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Short communication

N-Oxide formation causes loss of aromatase inhibitory activity of pyridyl-substituted tetrahydronaphthalenes

M Mitrenga, RW Hartmann*

Fachrichtung, 12.1, Pharmazeutische Chemie, Universität des Saarlandes, D-66041 Saarbrücken, Postfach 151150, Germany

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Summary — *N*-Oxidation plays a role in the metabolism of numerous pyridine-substituted drugs. In order to examine the effect of a possible *N*-oxidation on the activity of nonsteroidal aromatase inhibitors, three potent pyridine-substituted tetrahydronaphthalenes were converted to their corresponding *N*-oxides. Using human placental aromatase, it was shown that *N*-oxidation causes a nearly complete loss of aromatase inhibitory activity.

aromatase inhibitor / pyridine-*N*-oxide / tetrahydronaphthalene / estrogen-dependent disease

Introduction

Inhibition of the enzyme aromatase is a modern strategy for the therapy of estrogen-dependent diseases, such as breast cancer. Aromatase (CYP 19) is a cytochrome P 450 enzyme catalyzing the final step of estrogen biosynthesis. Selective inhibition of aromatase would lead to a reduction of estrogen formation without affecting the biosyntheses of other steroid hormones.

Potent and rather selective nonsteroidal aromatase inhibitors are presently in clinical trials, such as fadrozole [1], vorozole [2] and arimidex [3]. However, the only commercially available, nonsteroidal inhibitor still is aminoglutethimide (AG, fig 1). Because of its lack of specificity and its moderate tolerability the compound is far from being an optimal drug [4]. The replacement of the 4-aminophenyl substituent of AG by a 4-pyridyl group to give rogletimide results in a decrease of inhibitory potency toward aromatase but also in an increase in selectivity (fig 1) [6].

Nonsteroidal aromatase inhibitors exert their activity a) by complexing the heme iron of the P 450 enzyme with their basic nitrogen, and b) by interacting with the substrate binding site at the apoprotein moiety. Pyridin-*N*-oxide has been shown to complex the central iron of synthetic porphyrins [5].

N-Oxidation of the pyridyl group of rogletimide leads to a complete loss of inhibitory activity [6]. In biotransformation studies, the *N*-oxide was identified as the major urinary metabolite from rogletimide [7, 8].

Investigations by our group led to the pyridyl-substituted tetrahydronaphthalenes **1–3**. These compounds are potent inhibitors of aromatase surpassing the *in vitro* inhibitory activity of AG by far [9, 10], *eg*, in the case of compound **3** by a factor of 300. The aforementioned *N*-oxidation should be a possible metabolic pathway for these compounds as well. This study describes the effect of *N*-oxidation on the *in vitro* activity of inhibitors **1–3**.

Results

Chemistry

Compound **1** was obtained by the acid-catalyzed aldol condensation of the commercially available compounds 7-methoxy-1-tetralone and 4-pyridinecarbaldehyde. Hydrogenation of **1** using palladium on charcoal gave compound **2** [9], while the cyclopropyl compound **3** was synthesized by a Wolff-Kishner-type reduction of **1** [10] (fig 2). *N*-Oxidation of **1** and **2** was performed according to Ochiai's method [12] and gave compounds **4** and **5**. In case of the cyclopropyl compound, neutral conditions had to be applied to prevent ring opening. Thus, using the method of Payne *et al* [13], compound **6** was obtained.

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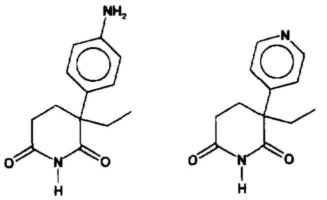


Fig 1. Structures of aminoglutethimide (left) and rogletimide (right).

Biological results

The inhibitory activities of the test compounds **1–6** toward aromatase were determined *in vitro* using human, placental microsomes and [1β, 2β, ^{3}H]testosterone according to the previously described procedure [11].

The IC_{50} values and potencies of the compounds, relative to AG are given in table I. In all cases *N*-oxidation leads to a dramatic decrease of the inhibitory potencies of the compounds: **4** and **5** are inactive; **6** shows a relative potency of 0.42. This is a decrease by a factor of 700 compared with the parent compound **3**.

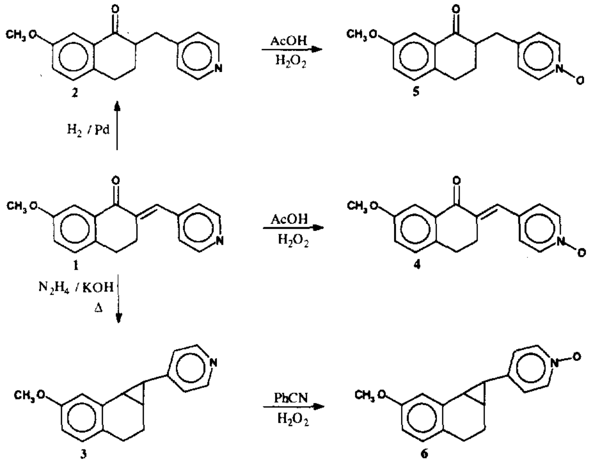


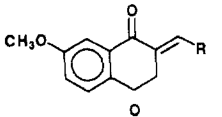
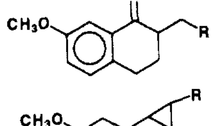
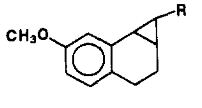
Fig 2. Synthesis of compounds **2–6**.

