A Simple Route Toward New Clomiphene Metabolites

Bruno C. Vitoriano,[a] Luísa C. R. Carvalho,[a] Mónica S. Estevão,[a] Michael H. Sekera,[b] M. Manuel B. Marques*[a]

[a] REQUIMTE/CQFB, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
Fax +351(21)2948550; E-mail: mmbmarques@dq.fct.unl.pt

[b] Laboratório de Análises de Dopagem, Autoridade Antidopagem de Portugal, Av. Prof. Egas Moniz (Estádio Universitário), 1600-190 Lisboa, Portugal

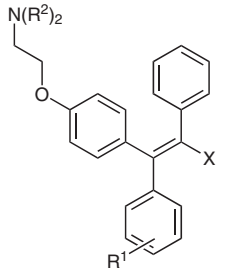
Received 25 November 2009

This work is dedicated to Professor Sundaresan Prabhakar, extraordinary teacher and mentor, for his dedication to chemistry.

Abstract: A new clomiphene metabolite, extremely useful for doping analysis, was synthesized in 19% overall yield. The approach involved a Grignard reaction via a *N*-acylbenzotriazole intermediate to afford a key aromatic ketone and a HWE reaction. Both stereoisomers were separated and identified.

Key words: clomiphene, Grignard reactions, *N*-acylbenzotriazole, olefination, drugs

The nonsteroidal anti-estrogens tamoxifen (**1**) and clomiphene (**2**, Figure 1) are synthetic triphenylethylene derivatives and have been widely used in the treatment of estrogen-dependent cancers.[1] Moreover, they have been found to act like as estrogen mimetic in prevention of bone loss in postmenopausal women.[2]



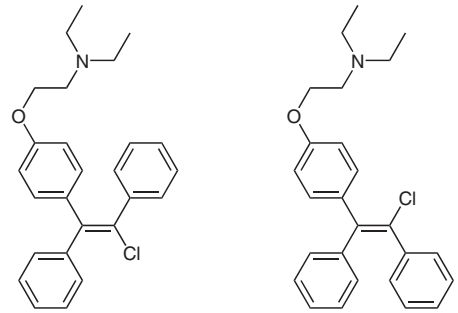
tamoxifen (**1**) R^1 = H, R^2 = Me, X = Et
clomiphene (**2**) R^1 = H, R^2 = Et, X = Cl
4-hydroxy-3-methoxytamoxifen (**3**) R^1 = 4-OH, 3-OMe, R^2 = Me, X = E
4-hydroxy-clomiphene (**4**) R^1 = 4-OH, R^2 = Et, X = Cl
4-hydroxy-3-methoxyclomiphene (**5**) R^1 = 4-OH, 3-OMe, R^2 = Et, X = C

Figure 1 Structures of tamoxifen (**1**), clomiphene (**2**) and its metabolites

Tamoxifen (**1**) is an important estrogen for the treatment,[3] and more recently, prevention of breast cancer.[4] Clomiphene citrate (CC), structurally related to diethylstilbestrol, is since the early 1960s, used in the treatment of women with absent or irregular ovulation by inducting ovulation.[5] In 1961 were reported the first results of CC on ovulation induction.[6] Clomiphene citrate is administrated as a mixture of two geometric isomers, enclomiphene (*E*) and zuclomiphene (*Z*) (Figure 2) in the ratio 62:38.[7]

SYNLETT 2010, No. 5, pp 0753–0756
Advanced online publication: 10.02.2010
DOI: 10.1055/s-0029-1219383; Art ID: G36509ST

Due to the anti-estrogenic activity, tamoxifen (**1**) and related anti-estrogens induce a decrease in side effects after abusive consumption of anabolic androgenic agents. Thus, since 2000, these compounds have been banned in male athletes by the International Olympic Committee and by the World Anti-Doping Agency. The presence of tamoxifen (**1**) and clomiphene (**2**) metabolites in urines is a proof of doping. Indeed, some metabolites have been detected and identified, such as **3** (Figure 1).[8]



