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Preparation and Pharmacological Profile of 7-(α-Azolylbenzyl)-1*H*-indoles and Indolines as New Aromatase Inhibitors

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Abstract—Aromatase (P450 arom) is a target of pharmacological interest for the treatment of breast cancer. New series of 7-(α-azolylbenzyl)-1*H*-indoles and indolines were synthesized as non-steroidal inhibitors of P450 arom. Selectivity was studied towards P450 17α enzyme. The most active compound, 1-ethyl-7-[(imidazol-1-yl)(4-chlorophenyl)methyl]-1*H*-indole **12c** exhibited promising relative potency (rp) of 336 (rp of aminoglutethimide = 1) and most of the described azoles were active and selective towards P450 arom.

Introduction

Aromatase (P450 arom) is a microsomal enzyme complex consisting of a cytochrome P450 hemoprotein and a NADPH cytochrome reductase.[1] It catalyses the conversion of androgens into estrogens by aromatization of the steroid A ring. Inhibitors of this enzyme are potential therapeutics for the treatment of estrogen dependent diseases, such as breast cancer.[2] Among the non-steroidal compounds, azoles appeared to be very potent and some of them have already been marketed (fadrozole,[3] anastrozole,[3] letrozole[4]) or are in phase III clinical trial (vorozole[5]) (Fig. 1).

During our investigation towards the synthesis of new non-steroidal aromatase inhibitors, we synthesized a series of 3-(azolylmethyl)-1*H*-indoles[6,7] and 2-, 3- and 5-(α-azolylbenzyl)-1*H*-indoles.[8,9] Thus, we demonstrated that most of our compounds display a high level of estrogen biosynthesis inhibitory activity. Among them, the 1-ethyl-5-[(imidazol-1-yl)(4-fluorophenyl)-methyl]-1*H*-indole **I** is the most active with IC_{50} of 0.041 μM while the IC_{50} of aminoglutethimide (AG) (Fig. 1), the first non-steroidal marketed drug, was measured at 18.5 μM. Our molecules also exhibited a good selectivity profile toward the P450 17α which converts progestins to androgens.

With the aim to check the relationships between pharmacological activity and position of the azolylbenzyl chain on the benzene part of the indole ring we have developed a new series of 7-(α-azolylbenzyl)-1*H*-indoles. Moreover, the indoline analogues were also tested for inhibitory activity towards P450 arom and P450 17α enzymes.

Chemistry

The compounds synthesized for this study were prepared by two general routes (Scheme 1). By modification of the method of Sugasawa et al.,[10] *ortho*-benzoylation of indoline **1**, with suitable benzonitriles as acylating agents in the presence of boron trichloride and aluminum trichloride, gave the desired 7-benzoylindolines **1a–d** as starting materials for both indoline and indole series. In the first series, halogenation in the 5-position of indoline ring was achieved using either *N*-bromosuccinimide or *N*-chlorosuccinimide in methylene choride.[11] *N*-alkylation was carried out with ethyl iodide in dry dimethyl

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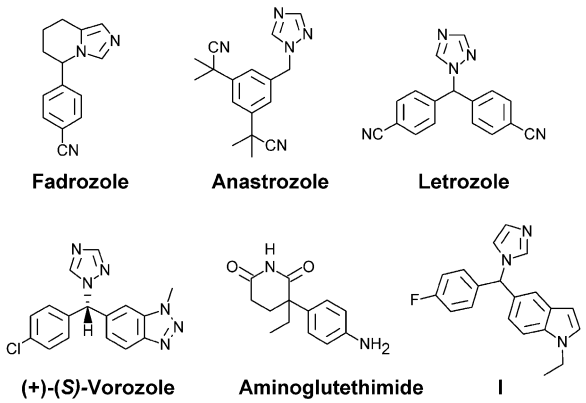


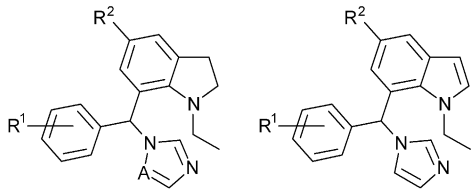
Figure 1. Non-steroidal aromatase inhibitors.

