The product was purified by chromatography using 1 : 50 methanol–dichloromethane (R_f = 0.37 after two developments) to provide 71 mg of **11eb** (68%). mp 234–236 °C (decomp). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.06 (s, 1H), 3.52 (t, J = 5.5 Hz, 2H), 3.23 (q, J = 5.9 Hz, 4H), 2.83 (t, J = 5.5 Hz, 2H), 2.72 (q, J = 6.2 Hz, 4H), 2.28–2.12 (m, 4H), 2.1 (s, 3H), 2.08–1.98 (m, 2H), 1.89 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.51, 159.14, 148.84, 147.32, 144.91, 121.89, 121.26, 117.92, 107.16, 104.81, 70, 49.15, 48.65, 42.21, 38.77, 27.63, 27.13, 25.78, 25.01, 21.05, 20.16, 19.81. HRMS (ESI) calcd for $C_{24}H_{29}N_2O_3$ [MH^+]: 393.2173. Found: 393.2158.

3′-Acetyl-2′,3′,8′,9′,12′,13′-hexahydro-7′*H*,11′*H*-spiro[cyclo-hexane-1,4′-pyrido[3,2,1-*ij*]pyrido[4′,3′:4,5]pyrano[2,3-*f*]quinolin]-5′(1′*H*)-one (11ec)

To 110 mg (0.23 mmol) of 2′,3′,8′,9′,12′,13′-hexahydro-7′*H*,11′*H*-spiro[cyclohexane-1,4′-pyrido[3,2,1-*ij*]pyrido[4′,3′:4,5]pyrano[2,3-*f*]-quinolin]-5′(1′*H*)-one 2,2,2-trifluoroacetate in 2 mL of dichloromethane at 0 °C was added 51 mg (0.51 mmol, 2.2 eq.) of triethylamine followed by 22 mg (0.28 mmol, 1.2 eq.) of acetyl chloride. The mixture was stirred at 25 °C for 2 h, poured into water, and extracted with dichloromethane. The organic layers were dried over anhydrous $MgSO_4$ and concentrated. The product was purified by chromatography using 1 : 20 methanol–dichloromethane (R_f = 0.37 after two developments) to provide 59 mg of **11ec** (63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (s, 1H), 3.81 (t, J = 6.2 Hz, 2H), 3.29–3.17 (m, 4H), 2.84 (q, J = 6.7 Hz, 4H), 2.74 (t, J = 6.4 Hz, 2H), 2.67–2.53 (m, 4H), 2.24 (s, 3H), 2.06–1.9 (m, 4H), 1.77–1.62 (m, 2H), 1.51–1.38 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.15, 160.01, 149.92, 146.37, 145.21, 123.36, 120.58, 118.01, 108.62, 106.3, 62.36, 50.01, 49.66, 39.76, 33.83, 27.97, 26.71, 25.42, 25.41, 22.56, 21.86, 20.9, 20.46. HRMS (ESI) calcd for $C_{25}H_{31}N_2O_3$ [MH^+]: 407.2329. Found: 407.2314.

2,2′,3,3′,5′,6′,8,9,12,13-Decahydro-7*H*,11*H*-spiro[pyrido[3,2,1-*ij*]-pyrido[4′,3′:4,5]pyrano[2,3-*f*]quinoline-4,4′-thiopyran]-5(1*H*)-one 2,2,2-Trifluoroacetate (11ee)

Condition B: mp >220 °C; yield 81%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.7 (br. s, 2H), 7.15 (s, 1H), 3.44–3.33 (m, 2H), 3.26 (q, J = 6.1 Hz, 4H), 3.19 (t, J = 13.2 Hz, 2H), 3.09 (t, J = 6.2 Hz, 2H), 2.93 (td, J = 14.3, 4 Hz, 2H), 2.73 (q, J = 6.9 Hz, 4H), 2.57–2.48 (m, 2H), 2.15 (br. d, J = 14.6 Hz, 2H), 1.87 (q, J = 5.4, 5 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.49, 149.32, 147.75, 145.72, 121.7, 118.47, 106.25, 104.71, 64.91, 49.17, 48.66, 34.51, 30.23, 27.09, 21.32, 20.9, 19.99, 19.72, 15.17. HRMS (ESI) calcd for $C_{22}H_{27}N_2O_2S$ [MH^+]: 383.1788. Found: 383.1791.