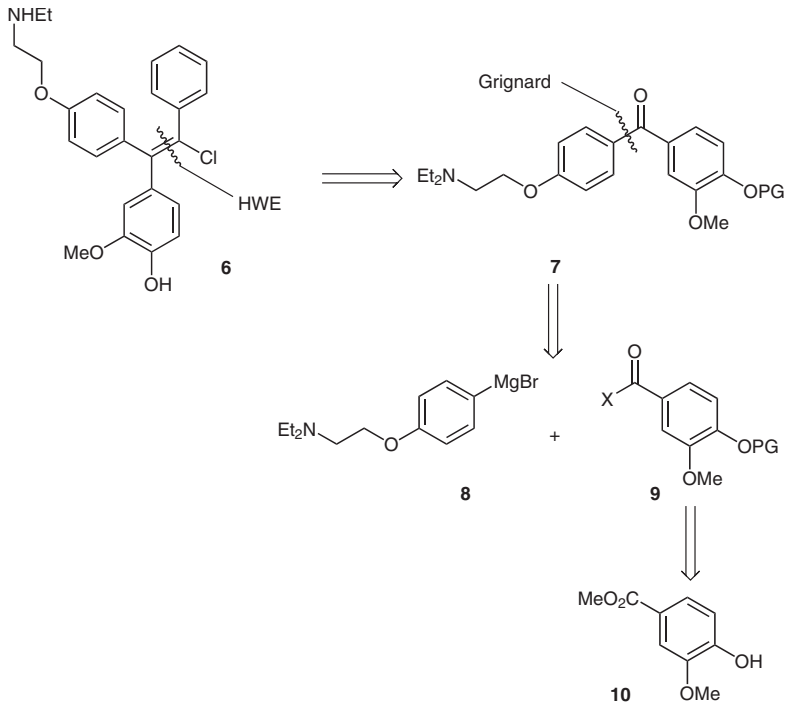
enclomiphene (*E*) zuclomiphene (*Z*)

Figure 2 The two geometric isomers of clomiphene (**2**)

In order to study the molecular action of synthetic nonsteroidal anti-estrogens protocols have been developed to address the synthesis of clomiphene (**2**)[9] and its metabolites.[9c,10] Concerning the preparation of clomiphene metabolites, some general synthetic approaches to establish the carbon skeleton have been reported, such as: the use of organometallic reagents, in particular Grignard reagents; and the McMurry coupling, also applied to tamoxifen (**1**) synthesis,[11] which has the disadvantage of generating a mixture of the hetero-ketone coupling and the undesired homo-ketone coupling.

Recently, analysis of human urine, taken after clomiphene (**1**) administration, revealed the presence of a metabolite with *m/z* = 424, compatible with the structure of **6a/6b**.[12] However, no authentic sample was available and structural confirmation could not be achieved.

The therapeutic application of the anti-estrogens as well as the interest in the identification of novel metabolites for detection in doping analysis has stimulated us to synthesize novel clomiphene metabolites. Thus, we now report a

