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4'-SUBSTITUTED ANALOGUES OF IDOXIFENE: ANTIESTROGENS AND CALMODULIN ANTAGONISTS

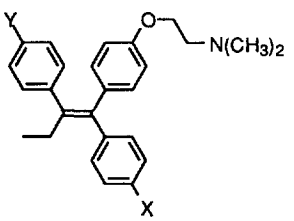
Ian R. Hardcastle*, Martin G. Rowlands, John Houghton and Michael Jarman

CRC Laboratories, CRC Centre for Cancer Therapeutics at the Institute of Cancer Research, Cotswold Road, Sutton, Surrey, SM2 5NG. UK

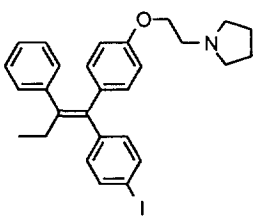
Abstract. 4'-substituted analogues of the antiestrogen idoxifene have been prepared. All the compounds were assayed for antagonism of calmodulin dependent c-AMP phosphodiesterase and for binding to rat uterine estrogen receptor. The 4'-amino compound was the most potent antiestrogen (RBA = 47) whilst retaining a similar calmodulin antagonism to idoxifene.

The widespread use of non-steroidal antiestrogens such as tamoxifen **1** for the treatment of breast cancer[1,2] has prompted much research into the development of more potent compounds which may overcome the problems of acquired resistance from which **1** suffers. In addition to displacing the growth promoting hormone estrogen from the protein receptor[3], **1** displays a number of hormone independent effects[4-7] including the antagonism of the calcium-binding protein calmodulin[8,9]. Idoxifene **2**, developed in our laboratories[10,11] and now in clinical trials[12], is a more potent antiestrogen and displays greater antagonism of calmodulin than **1**.

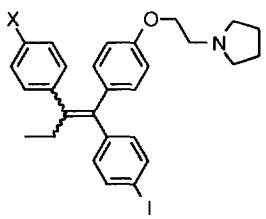
The 4'-position of tamoxifen **1** has not been extensively elaborated. Ogawa and co-workers prepared TAT-59 **3**, which has a 4'-isopropyl substituent, and its metabolites[13]. 4'-Amino tamoxifen **4a** and 4'-iodotamoxifen **4b** have been prepared by Strickland and co-workers[14] for use in radiochemical imaging. Preclinical studies[15,16] on **2** identified the 4'-hydroxylated derivative **5a** as a major metabolite in rat hepatocytes and an authentic sample was required for comparison and biological evaluation. The need for an authentic sample of 4-hydroxyidoxifene **5**, and the possibility of discovering more potent antiestrogens, led us to explore the synthesis of some 4'-substituted derivatives of idoxifene **2**. Thus, we have prepared several 4'-substituted analogues of idoxifene **2** and now report that the 4'-amino compound **6** is a potent antiestrogen and displays similar calmodulin antagonism to **2**.



