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1-[(Benzofuran-2-yl)phenylmethyl]-Triazoles and -Tetrazoles - Potent Competitive Inhibitors of Aromatase

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Abstract: The synthesis of a series of novel 1-[(benzofuran-2-yl)phenylmethyl]-triazoles and -tetrazoles is described. The compounds were tested for human placental aromatase inhibition *in vitro*, using [1β-^{3}H]-androstenedione as the substrate for the aromatase enzyme, the percentage inhibition and IC_{50} data is included.
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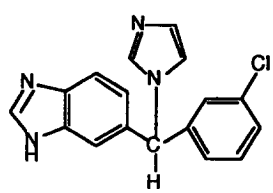
The enzymes involved in the biosynthesis of steroidal hormones are proving to be major targets for therapeutic intervention. One of these enzymes is aromatase (P450Arom), an enzyme complex consisting of a cytochrome P450 haemoprotein and an NADPH-cytochrome P450 reductase, which catalyses the final step in the steroidogenic pathway for the synthesis of oestrogens from cholesterol.

Aromatase inhibitors have been shown to be useful in the second-line therapy of oestrogen-dependent breast cancer in postmenopausal women.[1] Non-steroidal P450Arom inhibitors of interest include Liarazole® (**1**), an azolyl-substituted benzimidazole,[2] and the 1-[(benzofuran-2-yl)phenylmethyl]-imidazoles[3] (**2**) which are active at IC_{50} < 10nM. Our interest was in synthesizing compounds with a similar general structure however using triazole (**3**) and tetrazole (**4**) substituted 1-(benzofuran-2-yl)phenylmethyl compounds (Fig.1) which enabled an *in vitro* comparison between the relative potencies of the triazoles (**3**) and tetrazoles (**4**) with the known imidazoles (**2**).

Figure 1



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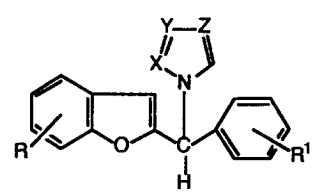

2, X = Z = CH, Y = N
3, X = Z = N, Y = CH
4, X = Y = Z = N

All the ketones **8a-f** were prepared by a modification of the procedure described by Pestillini *et al.*[4] and involved reaction of salicyclaldehydes **6** (R^1 = H, NO_2) with the α-bromoketones **7** (R^2 = H, F, Cl, Me; R^3 = H, Cl). Reduction of the ketones with sodium borohydride gave the secondary alcohols **9a-f** in quantitative yields, which were pure enough after an aqueous extraction to be used in the following reactions. The alcohols were

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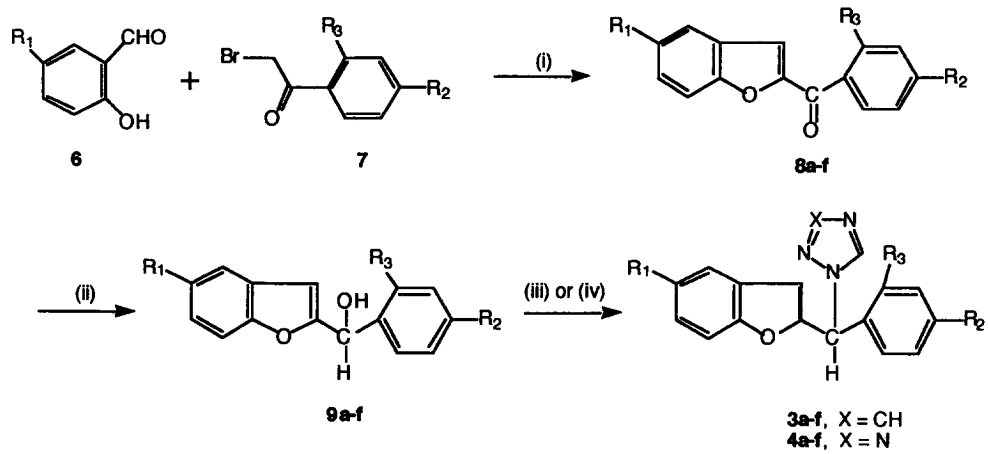
treated with either *N*, *N'*-thiionylditriazole [(triazole)$_2$S=O] or *N*, *N'*-thionylditetrazole [(tetrazole)$_2$S=O], prepared by reaction of either triazole or tetrazole with one equivalent of thionyl chloride at -10°C in acetonitrile for one hour, in the presence of anhydrous potassium carbonate at room temperature for four days. The triazoles **3a-f** and tetrazoles **4a-f** were obtained after purification by column chromatography in moderate to good yields, however in all cases it was possible to recover the unreacted starting material.