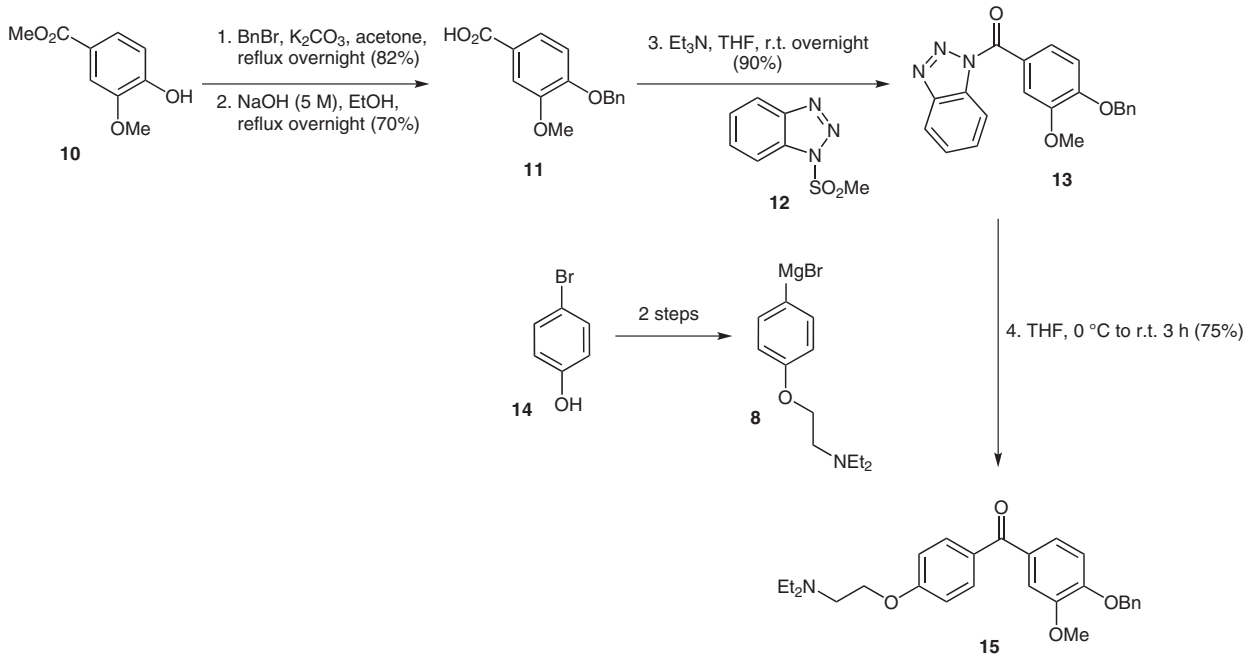


Scheme 1 Retrosynthetic analysis of clomiphene metabolite **6**

modified and alternative synthetic approach toward the triphenylethylene moiety.

Our strategy for the synthesis of the novel clomiphene metabolite **6** is outlined in Scheme 1. The preparation of tetrasubstituted olefins[13] from ketones has been addressed by the Horner–Wadsworth–Emmons (HWE) approach,[14] however, due to ketone lower reactivity and steric bulkiness this route has been barely applied. According to Kumara Swamy et al.[15] cyclic phosphonates show enhanced reactivity when compared to acyclic ones,[16] and the authors have used this phosphonates to prepare trisubstituted vinyl chlorides, via a HWE reaction.

A similar strategy was followed to prepare the vinyl chloride **6**. The use of easily accessible and stable *N*-acylbenzotriazoles, as acylating agents,[17] has been successfully applied for the preparation of aromatic ketones via organometallic reactions, such as the Grignard reaction.[18] In order to avoid the difficulties associated with the use of a carboxylic acid or acid chloride, we applied a Grignard approach reacting a *N*-acylbenzotriazole derivative **9** with



Scheme 2 Preparation of the ketone **15** via a Grignard reaction with a *N*-acylbenzotriazole intermediate

the known Grignard reagent **8**[19] to obtain the ketone **7** via a mild, simple, and effective procedure. Thus, our approach started with protection of the commercial methyl vanillate **10** with the benzyl group, followed by hydrolysis to afford the acid **11**[20] in 57% yield (2 steps, Scheme 2).

First attempts to obtain ketone **15** via Grignard reaction using the corresponding acid chloride of **11** failed. In order to overcome the problems associated with the use of the acid chloride, the *N*-acylbenzotriazole derivative **13** was prepared in 90% yield, by treatment of the carboxylic acid **11** with the *N*-(1-methanesulfonyl)benzotriazole (**12**, readily prepared from benzotriazole and methanesulfonyl chloride).[21] The next step consisted on the Grignard reaction of *N*-acylbenzotriazole **13** with the known magnesium reagent **8** [readily prepared from 4-bromophenol (**14**) in 2 steps].[19] The reaction proceeded under very mild conditions and ketone **15** was isolated in 75% yield, after chromatographic purification, and the benzotriazole could be recovered.