Tamoxifen 1 X, Y = H
3 X = OPO_3H, Y = *i*-Pr
4a X = H, Y = NH_2
4b X = H, Y = I



Idoxifene 2

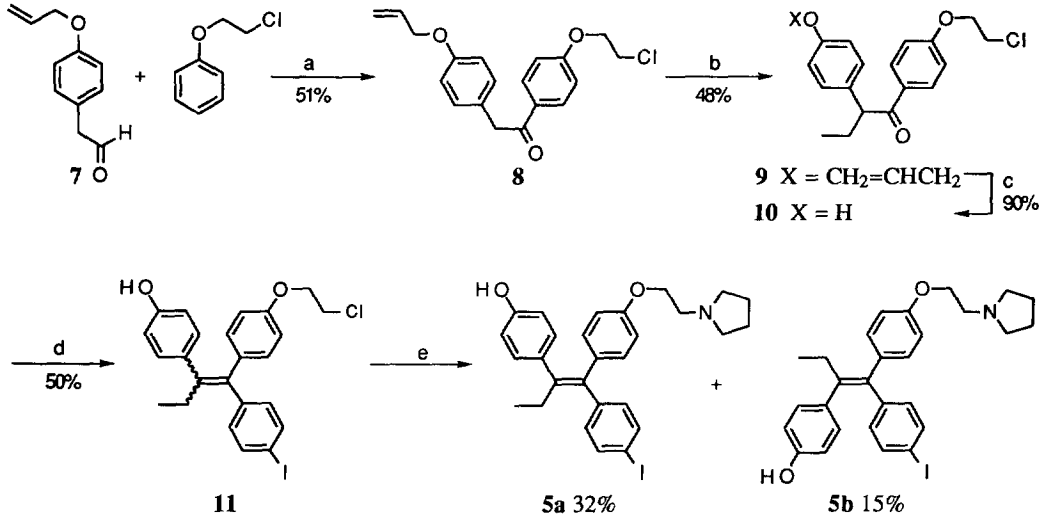


5a X = OH (*trans*)
5b X = OH (*cis*)
6 X = NH_2 (*trans*)
12 X = I (*trans*)

The 4'-hydroxy metabolites **5a+b** were prepared according to Scheme 1. 2-[4-(Prop-2-enoxy)phenyl]ethanoic acid **7** was prepared and reacted with 2-chloroethoxyphenyl ether to give the ketone **8** (51% yield). Alkylation of **8** gave the mono-ethyl compound **9** (48% yield). Deprotection was accomplished by stirring **9** with palladium on charcoal, *p*-toluenesulphonic acid, and methanol to give **10** in 90% yield. Reaction of the

resulting phenol **10** with an excess of the lithio reagent followed by dehydration afforded **11** as a mixture of geometric isomers (50% yield). Treatment of **11** with pyrrolidine gave the desired 4'-hydroxy compound **5a** as well as its isomer **5b** which were separable by chromatography.

Scheme 1



a) TFAA; b) NaH, EtI, THF; c) TsOH, Pd/C, MeOH; d) i) IC_6H_4Li, THF, -78 °C, ii) EtOH, HCl; e) pyrrolidine, EtOH.

Strickland[14] prepared 4'-iodotamoxifen **4b** from the diazo-salt of 4'-aminotamoxifen **4a**. Both these compounds had estrogen receptor binding affinities greater than that of tamoxifen. We considered that a route similar to that used for these compounds would provide us with the 4'-amino and 4'-iodo derivatives **6** and **12** (Scheme 2). Thus, 4-nitrophenylethanoic acid reacted with chloroethoxyphenyl ether to give the ketone **13** (48% yield). Alkylation (EtI, NaH, THF) afforded the mono-ethyl compound **14** in good yield. The pyrrolidino group was introduced at this stage in excellent yield to give **15**. Reduction of the nitro-group with hydrogen and palladium-charcoal afforded **16** in excellent yield. Protection of the amino-function as the sodium-salt of the corresponding trifluoroacetamide for use in the Grignard reaction as reported proved ineffective so we decided to employ the bis(dimethylsilyl)ethyl group[17]. Thus, treatment of **16** with bis(chlorodimethylsilyl)ethane gave the bis-protected compound **17**. Reaction of **17** with 4-iodophenyl-lithium followed by dehydration and concomitant deprotection gave the 4-iodo-compound **6** as a mixture of isomers. Separation of the isomers was achieved by chromatography to give the pure *E*-isomer **6** (45% yield). The diazonium salt **18** was prepared under standard conditions and isolated as the hexafluorophosphate. Addition of **18** to a refluxing solution of sodium iodide and sodium thiosulfate in acetonitrile afforded the diiodo derivative **12** in good yield.