Scheme 1. *Reagents and conditions* : i. NaH, DMF, 70°C, 1.5hr then NaOMe added, 70°C, 1.5h; ii. $NaBH_4$, dioxane, r.t., 2h; iii. triazole, $SOCl_2$, CH_3CN, K_2CO_3, r.t., 4 days; iv. tetrazole, $SOCl_2$, CH_3CN, K_2CO_3, r.t., 4 days.
a, $R^1 = R^2 = R^3 = H$; **b**, $R^1 = NO_2$, $R^2 = R^3 = H$; **c**, $R^1 = R^3 = H$, $R^2 = Cl$; **d**, $R^1 = R^3 = H$, $R^2 = F$; **e**, $R^1 = R^3 = H$, $R^2 = Me$; **f**, $R^1 = H$, $R^2 = R^3 = Cl$.

The compounds were tested for aromatase inhibitory activity according to the literature procedure.[5] The microsomal fraction of freshly delivered human term placenta provided the source of the aromatase enzyme, and aromatase activity was determined by the measurement of ^3H_2O released from [1β-^{3}H]androstenedione. Separation of ^3H_2O was achieved by adding charcoal and after centrifugation, the activity of the supernatant sample was counted with a liquid scintillation counter.

The results are expressed as percentage inhibition of the enzyme compared with a control value determined in the absence of inhibitor. To determine IC_{50} values, compounds were tested in six appropriate concentrations with each experiment performed in duplicate, the IC_{50} value shown (Table 1) is an average of these two values. The percent inhibition was plotted versus the logarithm of concentration, from this the molar concentration causing 50% inhibition was determined using Cricket Graph®. The relative potencies of the triazoles and tetrazoles were determined by comparison with the well known aromatase inhibitor aminoglutethimide (AG).[6]

In all cases the triazoles exhibited greater inhibitory activity than the corresponding tetrazole analogues. Substitution in the phenyl ring (*i.e.* substitution at R^2 or R^3), in particular substitution at R^2, resulted in improved activity compared with the parent compound (**a**, $R^1 = R^2 = R^3 = H$), substitution in the benzofuran ring (*i.e.* substitution at R^1) had an adverse effect on *in vitro* activity.

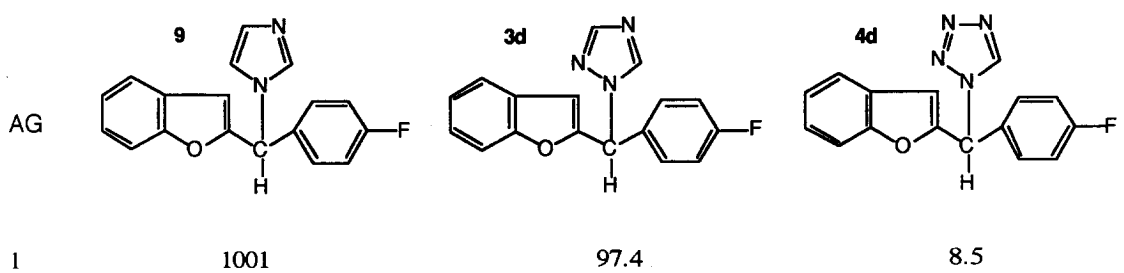
Table 1. Inhibition of human placental aromatase by 1-[(benzofuran-2-yl)phenylmethyl]triazoles (**3a-f**) and tetrazoles (**4a-f**).