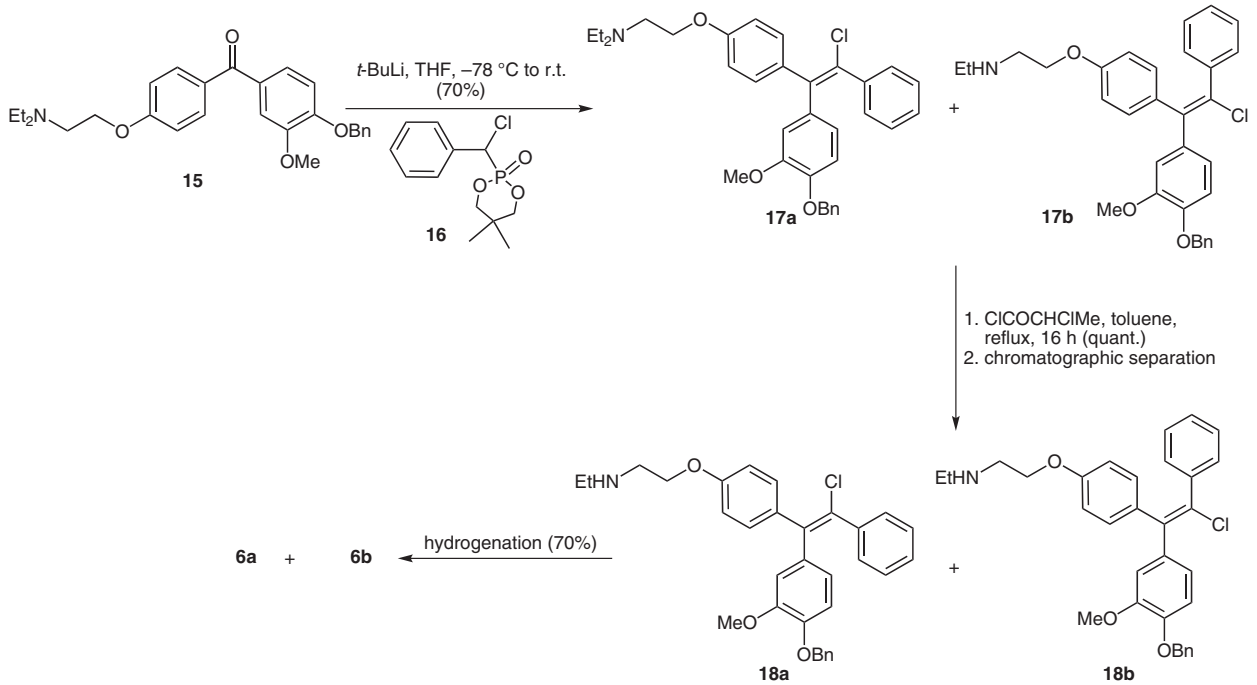
In order to avoid a stepwise installation of the trisubstituted vinyl chloride moiety (formation of the double bond followed by chlorination), the use of a proper α-chlorophosphonate was required, allowing formation of the double bond and insertion of a chlorine atom simultaneously. Thus, having in hand useful quantities of ketone **15**, the assembly of the olefinic system was next undertaken via a HWE reaction using the chlorophosphonate **16** (prepared in 2 steps from the commercially available 5,5-dimethyl-1,3,2-dioxaphosphorinan-2-one)[15,22] (Scheme 3). Compound **17** was obtained as a mixture of two isomers **17a** and **17b** (*E*/*Z*, 60:40 by ^{1}H NMR) in 70% yield. Other protocols previously described for clomiphene (**2**) synthesis, involving the use of acyclic chlorophosphonates,[9a] were also tested. However, these conditions proved to be insufficient when applied to the ketone **15**. Subsequent mono-N-dealkylation of isomers **17a/17b** using the procedure described by Olofson et al.[23a] afforded the corresponding secondary amines **18a/18b** as a mixture of both *E*/*Z* isomers that were next separated by preparative thin-layer chromatography.

