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Friedländer-Type Reaction of 4-Cholesten-3-one with 2′-Aminoacetophenone: Angular versus Linear Quinoline-Fused Steroids

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Abstract: To optimize the experimental conditions used for the Friedländer-type condensation, an angular fused 4-substituted quinoline steroid has been obtained in very high yield and regioselectivity using readily available 4-cholesten-3-one and 2′-aminoacetophenone. Moreover, by varying the reaction conditions and the catalyst, the corresponding linear regioisomer was also achieved with an acceptable isolated yield and high chemoselectivity. Both structures have been definitively elucidated via 2D-NMR and fully characterized.

Keywords: fused steroid quinoline polycycles; Friedländer's condensation; angular cholestanoquinoline; linear cholestanoquinoline

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1. Introduction

The quinoline nucleus is ubiquitous in nature and plays a significant role in the development of novel heterocyclic compounds that have important pharmacological features, such as antitumoral activities [1,2]. Over time, studies of the biological activity of quinoline derivatives on diverse cancer cell lines have revealed a wide range of action mechanisms, paving the way for the development of more effective quinoline-derived drugs [3,4]. Figure 1 depicts a non-exhaustive list of commercialized anticancer drugs that use the quinoline ring as their primary component.

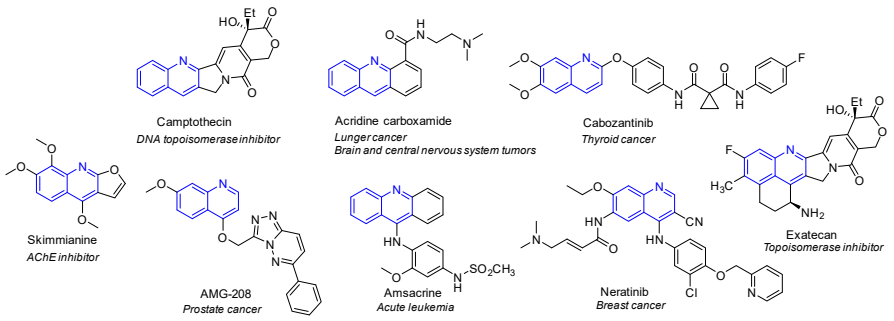


Figure 1. Selected therapeutic agents that have a quinoline nucleus.

In addition, the incorporation of the quinoline nucleus into the steroid nucleus [5] has emerged as a promising strategy for the identification of new antitumor agents (Figure 2) [6–8].