Compound	R^1	R^2	R^3	% Inhibition[a]	IC_{50} (μM)	Relative potency[b]
3a	H	H	H	85.7	-	-
3b	NO_2	H	H	58.4	-	-
3c	H	Cl	H	98.6	0.20	92.5
3d	H	F	H	98.8	0.19	97.4
3e	H	Me	H	96.8	0.59	31.4
3f	H	Cl	Cl	97.7	0.88	21.0
4a	H	H	H	35.0	-	-
4b	NO_2	H	H	14.4	-	-
4c	H	Cl	H	90.3	3.0	6.2
4d	H	F	H	92.6	2.17	8.5
4e	H	Me	H	67.2	-	-
4f	H	Cl	Cl	82.3	52.02	0.34
AG				32.5	18.5	1

[a]Concentration of androstenedione, 0.6μM, concentration of inhibitor, 20μM, values are the average of two experiments.
[b]Relative potency, calculated from the IC_{50} values and related to aminoglutethimide (IC_{50} 18.5μM).

The relative potencies of the 4'-fluoro-substituted imidazole-, triazole- and tetrazole-derivatives, the most active in each series, were compared relative to aminoglutethimide (Fig.2). As can be seen from Fig.2, *in vitro* the imidazole substituted benzofurans are the most active. On exchanging the imidazole heterocycle with a triazole or tetrazole heterocycle, a 10 fold and 100 fold reduction is observed respectively, with the tetrazoles exhibiting comparable activity with AG itself. The imidazole interacts through a N: → Fe^{3+} - Haem coordinate link, *via* the N-3. Introduction of an additional nitrogen in the heterocyclic ring reduces the coordination potential *via* the N: as a result of the electron withdrawing effect of the additional electronegative nitrogen, this effect is enhanced with two additional nitrogens pulling electron density from either side of the coordinating nitrogen.

Figure 2 Relative Potencies of **9**, **3d**, **4d** compared with Aminoglutethimide (AG)



It is probable that the relationship between relative activity and co-ordination potential observed in this particular series of aromatase inhibitors, would also apply to other competitive inhibitors of aromatase which contain a nitrogen heterocyclic moiety. However, it should be noted that there are several clinically used compounds which containing a triazole moiety which are extremely potent inhibitors of aromatase, *e.g.* vorazole,

letrozole and anastrozole, which would indicate that it is not solely the nitrogen containing heterocycle which determines activity but the overall binding capability and tightness of fit of the entire structure in the P450 aromatase active site.

Experimental Procedures

General method for the preparation of the alcohols 9a-f: To a suspension of the ketone (**8a-f**, 3mmol) in dry dioxane (7ml) was added sodium borohydride (3mmol) and the reaction stirred at room temperature under nitrogen for 2 hours. The reaction was concentrated under reduced pressure and 2M aqueous hydrochloric acid (approx. 10ml) added to the resulting syrup. This solution was extracted into diethyl ether (100ml), washed with water (2 x 25ml), dried ($MgSO_4$) and concentrated under reduced pressure to give the required product. T.l.c. system : petroleum ether-ethyl acetate 3:1 v/v.

General method for the preparation of the triazoles (3a-f) and tetrazoles (4a-f): To a cooled (10°C) suspension of either 1,2,4-triazole or 1*H*-tetrazole (6mmol) in dry acetonitrile (4ml) was added a solution of thionyl chloride (1.5mmol) in dry acetonitrile (2ml) and the reaction stirred at 10°C for one hour. Activated potassium carbonate (1.5mmol) was then added, followed by a solution of the alcohol (**8a-f**, 1.5mmol) in dry acetonitrile (4ml). The reaction was stirred under nitrogen at room temperature for four days then the reaction mixture was filtered to remove solid residues. The filtrate was concentrated under reduced pressure then redissolved with dichloromethane (100ml) and this solution washed with water (2 x 50ml), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petroleum ether-ethyl acetate 80:20 v/v increasing to 60:40 v/v). T.l.c. system : petroleum ether-ethyl acetate 1:1 v/v.

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