sulfoxide in the presence of sodium hydride. The reduction of the ketones was performed with sodium borohydride in methanol and the crude alcohols, so obtained, were treated with 1,1′-carbonyldiimidazole (CDI)[12] in tetrahydrofuran to afford imidazole derivatives **5a–b**, **6a** and **6′a**. A triazole analogue **7a** was prepared by reaction of the corresponding carbinol with 1,1′-sulfinylditriazole (SDT)[13] in acetonitrile. In the first step of the second series, the 7-benzoylindolines **1a–d** and **2a** were converted to the indole analogues **8a–d** and **9a** by dehydrogenation with activated manganese dioxide[11] in high yields. As described before, subsequent *N*-alkylation, reduction and fixation of the imidazole moiety led to the corresponding 7-(α-imidazolylbenzyl)-1*H*-indoles **12a–d** and **13a**.

Results and Discussion

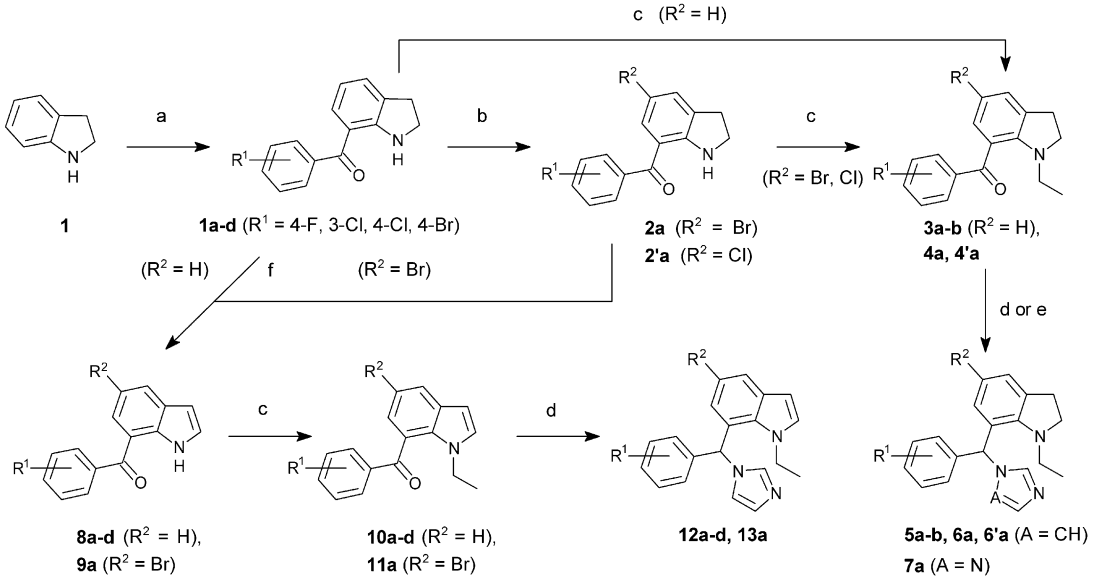
The method of Thompson and Siiteri,[14] that is human placental microsomes and [1β,2β-^{3}H]testosterone, was used for the determination of P450 arom inhibitory activities. Table 1 shows the IC_{50} values and the inhibitory potencies of the compounds relative to AG (rp values). The assay of P450 17α inhibition was performed according to the literature procedure[15] using rat testicular microsomes as source of the enzyme and non-labeled progesterone as substrate.