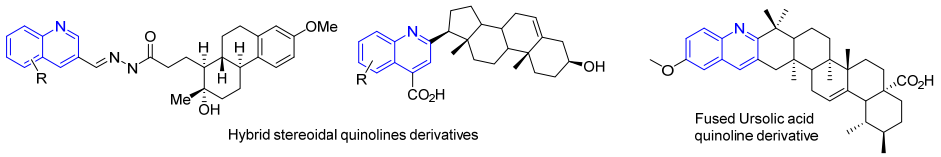


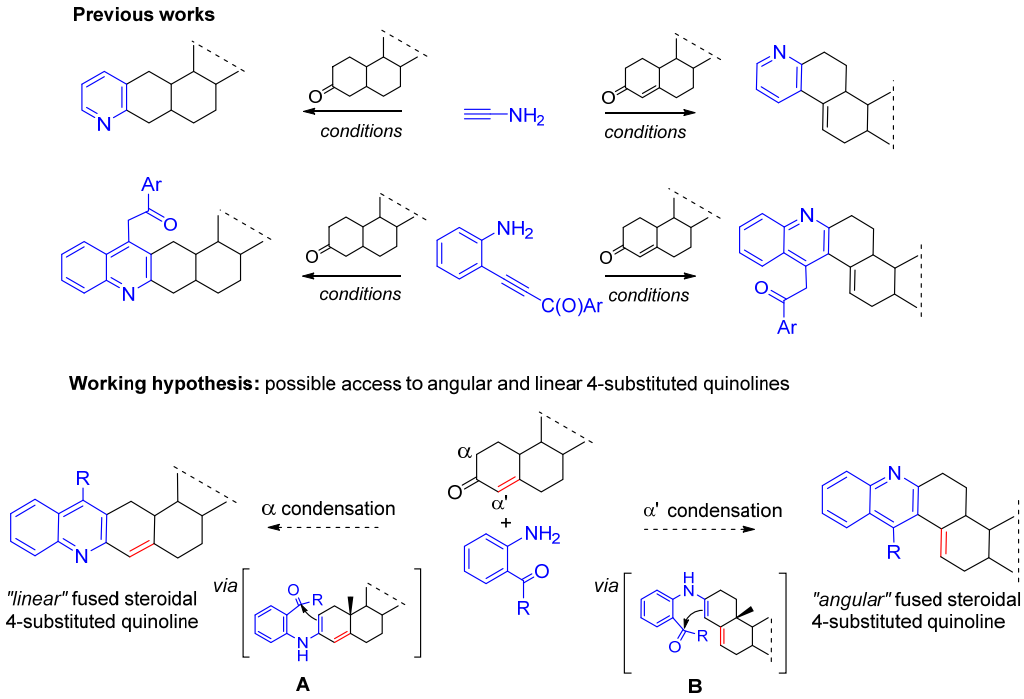
Figure 2. Selected hybrid and fused steroidal quinolines tested for anticancer activity.

So far, the Friedländer reaction has proved to be one of the most powerful methods of performing diversity-oriented synthesis of bioactive heterocyclic scaffolds based on the quinoline nucleus [9–11]. Indeed, the Friedländer condensation of 2-formyl- or 2-acyl anilines with keto-steroids provided a simple way to achieve A-ring and D-ring-fused quinolines [12,13].

In this field, as part of our ongoing research into heterocyclic scaffolds that have potential biological activity, two members of our team successfully synthetized pyridines and quinolines via cascade amination/cyclization/aromatization reactions of propargylamine [14] or β-(2-aminophenyl)-α,β-ynones [15], with the ketones using metal or Brønsted acid catalysis, respectively. These methodologies also enabled access to linear and angular polycyclic steroidal derivatives using cholestanones or cholestenones, respectively, as reaction partners (Scheme 1).

Due to the importance of substituent and skeleton variations in drug design [16], we became interested in implementing the synthesis of fused quinoline steroids using readily available and inexpensive 2-acyl-substituted anilines as convenient alternative starting materials in Friedländer-type condensation with α,β-unsaturated keto-steroids.

Here we report a preliminary optimization study of the model system 2′-aminacetophenone and 4-cholesten-3-one, which aimed to obtain novel 4-alkyl-substituted quinolines, as well as address the α/α′ regioselectivity issue.



Scheme 1. Previous approaches to studying polycyclic pyridines and quinolines considered in this work.

2. Results and Discussion

Synthesis

Due to the possibility of forming two regioisomeric enamine intermediates (type **A** and **B**), the reaction between 2′-aminoacetophenone **1** and 4-cholesten-3-one **2** poses a regioselectivity issue, namely the competition between the condensations at the α and α′ positions (Figure 3) [17].

Thus, following our previous studies of this matter, we performed the reaction under a variety of experimental conditions, using both gold(III) catalysis and Brønsted acid catalysis. The effects of the catalytic loading, the nature of the solvent and the temperature were also investigated (Table 1).