Discussion

As observed for rogletimide, *N*-oxidation of **1–3** results in a nearly complete loss of inhibitory potencies of the compounds toward aromatase. There are two possible explanations of this result.

First, the molecules cannot complex the heme iron of the cytochrome P 450 enzyme aromatase per se. At the first glance this is in contrast to the observation of Mashiko *et al* [5], who have demonstrated complexation of pyridine-*N*-oxide with synthetic porphyrins. These studies, however, were performed in THF. In case of the cytochrome P 450 enzyme aromatase, the nonsteroidal inhibitors are supposed to displace water

Table I. Inhibition of human, placental aromatase by compounds **1–6**.

Compound	*Structure*	*R*	*IC_{50} (μM)*[a]	*RP*[b]
1		4-Pyridyl	6.5	2.8
4		4-Pyridyl-*N*-oxide	246	0.075
2		4-Pyridyl	3.95	4.7
5		4-Pyridyl-*N*-oxide	–[c]	–[c]
3		4-Pyridyl	0.062	300
6		4-Pyridyl-*N*-oxide	44.0	0.42

[a] IC_{50} is the concentration of inhibitor required to give 50% inhibition. Concentration of testosterone used: 2.5 μM. [b] Relative potency, calculated from the IC_{50} values and related to AG ($IC_{50}AG$ = 37 μM). [c] No inhibition at an inhibitor concentration ≤ 250 μM.

ligands from the heme iron before complexation [14]. Interestingly, it is known that several *N*-oxides cannot replace water from the primary coordination sphere of transition metal ions [15].

Second, the molecules do not fit into the active site of the enzyme. *N*-Oxidation of **1–3** causes an elongation of the compounds by the N-O bondlength. This possibly prevents an optimal fitting of **4–6** into the active site of the enzyme.

Experimental protocols

Chemistry

Chemical analysis was carried out using the following instruments: melting point (uncorrected): Kofler melting point apparatus Thermopan (Reichert); IR spectra: Perkin-Elmer 398; ^{1}H-NMR spectra ($CDCl_3$ and DMSO; TMS internal standard): Bruker AW-80, Bruker AW-400; EI-MS: MAT 90 (Finnigan), MAT 311 (Varian).

*E-7-Methoxy-2-(4-pyridylmethylene)-1-tetralone **1***

4-Pyridinecarbaldehyde (4 g, 37.34 mmol) and 7-methoxy-1-tetralone (4 g, 22.72 mmol) were stirred in 40 ml of 54% sulfuric acid for 3 h at 80°C. After cooling the resulting precipitate was suspended in water and treated with 10% NaOH solution until the mixture showed an alkaline reaction. The solid was filtered off and recrystallized from ethyl acetate to yield 5.4 g (90%) of **1** (mp 134°C (lit [9] 134.5–135.5°C)).

^{1}H-NMR (80 MHz, $CDCl_3$), δ (ppm): 2.76–3.26 (m, 4H, $-CH_2-$); 3.88 (s, 3H, $-OCH_3$); 7.01–7.22 (m, 2H, arom H-5/6); 7.31 (d, 2H, AA'XX', pyridyl H, $^3J \approx 6$ Hz); 7.66 (d, 1H, arom H-8, $^4J = 3$ Hz); 7,75 (s, 1H, =CH-); 8.72 (d, 2H, AA'XX', pyridyl H, $^3J \approx 6$ Hz). IR (KBr): 3060 cm^{-1} (Ar-H); 2950 (CH); 2840 (OCH_3); 1680 (C=O). Anal $C_{17}H_{15}NO_2$ (C, H, N).

*General procedure for the synthesis of compounds **4** and **5***

The corresponding pyridine compound (7.54 mmol) was dissolved in 20 ml acetic acid. Hydrogen peroxide urea adduct (1.3 g, 13 mmol, 35%) was added and the mixture was stirred for 5 h at 70°C.

*E-7-Methoxy-2-(4-pyridylmethylene-N-oxide)-1-tetralone **4**.*

The preparation started from 2 g of **1**. The mixture was diluted with water and the product was precipitated by alkalizing with 10% NaOH solution. Purification by column chromatography (SiO_2; ethyl acetate/ethanol, 8:2) yielded 1.48 g (70%) of **4** (mp 174–175°C).