Table 1. In vitro activity of 7-(α-azolylbenzyl)-1*H*-indoles and indolines



Compd	R^1	R^2	P450 arom		P450 17α
			IC_{50}, μM[a]	rp[c]	% Inhibition[e]
5a	4-F	H	0.0615	300.8	37
5b	3-Cl	H	0.11	164	38
6a	4-F	Br	0.09	205.6	8
6′a	4-F	Cl	0.063	293.6	23
7a	4-F	H	2.90[b]	10[d]	ND[f]
12a	4-F	H	0.062	298.4	37
12b	3-Cl	H	0.17	108.8	43
12c	4-Cl	H	0.055	336	29
12d	4-Br	H	0.070	264	29
13a	4-F	Br	0.20	92.5	9

[a]IC_{50} is the concentration of inhibitor required to give 50% inhibition. Concentration of testosterone: 2.5 μM. The given values are mean values of at least three experiments. The deviations were within ±5%.
[b]Concentration of androstenedione: 0.5 μM.
[c]Relative potency, calculated from IC_{50} values and related to AG (IC_{50} of AG: 18.5 μM).
[d]IC_{50} of AG: 29.5 μM.
[e]Concentration of progesterone: 25 μM. Concentration of inhibitor: 2.5 μM. Under identical experimental conditions ketoconazole caused an inhibition of 70%. All values are the mean of at least two determinations.
[f]Not determined.



Scheme 1. Reaction conditions and yields: (a) R^1-benzonitrile, $BCl_3/AlCl_3$, toluene, reflux, 61–73%; (b) NBS or NCS, CH_2Cl_2, rt, 69–75%; (c) NaH, EtI, DMF, rt, 52–91%; (d) (1) $NaBH_4$, MeOH, reflux; (2) CDI, THF, reflux, 20–70%; (e) (1) $NaBH_4$, MeOH, reflux; (2) SDT, CH_3CN, reflux, 17%; (f) MnO_2, CH_2Cl_2, reflux, 86–96%.

Considering the aromatase inhibitors carrying the imidazolyl substituent, it is evident that the oxidation of the indoline to indole had no pharmacological effect and both compounds **5a**, **12a** and **5b**, **12b** exhibited the same range of activity (Table 1). The most active compound was the 1-ethyl-7-[(imidazol-1-yl)(4-chlorophenyl) methyl]-1*H*-indole **12c** with IC_{50}: 0.055 μM and rp: 336 but all compounds bearing halogen atom in the 4-position of phenyl nucleus also remained potent aromatase inhibitors, except **13a**. Moreover, the nature of the halogen did not appear to be an essential requirement for inhibitory activity as showed the close and high rp values of **12a** (R^1 = 4-F, rp: 298.4), **12c** (R^1 = 4-Cl, rp: 336) and **12d** (R^1 = Br, rp: 264).

On the one hand, in the indole series, the introduction of a bromine into the 5-position led to markedly decrease the P450 arom inhibition (compound **13a**, rp: 92.5). On the other hand, in the indoline series, the replacement of an hydrogen by a bromine (**6a**, rp: 205.6) had a negative effect on inhibitory aromatase activity and the presence of a chlorine (**6′a**, rp: 293.6) influenced only moderately this activity by comparison with **5a** showing a relative potency of 300.8.

For the triazole compound **7a**, the aromatase assay was performed according to described methods,[16,17] monitoring enzyme activity by measuring ^3H_2O formed from [1β-^{3}H]androstenedione during aromatization. The replacement of the imidazolyl group of compound **5a** by a triazolyl substituent (compound **7a**) strongly decreased the inhibition of P450 arom.

Percent inhibition values of P450 17α indicate that all compounds do not significantly inhibit the enzyme or show only marginal inhibitory activity at 2.5 μM.

Conclusion

We have discovered new 7-(α-azolylbenzyl)-1*H*-indoles and indolines as potent and selective aromatase inhibitors but less active than an analogue in the 5-position previously synthesized and tested by us[9] (compound **I**, rp: 451.2).

The next step of the work will be the separation of the most active racemic compounds **5a**, **6′a**, **12a** and **12c**, by chiral HPLC, so as to enhance the P450 arom inhibitory activity of our imidazoles by testing enantiomerically pure compounds as vorozole, for instance.

The triazole analogue **7a** was prepared with the aim of exploring the effects of the variation of the aza-heterocycle. It has been reported that the replacement of the imidazole moiety with a triazole one can lead to higher in vivo activity.[18]

Further experiments on the in vivo inhibitory activity and on the selectivity towards other steroidogenic P450 enzymes such as P450 SCC will be performed.

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