8-(Dimethylamino)-1′-methyl-2,3-dihydrospiro[chromeno-[3,4-*c*]-pyridine-4,4′-piperidin]-5(1*H*)-one (11bf)

Condition B: mp >220 °C; yield 73%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48 (d, J = 9.2 Hz, 1H), 6.72 (dd, J = 9, 2.4 Hz, 1H), 6.49 (d, J = 2.4 Hz, 1H), 3 (s, 6H), 2.9 (t, J = 6.8 Hz, 2H), 2.73–2.66 (m, 4H), 2.62–1.58 (m, 4H), 2.3 (s, 3H), 1.36 (d, J = 12.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.22, 153.86, 152.16, 150.31, 125.05, 122.14, 109.1, 108.67, 96.71, 50.97, 49.93, 44.9, 35.8, 30.32, 26. HRMS (ESI) calcd for $C_{19}H_{26}N_3O_2$ [MH^+]: 328.2020. Found: 328.2023.

1-Methyl-2′,3′,8′,9′,12′,13′-hexahydro-7′*H*,11′*H*-spiro[piperidine-4,4′-pyrido[3,2,1-*ij*]pyrido[4′,3′:4,5]pyrano[2,3-*f*]quinolin]-5′(1′*H*)-one (11ef)

Condition B: mp = 144–146 °C (decomp); yield 84%; chromatographic solvent: 1 : 10 methanol–dichloromethane (R_f 0.13). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.41 (s, 1H), 7.1 (s, 1H), 3.31–3.11 (m, 8H), 2.9 (t, J = 6.6 Hz, 2H), 2.84 (dt, J = 14.8, 13.8, 4.5 Hz, 2H), 2.76 (s, 3H), 2.75–2.69 (m, 4H), 2.67 (t, J = 5.6 Hz, 2H), 2.02–1.8 (m, 4H), 1.6 (d, J = 14.2 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.5, 157.92, 157.62, 151.09, 149.26, 145.05, 121.32, 119.13, 118.87, 118.03, 115.87, 107.72, 104.82, 49.96, 49.16, 48.66, 35.97, 27.12, 25.74, 21.05, 20.17, 19.83. HRMS (ESI) calcd for $C_{23}H_{30}N_3O_2$ [MH^+]: 380.2333. Found: 380.2337.

(4*R*,5′*S*,8′*S*,9′*S*,10′*S*,13′*S*,14′*S*)-8-Amino-10′,13′-dimethyl-1′,2,2′,3,4′,5′,6′,7′,8′,9′,10′,11′,12′,13′,14′,15′,16′,17′-octadecahydrospiro[chromeno[3,4-*c*]pyridine-4,3′-cyclopenta[*a*]phenanthren]-5(1*H*)-one (11ag)

Condition C: yield 21%. ^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.22 (br.d, J = 38.9 Hz, 3H), 7.45 (d, J = 8.8 Hz, 1H), 6.61 (dd, J = 8.8, 2.2 Hz, 1H), 6.40 (d, J = 2.2 Hz, 1H), 6.26 (s, 2H), 3.13–2.93 (m, 2H), 2.76–2.57 (m, 2H), 1.97–1.78 (m, 2H), 1.78–1.72 (m, 1H), 1.71–1.56 (m, 3H), 1.57–1.43 (m, 6H), 1.44–1.03 (m, 8H), 0.97 (s, 3H), 0.95–0.74 (m, 4H), 0.70 (s, 3H). Calcd for $C_{30}H_{41}N_2O_2$ [MH$^+$]: 461.3163. Found: 461.3167.

(4*R*,5′*S*,8′*S*,9′*S*,10′*S*,13′*S*,14′*S*)-8-(Dimethylamino)-10′,13′-dimethyl-1′,2,2′,3,4′,5′,6′,7′,8′,9′,10′,11′,12′,13′,14′,15′,16′,17′-octadecahydrospiro[chromeno[3,4-*c*]pyridine-4,3′-cyclopenta[*a*]phenanthren]-5(1*H*)-one hydrochloride (11bg)