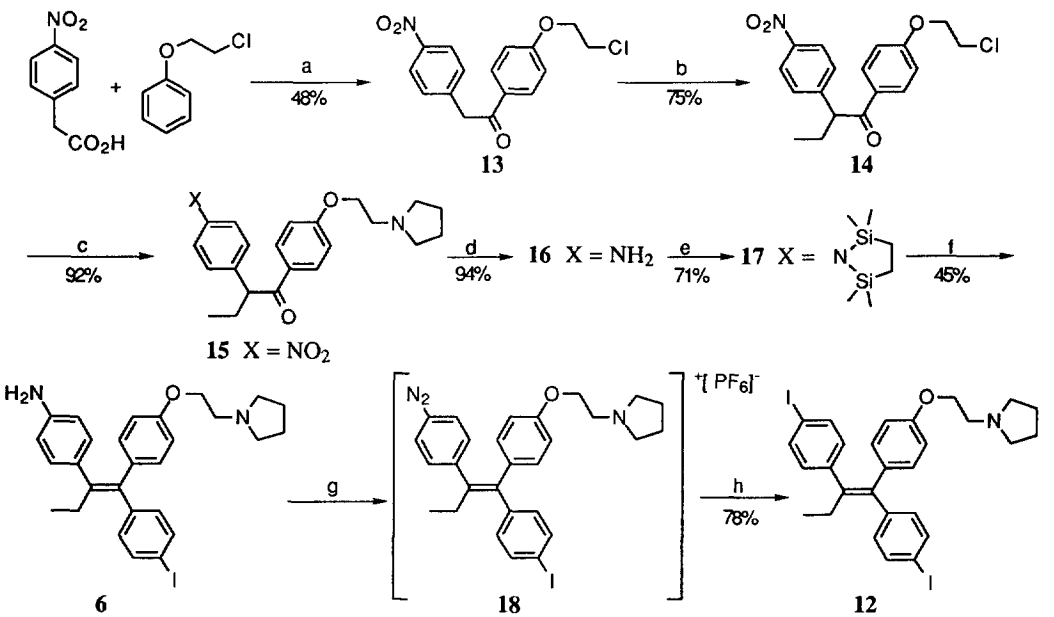
Compounds **5a**, **5b**, **6** and **12** were assayed both for antagonism of calmodulin-dependent cyclic-AMP phosphodiesterase[18] and rat uterine estrogen receptor binding affinity[19], according to the published procedures, and the results are presented in the Table. All the compounds assayed retained estrogen receptor binding, and calmodulin antagonistic properties. Interestingly, the activity of **5a** in these tests was almost identical with

those of **2**. Hence metabolism of **2** to **5a** is essentially neutral from the point of view of biological activity, although of course it may have pharmacokinetic consequences. Isomers **5a** and **5b** may be useful probes in studies of the interactions of the estrogen receptor and calmodulin in the presence of non-steroidal antiestrogens[20] as they possess similar calmodulin antagonistic properties but differing receptor affinities in structurally similar molecules. The presence of a second iodine atom at the 4'-position provided a compound **12** which was inferior to **2**. The 4'-amino compound **6** displayed a high affinity for the receptor (RBA = 47) whilst retaining calmodulin antagonistic properties similar to those of **2**. This compound may prove to be an even more potent therapeutic agent than **2**.

Table

Compound[21]	Antagonism of cAMP phosphodiesterase (IC_{50} μM±SE)[22]	Estrogen Receptor Binding Affinity (RBA)[23]
2	1.5 ± 0.1	12
5a	1.8 ± 0.2	15
5b	2.2 ± 0.2	2
6	2.6 ± 0.3	47
12	5.0 ± 0.5	7

Scheme 2



a) TFAA; b) NaH, EtI, THF; c) pyrrolidine, EtOH; d) H_2,Pd/C, EtOAc;
e) $Cl(Me)_2Si(CH_2)_2Si(Me)_2Cl$, Et_3N, toluene; f) i) LiC_6H_4I, THF, -78°C ii) EtOH, HCl;
g) $NaNO_2$, H_2SO_4, NH_4PF_6; h) NaI, $Na_2S_2O_3$, MeCN.

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(21) All new compounds gave satisfactory nmr, ms and analytical data.
(22) The IC_{50} value is the concentration at which 50% of the enzyme activity was inhibited.
(23) RBA is the binding affinity of the compound for the receptor relative to that of estradiol which is 100.

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