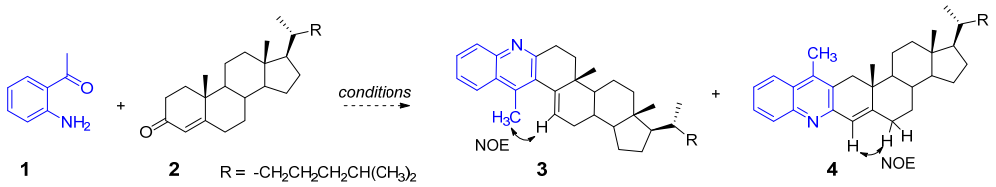


Figure 3. Reaction scheme and key spectral differences between regioisomers **3** and **4**.

Using previously reported conditions for sequential condensation/annulation reaction of active methylene ketones with 2-aminocarbonyls, the gold(III)-catalyzed reaction produced a mixture of angular and linear quinolines **3** and **4** in a 1:2 ratio with a modest overall yield of 40% after 24 h (entry 1). Despite the fact that 66% of the starting material 2 could be recovered when the reaction was terminated, NMR analysis of the crude after a shorter reaction time (5 h) revealed a comparable conversion of 2 (note c, entry 1). Due to the low regioselectivity, the catalyst loading was not further investigated.

An increase in the temperature resulted in a slight increase in overall yield (45%) and substantially unchanged regio- and chemo-selectivity (i.e., global quinoline-isolated yield vs. starting material conversion) (entry 2).

Moreover, 2D-NMR experiments performed using isolated products definitively disambiguated the structures of the two regioisomers. As detailed in the Supplementary Materials document, the key difference between the two types is the observation of NOE between vinylic H and the methyl group using the quinoline moiety in **3** vs. the lack of NOE between vinylic H and the same methyl group using quinoline **4**, which, conversely, presents a strong transient NOE with methylene 1 (See Figure S22).

In contrast, substantial improvements in efficiency and overall yield were obtained when using *p*-toluenesulfonic acid monohydrate (*p*-TsOH·H_2O) as the catalyst. In fact, under the conditions described in entry 3, the quinolines **3** and **4** were isolated in 60% and 30%, respectively.

A remarkable enhancement of the regioselectivity in favor of the angular quinoline was achieved using non-polar toluene as the solvent rather than polar protic EtOH, so much so that, with a stoichiometric amount of *p*-TsOH·H_2O in toluene at 80 °C, the starting material **2** was completely converted within 5 h, and the angular quinoline **3** was isolated as a single regioisomer in nearly quantitative yield (entry 8).

A control experiment performed using $NaAuCl_4 \cdot 2H_2O$ in toluene confirmed the crucial effect of this solvent in preferentially directing the regioselectivity toward the angular quinoline. In fact, despite a poor overall yield of 15%, the regioisomer **3** was isolated in a large majority of cases (entry 9). In these cases, toluene strongly impacts the chemoselectivity of the reaction, probably due to the well-known sensitivity of the catalyst to aromatic compounds [18].

Table 1. Friedländer condensation of 2′-aminoacetophenone and 4-cholesten-3-one under various conditions.

Entry	Catalyst (%)	T (°C)	Solvent	Time (h)	**2** [a] Conversion (%) [a]	**3** Yield (%) [b]	**4** Yield (%) [b]
1 [c]	$NaAuCl_4 \cdot 2H_2O$ (5%)	80	EtOH	24	54	13	27
2	$NaAuCl_4 \cdot 2H_2O$ (5%)	110	EtOH	5	62	15	30
3	*p*-TsOH·H_2O (20%)	110	EtOH	2	Quantitative	60	30
4 [d]	*p*-TsOH·H_2O (20%)	80	Toluene	24	55	45	Traces
5	*p*-TsOH·H_2O (20%)	80	Toluene	48	85	67	Traces
6	*p*-TsOH·H_2O (20%)	110	Toluene	5	Quantitative	76	7
7	*p*-TsOH·H_2O (1 eq)	110	Toluene	5	Quantitative	81	14
8 [e]	*p*-TsOH·H_2O (1 eq)	80	Toluene	5	Quantitative	>98	Traces
9	$NaAuCl_4 \cdot 2H_2O$ (5%)	110	Toluene	24	75	12	<3

(a) Conversion was calculated using recovered 4-cholesten-3-one. (b) Yield refers to isolated and chromatographically pure products. (c) **2**/(**3** + **4**) NMR ratio calculated after 5 h at 80°C was 1:1. (d) In this entry, conversion of **2** and yield in **3** were estimated through ^{1}H-NMR. (e) In this entry, 2.5 equivalent of 2′-aminoacetophenone **1** was used.