Condition C: yield 70%; purified by trituration with hot methanol. ^{1}H NMR (400 MHz, CF_3COOD) δ 7.96 (d, J = 8.8 Hz, 1H), 7.87 (br. s, 1H), 7.77 (d, J = 2.3 Hz, 1H), 7.73 (dd, J = 8.9, 2.1 Hz, 1H), 3.86–3.69 (m, 2H), 3.45 (s, 6H), 3.11 (t, J = 13.8 Hz, 1H), 2.94 (t, J = 13.9 Hz, 1H), 1.98–1.9 (m, 1H), 1.9–1.77 (m, 2H), 1.72 (d, J = 12.6 Hz, 1H), 1.67–1.48 (m, 5H), 1.47–1.32 (m, 5H), 1.32–1.23 (m, 3H), 1.22–1.1 (m, 3H), 1.09 (s, 3H), 1.04–0.87 (m, 2H), 0.78 (dt, J = 11.7, 11, 3.8 Hz, 1H), 0.69 (s, 3H). ^{13}C NMR (100 MHz, CF_3COOD) δ 162.54, 154.54, 149.99, 146.98, 129.77, 126.7, 122.58, 120.00, 111.85, 64.46, 56.5, 49.28, 43.36, 42.64, 41.94, 40.43, 38.2, 37.98, 37.9, 35.14, 34.58, 33.80, 29.71, 28.46, 26.96, 25.12, 22.68, 21.76, 18.15, 12.68. HRMS (ESI) calcd for $C_{32}H_{45}N_2O_2$ [MH$^+$]: 489.3476. Found: 489.3478.

(3*R*,5*S*,8*S*,9*S*,10*S*,13*S*,14*S*)-10,13-Dimethyl-1,2,2′,3′,4,5,6,7,8,8′,9,9′,10,11,12,12′,13,13′,14,15,16,17-docosahydro-7′*H*,11′*H*-spiro[cyclopenta[*a*]phenanthrene-3,4′-pyrido[3,2,1-*ij*]pyrido[4′,3′:4,5]pyrano[2,3-*f*]quinolin]-5′(1′*H*)-one hydrochloride (11eg)

Condition C: yield 86%; purified by trituration with hot methanol. ^{1}H NMR (400 MHz, CF_3COOD) δ 7.98–7.43 (m, 1H), 3.94–3.69 (m, 6H), 3.5 (t, J = 6.2 Hz, 2H), 3.25 (t, J = 6.9 Hz, 4H), 3.05 (t, J = 13.7 Hz, 1H), 2.61–2.41 (m, 4H), 2.03 (d, J = 14 Hz, 1H), 1.98–1.86 (m, 2H), 1.81 (d, J = 12.6 Hz, 1H), 1.77–1.57 (m, 5H), 1.57–1.43 (m, 5H), 1.43–1.31 (m, 3H), 1.3–1.2 (m, 3H), 1.19 (s, 3H), 1.12–0.96 (m, 2H), 0.87 (dt, J = 12.3, 11.8, 4.5 Hz, 1H), 0.79 (s, 3H). ^{13}C NMR (100 MHz, CF_3COOD) δ 162.99, 150.42, 150.15, 137.1, 131.52, 126.21, 123.77, 121.25, 64.47, 56.55, 56.27, 43.42, 42.67, 41.98, 40.47, 38.29, 38, 37.94, 35.25, 34.63, 33.84, 29.74, 28.5, 27, 26.84, 25.12, 22.71, 21.8, 21.55, 21.36, 20.57, 18.19, 12.7. HRMS (ESI) calcd for $C_{36}H_{49}N_2O_2$ [MH$^+$]: 541.3789. Found: 541.3790.

(3*R*,5*S*,8*S*,9*S*,10*S*,13*S*,14*S*)-10,13-Dimethyl-1,2′,3′,4,5,6,7,8,8′,9,9′,10,11,12,12′,13,13′,14,15,16-icosahydro-7′*H*,11′*H*-spiro[cyclopenta[*a*]phenanthrene-3,4′-pyrido[3,2,1-*ij*]pyrido[4′,3′:4,5]pyrano[2,3-*f*]quinoline]-5′,17(1′*H*,2*H*)-dione (11eh)