^{1}H-H-NMR (400 MHz, $CDCl_3$) δ (ppm): 2.94 (t, 2H, Ar-CH_2CH_2-, $^3J = 6$ Hz); 3.10 (t, 2H, Ar-CH_2CH_2-, $^3J = 6$ Hz); 3.87 (s, 3H, $-OCH_3$); 7.10 (dd, 1H, arom H-6, $^3J = 8.4$ Hz, $^4J = 2.8$ Hz); 7.20 (d, 1H, arom H-5, $^3J = 8.4$ Hz); 7.34 (d, 2H, AA'XX', pyridyl H, $^3J = 7.0$ Hz); 7.60 (d, 1H, arom H-8, $^4J = 2.8$ Hz); 7.66 (s, 1H, =CH-); 8.22 (d, 2H, AA'XX', pyridyl H, $^3J = 7$ Hz). IR (KBr): 3100 cm^{-1} (Ar-H); 2950 (CH); 2840 (OCH_3); 1660 (C=O); 1300 (arom *N*-oxide); 830 (arom *N*-oxide). MS (70 eV): *m/e* = 281 ($M^{+\bullet}$). Anal $C_{17}H_{15}NO_3$ (C, H, N).

*E-7-Methoxy-2-(4-pyridylmethyl-N-oxide)-1-tetralone **5**.*

The preparation started from 2 g of **2** [9]. After neutralization with Na_2CO_3 the precipitate was stirred with dichloromethane. The organic phase was washed with water and dried over Na_2SO_4. The crude product was purified by column chromatography on silica-gel (SiO_2; ethyl acetate/ethanol, 8:2) to give 1.7 g (74%) of **5** (mp 141°C).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.79–1.86 and 2.09–2.15 (2 m, 1H each Ar-CH_2CH_2-); 2.73–2.96 (m, 4H, Ar-CH_2- + -CH_2-py); 3.30–3.35 (m, 1H, -CH<); 3.83 (s, 3H, $-OCH_3$); 7.07 (dd, 1H, arom H-6, $^3J = 8.4$ Hz, $^4J = 2.8$ Hz); 7.12–7.19 (m, 3H, arom H-5 + AA'XX', pyridyl H); 7.50 (d, 1H, arom H-8, $^4J = 2.8$ Hz); 8.14 (d, 2H,AA'XX', pyridyl H, $^3J = 6.9$ Hz). IR (KBr): 3050 cm^{-1} (Ar-H); 2920 (CH); 2840 (OCH_3); 1680 (C=O); 1250 (arom *N*-oxide); 830 (arom *N*-oxide). MS (70 eV): *m/e* = 283 ($M^{+\bullet}$). Anal $C_{17}H_{17}NO_3 \cdot 0.53\ C_2H_5OH$ (C, H, N). Various methods were tried to remove the ethanol in **5** and **6**, but did not succeed.

*exo-6-Methoxy-1-(4-pyridyl-N-oxide)-1a,2,3,7b-tetrahydro-1H-cyclopropa[a]naphthalene **6***

Compound **3** [10] (5.2 g, 20 mmol) was dissolved in 5.4 ml of methanol. Benzonitrile (2.1 g, 20 mmol) and hydrogen peroxide urea adduct (3 g, 31 mmol) were added to the solution, which was adjusted to pH 8.5 with 0.1 N NaOH. After stirring the mixture for 2 h at room temperature the methanol was evaporated and 30 ml chloroform were added. The benzamide formed, which precipitated by cooling to –20°C, was filtered off and the solvent was evaporated under reduced pressure. Purification was performed by flash chromatography (SiO_2; ethyl acetate/ethanol, 7:3). The resulting yellow oil was dried *in vacuo* over P_2O_5 at 30°C to yield 3.6 g (67%) of **6** as a deliquescent solid.

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.62–1.65 (m, 1H, Ar-CH_2CH_2-); 1.99–2.02 (m, 1H, -CH_2CH<); 2.20–2.30 (m, 3H, ar-CH_2CH_2- and Ar-CH< and >CH-py); 2.52–2.71 (m, 2H, Ar-CH_2-); 3.78 (s, 3H, $-OCH_3$); 6.69 (dd, 1H, arom H-5, $^3J =$ 7 Hz, $^4J = 2.7$ Hz); 6.81 (d, 1H, arom H-7, $^4J = 2.7$ Hz); 6.97–7.00 (m, 3H, arom H-4 + pyridyl H); 8.11 (d, 2H, AA'XX', pyridyl H, $^3J = 7$ Hz). IR (KBr): 3050 cm^{-1} (Ar-H); 2920 (CH); 2840 (OCH_3); 1280 (arom *N*-oxide). MS (70 eV): *m/e* = 267 ($M^{+\bullet}$). Anal $C_{17}H_{17}NO_2 \cdot 0.81\ C_2H_5OH$ (C, H, N).

Biology

Aromatase inhibition in vitro

The compounds were tested for their inhibitory activity against aromatase according to the procedure described previously [11]. The microsomal fraction of freshly delivered human placenta provided the source of the enzyme.

Inhibition of aromatase by compounds **4–6** was determined by measuring the ^3H_2O liberated during the conversion of [1β 2β ^{3}H] testosterone to estradiol. Separation of the steroids was achieved by adding dextran-coated charcoal (DCC). After centrifugation the radioactivity of a supernatant aliquot was measured.

The IC_{50} values were determined by testing the compounds in six appropriate concentrations and plotting the percent inhibition against the concentration of inhibitor on a semilog plot.

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