3. Materials and Methods

3.1. General Information

The reactions were monitored via thin layer chromatography (TLC) using Merck Silica Gel 60 F254 plates (Milan, Italy) and visualized through fluorescence quenching at 254 nm.

The purification of products was carried out via flash chromatography using silica gel 60. The NMR spectra were recorded via Bruker Avance 400 spectrometers (400 MHz, 1H; 101 MHz, 13C; 135 MHz DEPT) (Milan, Italy). Spectra were referenced to the residual $CHCl_3$ (7.26 ppm, 1H; 77.00 ppm, 13C). A full characterization using the 2D-NMR spectra of both **3** and **4** can be found in the Supplementary Materials document.

Yields are given for isolated products. Purity was monitored via NMR spectra. High-resolution mass spectra (HRMS) were acquired using a Xevo G2-XS QTof (Waters Corporation, Milford, CT, USA). The samples were ionized in positive ion mode using an electrospray (ESI) ionization source. The IR spectra were recorded via a Perkin–Elmer spectrometer (Spectrum Two FT-IR) (Waltham, MA, USA) equipped with a universal attenuated total reflectance accessory (UATR). Optical rotations were measured using a ZUZI 412 Digital Polarimeter (tube length: 100 mm) (Auxilab, Navarra, Spain). Melting points were determined using STUART Melting Point Apparatus SMP30 (Vernon Hills, IL, USA).

3.2. Materials

All chemicals and solvents were obtained from commercial sources and used without further purification.

3.3. General Procedure for the Synthesis of Compound **3** *and* **4**

A mixture of 2-Aminoacetophenone **1** (0.8 mmol, 108 mg, 97 µL and 2 equivalent), (+)-4-cholesten-3-one 2 (0.4 mmol and 154 mg) and p-TsOH·H_2O (0.4 mmol, 76 mg and 1 equivalent) in Toluene (2 mL) was stirred at 80 °C for 5 h. After completion (reaction monitored via TLC eluting in pure CH_2Cl_2), the mixture was diluted using a saturated solution of $NaHCO_3$ and extracted using CH_2Cl_2 (25 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4. After the removal of the solvent at reduced pressure, the crude product was purified via flash chromatography using silica gel eluting with n-hexane/CH_2Cl_2 mixtures (from 9:1 to pure CH_2Cl_2) to give 189 mg of (3R,3aR,5bR)-3a,5b,13-trimethyl-3-((*R*)-6-methylheptan-2-yl)-2,3,3a,4,5,5a,5b,6,7,15,15a,15b-dodecahydro-1*H*-cyclopenta[5,6]naphtho[2,1-a]acridine (**3**) as a white solid (yield 98%; mp 127.5–128.0 °C; $[\alpha]_D = -10.78$ (c 0.1 in $CHCl_3$). IR (cm^{-1}) 2944; 2920; 2866; 2845; 1452; 1380; 765).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (dd, *J* = 12.2, 8.4 Hz, 2H); 7.62 (t, *J* = 7.5 Hz, 1H); 7.49 (t, *J* = 7.6 Hz, 1H); 5.60 (d, *J* = 3.3 Hz, 1H); 2.89–2.78 (m, 2H); 2.70 (s, 3H); 2.31 (dt, *J* = 17.5, 4.6 Hz, 1H); 2.19–2.05 (m, 2H); 1.94–0.99 (m, 21H); 0.93 (m, 12H) and 0.76 (s, 3H).