Condition C: yield 57%. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.04 (s, 1H), 3.21 (q, J = 5.5 Hz, 4H), 2.85 (t, J = 5.8 Hz, 2H), 2.71 (q, J = 6.1 Hz, 4H), 2.58 (t, J = 5.7 Hz, 2H), 2.46–2.3 (m, 3H), 2.08–1.95 (m, 1H), 1.93–1.78 (m, 5H), 1.74 (dd, J = 12.7, 3.3 Hz, 1H), 1.7–1.59 (m, 2H), 1.59–1.42 (m, 3H), 1.4–1.32 (m, 2H), 1.31–1.18 (m, 4H), 1.18–1.08 (m, 3H), 1.02–0.96 (m, 1H), 0.95 (s, 3H), 0.79 (s, 3H), 0.79–0.7 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 219.89, 159.52, 149.46, 149.08, 144.61, 122.46, 121.13, 117.71, 108.15, 104.87, 53.87, 53.64, 50.83, 49.16, 48.66, 47.14, 36.12, 35.77, 35.32, 34.63, 33.23, 31.45, 30.65, 28.01, 27.8, 27.12, 26.28, 21.38, 21.15, 20.27, 19.86, 13.49, 11.57. HRMS (ESI) calcd for $C_{36}H_{47}N_2O_3$ [MH$^+$]: 555.3581. Found: 555.3584.

(4*R*,5′*S*,8′*R*,9′*S*,10′*S*,13′*S*,14′*S*,17′*S*)-8-Amino-17′-hydroxy-10′,13′-dimethyl-1′,2,2′,3,4′,5′,6′,7′,8′,9′,10′,11′,12′,13′,14′,15′,16′,17′-octadecahydro-spiro[chromeno[3,4-*c*]pyridine-4,3′-cyclopenta[*a*]phenanthrene]-5(1*H*)-one (11ai)

The procedure described under Condition C was repeated using 87 mg (0.3 mmol, 1 eq.) of 5α-androstan-17β-ol-3-one and 100 mg (0.36 mmol, 1.2 eq.) of the dihydrochloride salt of **9a** in 3 mL of absolute ethanol and 0.3 mL of concentrated HCl to afford 94 mg (57%) of **11ai** as an dihydrochloride salt: ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.33 (br s, 3H), 7.43 (d, J = 8.6 Hz, 1H), 6.6 (dd, J = 8.8, 1.9 Hz, 1H), 6.4 (d, J = 2.1 Hz, 1H), 6.22 (br. s, 2H), 4.43 (d, J = 4.8 Hz, 1H), 3.57–3.4 (m, 1H), 3.13–2.93 (m, 2H), 2.76–2.57 (m, 2H), 1.9–1.78 (m, 2H), 1.78–1.72 (m, 1H), 1.71–1.56 (m, 3H), 1.57–1.43 (m, 4H), 1.44–1.03 (m, 8H), 0.96 (s, 3H), 0.95–0.74 (m, 4H), 0.64 (s, 3H). HRMS (ESI) Calcd for $C_{30}H_{41}N_2O_3$ [MH$^+$]: 477.3112. Found: 477.3093. The procedure described previously for the conversion of hydrochloride salts to free bases was repeated to afford, after chromatography on silica gel using 1 : 10 methanol–dichloromethane (R_f 0.38), **11ai** as a free base: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, J = 8.5 Hz, 1H), 6.55 (dd, J = 8.6, 2.3 Hz, 1H), 6.52 (d, J = 2.2 Hz, 1H), 4.05 (br s, 2H), 3.63 (t, J = 8.5 Hz, 1H), 3.08 (t, J = 5.9 Hz, 2H), 2.74–2.65 (m, 2H), 2.58 (t, J = 13.3 Hz, 1H), 2.13–1.97 (m, 2H), 1.84–1.76 (m, 1H), 1.69–1.61 (m, 1H), 1.57–1.39 (m, 4H), 1.38–1.34 (m, 1H), 1.34–1.3 (m, 1H), 1.3–1.26 (m, 2H), 1.26–1.22 (m, 4H), 1.19–1.12 (m, 2H), 1.11–1.06 (m, 1H), 1.04 (s, 3H), 1.01–0.86 (m, 3H), 0.82–0.73 (m, 1H), 0.74 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.1, 154.37, 149.31, 148.26, 125.34, 125, 111.77, 100.78, 82.22, 55.1, 54.48, 51.37, 43.18, 41.3, 37.02, 36.62, 36.31, 35.88, 35.76, 34.07, 31.83, 30.72, 29.86, 28.63, 28.34, 27.1, 23.54, 20.71, 11.98, 11.33. HRMS (ESI) calcd for $C_{30}H_{41}N_2O_3$ [MH$^+$]: 477.3112. Found: 477.3112.