The assignment of the mixture **17a/17b** as isomers *E* and *Z* was done by NMR. This assignment was confirmed by NMR analysis (COSY, NOESY, HMQC, HMBC) of the compounds **18a** and **18b**, after chromatographic separation. The ^{1}H NMR spectrum of the major isomer (*E*)-**18a** shows the H1 and H2 resonances shifted to upfield (δ = 6.44 and 6.41 ppm, respectively), while in the minor isomer (*Z*)-**18b** H1 and H2 proton resonances appear at δ = 6.93 and 6.61 ppm, respectively. This observation suggests that the ring current effect of ring A moves H1 and H2 proton resonances to higher frequency in the *E*-isomer (Figure 3).

Moreover, the NOE experiment of **18a** shows a correlation between H1 and H2 from ring B with the aromatic protons from ring A. This correlation is not observed in the NOE spectra of **18b** (Figure 3).

Finally, removal of the benzyl group by hydrogenation (H_2, 10% Pd/C, AcOH)[10a] afforded the desired metabolite **6** in 70% yield.

The LC-MS analysis of **6** gave a peak at *m/z* = 424 with retention time of 7.50 min. Subsequently a urine sample of clomiphene administration was analyzed under the same chromatographic conditions. Using multiple reaction monitoring, a peak was observed at retention time of 7.50 min with ion transitions (*m/z* = 424 – 71 and *m/z* = 424 – 317), consistent with the data of the authentic sam-



Scheme 3 Synthesis of the metabolites **6a/6b** via a HWE reaction using the chlorophosphonate **16**

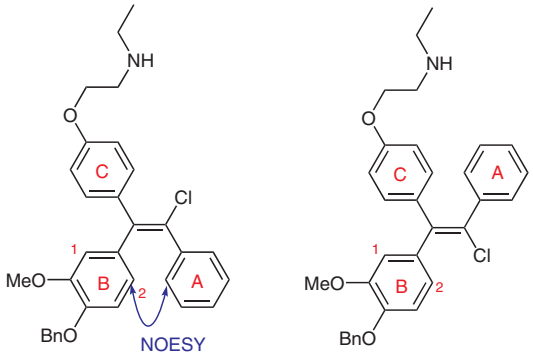


Figure 3 The two geometric isomers of the isolated metabolites **18a/18b**

ple **6**, suggesting that the observed metabolite[12] has the same structure.

In summary, we have prepared new clomiphene metabolites using a mild, simple, and straightforward route. Metabolites **6a** and **6b** were synthesized in 19% overall yield from commercially available starting materials.

The key steps consisted on the preparation of the ketone **15** using a *N*-acylbenzotriazole derivative as acylating agent and the HWE approach, using a cyclic α-chlorophosphonate, to establish the chloro vinyl skeleton. Both stereoisomers could be separated by chromatography and their structures assigned by NMR. Furthermore the synthesized compound **6** was used as control for analysis of the biological sample. This approach represents a simple and effective strategy to prepare novel and more substituted clomiphene metabolites useful for doping analysis.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

Acknowledgment

The authors acknowledge Fundação para a Ciência e Tecnologia for the fellowship to M. S. Estevão SFRH/BD/46234/2008 and Instituto do Desporto de Portugal for partial financial support. We would like to thank Prof. Ricardo B. Ferreira from Instituto Superior de Agronomia for the support running HPLC analysis. We acknowledge Fundação para a Ciência e Tecnologia (FCT) for the project REDE/1502/REM/2005 and M.C. Oliveira for providing preliminary data from the LC-MS at the Instituto Superior Técnico, Portugal.