$^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$) δ 161.3; 145.7; 139.8; 139.2; 132.4; 128.9; 128.6; 128.2; 128.1; 125.3; 124.4; 56.9; 56.3; 47.3; 42.6; 40.0; 39.5; 38.0; 36.2; 35.8; 34.6; 32.31; 32.30; 32.0; 28.3; 28.0; 24.2; 23.9; 23.7; 22.8; 22.6; 22.0; 18.7; 15.5 and 12.1. HRMS: *m*/*z* (MALDI-TOF) positive ion, which was calculated for $C_{35}H_{50}N$: $[M + H]^+$ 484.3943, found: 484.3941 (see Supplementary Materials).

A mixture of 2-Aminoacetophenone 1 (0.44 mmol, 59 mg, 53 µL and 1.1 equivalent), (+)-4-cholesten-3-one 2 (0.4 mmol, 154 mg and 1.0 equivalent) and $NaAuCl_4 \cdot 2H_2O$ (0.02 mmol, 8 mg and 0.05 equivalent) in EtOH (2 mL) was stirred at 110 °C for 5 h. After completion (reaction monitored via TLC elution in pure CH_2Cl_2), the mixture was diluted using a saturated solution of $NaHCO_3$ and extracted using CH_2Cl_2 (25 mL × 3). The combined organic layers were dried using anhydrous Na_2SO_4. After the removal of the solvent at reduced pressure, the crude was purified via flash chromatography (eluents: n-hexane/CH_2Cl_2 mixtures from 9:1 to pure CH_2Cl_2) to yield 30 mg of (1R,13aR,15aR)-12,13a,15a-trimethyl-1-((*R*)-6-methylheptan-2-yl)-2,3,3a,3b,4,5,13,13a,13b,14,15,15a-dodecahydro-1*H*-cyclopenta[5,6]naphtho[1,2-b]acridine (**4**) as a white solid (yield 30%; mp 136.3–138.8 °C; $[\alpha]_D$= 30.06 (c 0.02 in $CHCl_3$). IR (cm^{-1}): 2928; 2868; 1451; 1380; 761; 754.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, *J* = 22.8, 8.3 Hz, 2H); 7.56 (t, *J* = 7.6 Hz, 1H); 7.41 (t, *J* = 7.6 Hz, 1H); 6.45 (s, 1H); 3.26 (d, *J* = 15.3 Hz, 1H); 2.55 (s, 3H); 2.53–2.37 (m, 3H); 2.07 (t, *J* = 12.3 Hz, 1H); 1.91–0.98 (m, 21H); 0.97–0.82 (m, 12H) and 0.74 (s, 3H). $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$) δ 156.7; 153.5; 146.5; 139.1; 129.2; 128.0; 127.6; 125.7; 125.1; 123.6; 123.4; 56.3; 56.1; 53.6; 42.7; 39.9; 39.5; 39.2; 38.8; 36.2; 36.1; 35.8; 31.7; 31.5; 29.7; 28.2; 28.0; 24.3; 23.9; 22.8; 22.6; 21.9; 18.7; 18.4; 13.4 and 11.9. HRMS: *m*/*z* (MALDI-TOF) positive ion, which was calculated for $C_{35}H_{50}N$: $[M + H]^+$ 484.3943, found: 484.3945 (see Supplementary Materials).

4. Conclusions

In summary, we presented a direct means of accessing synthetically relevant fused angular- and linear 4-methyl-substituted quinoline derivatives of 4-cholesten-3-one. In addition to successfully adjusting the reaction parameters to maximize the yield of the angular regioisomer, the challenging linear derivative has been achieved in a significant isolated yield for the first time. The evaluation of the biological activity of both molecules will occur in due course in our laboratory.

Supplementary Materials: S1: NMR Spectra; S2: IR Spectra; S3: Mass Spectra; S4: **3** and **4** structure determination (2D-NMR spectra).

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