(4*R*,5′*S*,8′*R*,9′*S*,10′*S*,13′*S*,14′*S*,17′*S*)-8-(Dimethylamino)-17′-hydroxy-10′,13′-dimethyl-1′,2,2′,3,4′,5′,6′,7′,8′,9′,10′,11′,12′,13′,14′,15′,16′,17′-octadeca-hydrospiro[chromeno[3,4-*c*]pyridine-4,3′-cyclopenta[*a*]phenanthren]-5(1*H*)-one hydrochloride (11bi)

The procedure described under Condition C was repeated using 59 mg (0.2 mmol, 1 eq.) of 5α-androstan-17β-ol-3-one and 65 mg (0.24 mmol, 1.2 eq.) of the hydrochloride salt of **9b** in 2 mL of absolute ethanol and 0.2 mL of concentrated HCl to provide 94 mg (85%) of **11bi**. This product was triturated with hot methanol to achieve additional purification:

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.64–9.27 (m, 2H), 7.55 (d, J = 9 Hz, 1H), 6.77 (dd, J = 9.1, 2.5 Hz, 1H), 6.56 (d, J = 2.5 Hz, 1H), 4.44 (d, J = 4.8 Hz, 1H), 3.49–3.4 (m, 1H), 3.12–3.04 (m, 2H), 3.02 (s, 6H), 2.71–2.58 (m, 2H), 1.92–1.78 (m, 1H), 1.78–1.71 (m, 1H), 1.7–1.58 (m, 3H), 1.56–1.4 (m, 5H), 1.4–1.26 (m, 3H), 1.26–1.09 (m, 5H), 0.96 (s, 3H), 0.93–0.76 (m, 4H), 0.64 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 159.09, 154.34, 153.2, 125.94, 109.94, 97.07, 80.48, 53.32, 51.2, 43.03, 37.13, 35.75, 35.53, 31.62, 30.28, 28.01, 23.49, 20.58, 11.95, 11.82. HRMS (ESI) calcd for $C_{32}H_{45}N_2O_3$ [MH$^+$]: 505.3425. Found: 505.3405.

(3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,17*S*)-17-Hydroxy-10,13,17-trimethyl-1,2,2′,3′,4,5,6,7,8,8′,9,9′,10,11,12,12′,13,13′,14, 15,16,17-docosahydro-7′*H*,11′*H*-spiro[cyclopenta-[*a*]phenanthrene-3,4′-pyrido-[3,2,1-*ij*]pyrido[4′,3′:4,5]-pyrano[2,3-*f*]quinolin]-5′(1′*H*)-one (11ej) and (3*R*,5*S*,8*R*,9*S*,10*S*)-10,17,17-trimethyl-1,2,2′,3′,4,5,6,7,8,8′,9, 9′,10, 11,12,12′,13′,15,16,17-icosahydro-7′*H*,11′*H*-spiro[cyclopenta-[*a*]phenanthrene-3,4′-pyrido-[3,2,1-*ij*]pyrido[4′,3′:4,5]pyrano-[2,3-*f*]-quinolin]-5′(1′*H*)-one (14)

A suspension of 200 mg (0.62 mmol, 1.2 eq.) of **9e** and 160 mg (0.52 mmol, 1 eq.) of **10j** in 2 mL of absolute ethanol was stirred under reflux for 48 h to afford 240 mg of a crude as a mixture of **11ej** and **14** that was suspended in water and dichloromethane and washed with a saturated $NaHCO_3$ solution. The organic layers were dried over anhydrous Na_2SO_4, filtered, concentrated, and chromatographed using 1 : 10 methanol–dichloromethane (R_f 0.25) to provide 57 mg (19%) of **11ej**: ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.1–6.98 (m, 1H), 4.03 (s, 1H), 3.2 (q, J = 5.6 Hz, 4H), 2.85 (t, J = 5 Hz, 2H), 2.7 (q, J = 6 Hz, 4H), 2.58 (t, J = 5.5 Hz, 2H), 2.48–2.43 (m, 1H), 2.33 (t, J = 13 Hz, 1H), 1.93–1.78 (m, 5H), 1.72 (t, J = 10.7 Hz, 1H), 1.66–1.56 (m, 1H), 1.56–1.28 (m, 8H), 1.29–1.09 (m, 7H), 1.07 (s, 3H), 1.02–0.94 (m, 1H), 0.93 (s, 3H), 0.9–0.8 (m, 1H), 0.74 (s, 3H), 0.71–0.62 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 159.49, 149.36, 149.08, 144.6, 121.12, 117.7, 108.15, 104.87, 79.72, 53.94, 53.71, 50.45, 49.16, 48.67, 45.21, 38.38, 36.13, 36.07, 35.68, 35.22, 33.32, 31.65, 31.55, 28.22, 27.8, 27.13, 26.27, 26.19, 23.1, 21.15, 20.27, 19.87, 14.25, 11.61. HRMS (ESI) calcd for $C_{37}H_{51}N_2O_3$ [MH$^+$]: 571.3894. Found: 571.3902. In addition to **11ej**, chromatography on silica gel using 1 : 10 methanol–dichloromethane (R_f 0.48) afforded 58 mg (20%) of **14**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (s, 1H), 3.27–3.14 (m, 4H), 3.09–3 (m, 2H), 2.84 (t, J = 6.5 Hz, 2H), 2.75 (t, J = 12.5 Hz, 2H), 2.69–2.65 (m, 2H), 2.6 (t, J = 13.3 Hz, 1H), 2.28–2.15 (m, 1H), 2.12–1.98 (m, 2H), 1.99–1.88 (m, 5H), 1.88–1.72 (m, 2H), 1.67–1.62 (m, 1H), 1.61–1.57 (m, 3H), 1.56–1.48 (m, 2H), 1.4–1.27 (m, 3H), 1.27–1.16 (m, 2H), 1.16–1.08 (m, 1H), 1.08–1 (m, 2H), 1 (s, 3H), 0.98–0.96 (m, 1H), 0.93 (s, 3H), 0.92 (s, 3H). ^{13}C NMR (100 MHz, CDCl3) δ 160.71, 149.87, 148.5, 144.99, 141.18, 136.52, 123.18, 120.93, 118, 109.11, 106.38, 54.99, 51.95, 50.05, 49.68, 45.49, 41.33, 39.73, 36.86, 36.79, 36.25, 36.07, 33.96, 31.6, 29.89, 29.2, 28.26, 27.96, 27.19, 26.94, 26.64, 22.63, 22.55, 21.93, 21.01, 20.43, 11.4. HRMS (ESI) calcd for $C_{37}H_{49}N_2O_2$ [MH +]: 553.3789. Found: 553.3789. Anal. calcd for $C_{37}H_{50}N_2O_3$: C, 80.39; H, 8.75; N, 5.07. Found: C, 80.17; H, 8.94, N, 5.13.

(3*R*,5*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-10,13-Dimethyl-17-((*R*)-6-methyl-heptan-2-yl)-1,2,2′,3′,4,5,6,7,8,8′,9,9′,10,11,12,12′,13,13′,14,15, 16,17-docosahydro-7′*H*,11′*H*-spiro[cyclopenta-[*a*]phenanthrene-3,4′-pyrido[3,2,1-*ij*]pyrido[4′,3′:4,5]pyrano-[2,3-*f*]quinolin]-5′(1′*H*)-one hydrochloride (11ek)

Condition C: mp >230 °C; yield 78%. Purified by trituration with hot methanol. ^{1}H NMR (500 MHz, CF_3COOD) δ 7.57 (s, 1H), 3.84–3.76 (m, 1H), 3.76–3.61 (m, 4H), 3.42 (t, J = 6.9 Hz, 2H), 3.15 (t, J = 6.6 Hz, 4H), 3.13–3.07 (m, 1H), 3–2.9 (m, 1H), 2.52–2.34 (m, 4H), 3.48–3.38 (m, 1H), 2.11–2.01 (m, 3H), 1.98–1.73 (m, 2H), 1.64–1.54 (m, 4H), 1.53–1.43 (m, 7H), 1.42–1.3 (m, 3H), 1.19–1.11 (m, 4H), 1.1 (s, 3H), 1.07–0.91 (m, 4H), 0.89 (d, J = 6.4 Hz, 3H), 0.8 (dd, J = 6.7, 1.5 Hz, 6H), 0.78–0.72 (m, 2H), 0.68 (s, 3H). ^{13}C NMR (126 MHz, CF_3COOD) δ 163.02, 150.43, 150.18, 137.13, 131.54, 126.26, 126.23, 123.8, 121.28, 64.51, 58.58, 58.53, 56.61, 56.29, 44.6, 43.44, 41.89, 41.46, 38.32, 38.09, 37.99, 37.85, 37.66, 35.27, 34.61, 33.52, 29.9, 29.76, 28.52, 26.87, 25.78, 25.66, 25.15, 23.51, 23.27, 22.72, 21.59, 21.39, 20.61, 19.6, 12.9, 12.71. HRMS (ESI) calcd for $C_{44}H_{65}N_2O_2$ [MH$^+$]: 653.5041. Found: 653.5043.

4-(*p*-Tolyl)-8,9,12,13-tetrahydro-5*H*,7*H*,11*H*-pyrido[3,2,1-*ij*]pyrido-[4′,3′:4,5]pyrano[2,3-*f*]quinolin-5-one (13)

The procedure described under Condition B was repeated using 200 mg (0.62 mmol) of **9e** and 0.29 mL (2.49 mmol) of 4-toluylaldehyde to afford crude 4-(*p*-tolyl)-1,2,3,4,8,9,12,13-octahydro-5*H*,7*H*,11*H*-pyrido[3,2,1-*ij*]pyrido[4′,3′:4,5]pyrano[2,3-*f*]-quinolin-5-one 2,2,2-trifluoroacetate in 58% yield. To 100 mg (0.2 mmol) of this trifluoracetate salt in 6 mL of dimethyl sulfoxide was added 100 µL (0.7 mmol, 3.5 eq.) of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 94 mg (0.42 mmol, 2.1 eq.) of cupric bromide. The solution was stirred at 25 °C for 16 h. The mixture was poured into 20 mL of aqueous ammonia solution (5% w/w) and extracted with ethyl acetate. The organic layers were washed with brine dried over anhydrous $MgSO_4$, filtered and concentrated. The product was purified by chromatography using 1 : 50 methanol–dichloromethane (R_f 0.26) to provide 15 mg (20%) of **13** as a yellow powder: mp 216–218 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.67 (d, J = 5.6 Hz, 1H), 7.96 (d, J = 5.7 Hz, 1H), 7.73 (s, 1H), 7.38 (d, J = 8.1 Hz, 2H), 7.19 (d, J = 7.9 Hz, 2H), 3.31–3.22 (m, 4H), 2.79 (t, J = 6.3 Hz, 2H), 2.74 (t, J = 6.4 Hz, 2H), 2.37 (s, 3H), 1.9 (d, J = 6.8 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d$_6$) δ 162.9, 158.91, 151.24, 149.32, 146.12, 144.32, 138.54, 137.33, 128.87, 127.89, 121.57, 118.18, 113.2, 110.85, 105.61, 102.94, 49.22, 48.67, 27.03, 20.96, 20.93, 20.07, 19.99. HRMS (ESI) calcd for $C_{26}H_{23}N_2O_2$ [MH$^+$]: 383.1754. Found: 383.1756.

X-Ray crystallography

X-ray diffraction data were collected at 90.0(2) K on a Bruker D8 Venture κ-axis diffractometer using MoK(α) X-rays and using well-established, low-temperature crystal-handling techniques.[48] Raw data were integrated, scaled, merged and corrected for Lorentz-polarization effects using the APEX3 package (Bruker-AXS Inc., Madison, WI, US). Corrections for absorption were applied using SADABS.[49] The structure was solved by direct

methods (SHELXT[50]) and refinement was carried out against F^2 by weighted full-matrix least-squares (SHELXL[50]). Hydrogen atoms were found in difference maps placed at calculated positions and refined using riding models. Non-hydrogen atoms were refined with anisotropic displacement parameters. Absolute configuration was known due to the unchanging stereochemistry of the steroid ring system. Atomic scattering factors were taken from the International Tables for Crystallography. Experimental conditions, atomic coordinates, bond lengths and angles, as well as diffraction data and the crystallographic model were archived in the CIF, CCDC 1888376.†

NMR spectroscopy

NMR experiments were carried out at 25 °C using DMSO-d_6 on an Agilent 400 MHz spectrometer using a 5 mm probe equipped with a *z*-gradient optimized for ^{1}H detection. Chemical shifts were referenced relative to the residual protonated solvent signal set at 2.54 ppm.[51] A normal one-dimensional ^{1}H and ^{13}C (proton decoupled) and DEPT-135 (Distorsionless Enhancement of Polarization Transfer) spectra[52] were acquired and two-dimensional homonuclear (^{1}H–^{1}H) correlation spectroscopy (COSY and ROESY)[41,42] 2D-heteronuclear correlations (^{1}H–^{13}C) spectra (HSQC and HMBC) were acquired using standard pulse sequences from the Agilent library. Spectral widths of 4340 Hz for both dimensions with 1024 complex data points in $t2$ and 256 $t1$ increments were used for ROESY and COSY experiments. The relaxation delay between successive pulse cycles was set to 1 s and ROESY mixing time of 400 ms was used. Forty transients for each increment were collected for ROESY, and 16 transients for each increment were collected for COSY experiments. Phase sensitive two-dimensional spectra were obtained using the time-proportional phase incrementation method (TPPI). Spectra were zero-filled to 2048 × 1024 real data points along $f2$ and $f1$, respectively. Sine-bell π/3 window functions were used in both dimensions. HSQC adiabatic NMR spectrum was acquired with spectral widths of 4595 Hz (^{1}H) and 20 100 Hz (^{13}C) with 1 s of recycled delay to show direct ^{1}H–^{13}C connectivity and C–H correlation.

Computational modeling

The X-ray structure[35] of the androgen-ligand binding domain in human 17β-hydroxysteroid dehydrogenase type 5 (PDB: 1XF0) with 5α-androstane-3,17-dione was selected as the template to model the binding of the fluorescent adduct **11eh**. The initial enzyme structure was downloaded from the RCSB Protein Data Bank and was subsequently prepared for docking *via* Autodock Tools.[53] The adduct **11eh** was docked using Autodock Vina[54] into the position occupied by 5α-androstane-3,17-dione in 17β-HSD5. The binding poses of **11eh** obtained from Vina that contained high overlap with that of **11eh**. The binding pose was further refined by performing a series of energy minimization processes. Briefly, the AMBER14SB force field[55] and the second generation of the general AMBER force field (gaff2) were used for the proteins and ligands, respectively. Partial charges for **11eh** were generated *via* the Antechamber[56] program in AMBER 18 using the AM1-BCC model.[57] Two courses of minimization were conducted using a hybrid protocol of twenty five-hundred steps of steepest descent minimization followed by a conjugate gradient minimization until a maximum twenty five-hundred iteration steps was reached or the convergence criterion (the root-mean-square of the energy gradient is less than 1×10^{-4} kcal mol^{-1} Å^{-1}) was satisfied. During the first step of minimization, a force constant of 100 kcal mol^{-1} Å^{-2} was applied on the protein atoms. The second minimization step consisted of one thousand steps of steepest descent minimization, followed by fifteen hundred steps of conjugate gradient minimization, and this course of minimization had no restraints for either the ligand or the protein atoms.

Author contributions

James L. Mohler, Michael V. Fiandalo, Chunming Liu, Vitaliy M. Sviripa and David S. Watt conceived the overall experimental question, assisted with and contributed to the experimental design, and supervised data acquisition and analysis and manuscript preparation. Vitaliy M. Sviripa, Kristin L. Begley, Przemyslaw Wyrebek, Liliia Kril and David S. Watt planned and executed the synthetic chemistry. Vivekanandan Subramanian performed the 2D NMR studies. Sean R. Parkin performed the X-ray crystallography study. Xi Chen, Alexander H. Williams and Chang-Guo Zhan performed the pK_a calculations and the computational modeling studies. All authors reviewed the manuscript.

Conflicts of interest

CL and DSW have partial ownership in a for-profit venture, Epionc, Inc., that seeks to develop small-molecule inhibitors for cancer treatment. In accord with University of Kentucky policies, CL and DSW have disclosed this work to the University of Kentucky's Intellectual Property Committee and to a Conflict of Interest Oversight Committee.

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