References and Notes

(1) Ruenitz, P. C. *Burger's Medicinal Chemistry and Drug Discovery*, 5th ed., Vol.4; Wiley: New York, **1997**, 553–587.
(2) (a) Love, R. R.; Mazess, R. B.; Tormey, D. C.; Barden, H. S.; Newcomb, P. A.; Jordan, V. C. *Breast Cancer Treat.* **1988**, *12*, 297. (b) Young, R. L.; Goldzieher, J. W.; Elkind-Hirsch, K.; Hickox, P. G.; Chakraborty, P. K. *Int. J. Fertil.* **1991**, *36*, 167.
(3) Early Breast Cancer Trialists' Collaborative Group *Lancet* **1998**, *351*, 1451.
(4) Cuzick, J.; Powles, T.; Veronesi, U.; Forbes, J.; Edwards, R.; Ashley, S.; Boyle, P. *Lancet* **2003**, *361*, 296.
(5) Casper, R. F.; Mitwally, M. F. M. *J. Clin. Endocrinol. Metab.* **2008**, *91*, 760.
(6) Greenblatt, R. B.; Barfield, W. E.; Jungck, E. C.; Ray, A. W. *J. Am. Med. Assoc.* **1961**, *14*, 101.
(7) Ernest, S.; Hite, G.; Cantrell, J. S.; Richardson, A. Jr.; Benson, H. D. *J. Pharm. Sci.* **1976**, *65*, 148.
(8) Xin, Z.; Kairong, C.; Moutian, W. *Recent Adv. Doping Anal.* **2004**, *12*, 421.
(9) For the synthesis of **2** involving Horner–Emmons-type reaction, see: (a) Crenshaw, M. D.; Zimmer, H. *J. Org. Chem.* **1983**, *48*, 2782. Stannylcupration of diphenylacetylene, see: (b) Cummins, C. H. *Synth. Commun.* **1995**, *25*, 4071. Palladium-catalyzed cross-coupling, see: (c) Al-Hassan, M. I. *Synth. Commun.* **1987**, *17*, 1787 . Grignard reaction, see: (d) Palopoli, F. P.; Feil, V. J.; Allen, R. E.; Holtkamp, D. E.; Richardson, A. Jr. *J. Med. Chem.* **1967**, *10*, 84. (e) Rao, P. N.; Cessac, J. W. *J. Labelled Compd. Radiopharm.* **1985**, *22*, 245.
(10) (a) Ruenitz, P. C.; Arrendale, R. F.; Schmidt, W. F.; Thompson, C. B.; Nanavati, N. T. *J. Med. Chem.* **1989**, *32*, 192. (b) Ruenitz, P. C.; Bagley, J. R.; Watts, C. K. W.; Hall, R. E.; Sutherland, R. L. *J. Med. Chem.* **1986**, *29*, 2511.
(11) For recent references, see: (a) Detsi, A.; Koufaki, M.; Calogeropoulou, T. *J. Org. Chem.* **2002**, *67*, 4608. (b) Top, S.; Kaloun, E. B.; Vessières, A.; Laïos, I.; Leclercq, G.; Jaouen, G. *J. Organomet. Chem.* **2002**, 643. (c) Jaoun, G.; Top, S.; Vessières, A.; Pigneon, P.; Leclercq, G.; Laïos, I. *Chem. Commun.* **2001**, 383.
(12) Sample obtained at the Laboratório de Análises de Dopagem, Instituto do Desporto de Portugal for the Master degree of: Vitoriano, B. *Master Thesis*; New University of Lisbon: Portugal, **2009**.
(13) For preparation of tetrasubstituted olefins, see: Zhou, C.; Emrich, D. E.; Larock, R. C. *Org. Lett.* **2003**, *5*, 1579; and references cited therein.
(14) (a) Petrova, J.; Coutrot, P.; Drefux, M.; Savignac, P. *Synthesis* **1975**, 658. (b) Crenshaw, M. D.; Zimmer, H. *J. Org. Chem.* **1983**, *48*, 2782. (c) Gajda, T. *Synthesis* **1970**, 717. (d) Morise, X.; Savignac, P.; Denis, M. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2179.
(15) Muthiah, C.; Kumar, P.; Kumaraswamy, S.; Kumara Swamy, K. C. *Tetrahedron* **1998**, *54*, 14315.
(16) Larsen, R. O.; Aksnes, G. *Phosphorus, Sulfur Relat. Elem.* **1983**, *16*, 339.
(17) For a review of *N*-acylbenzotriazoles as acylating agents, see: Katritzky, A. R.; Suzuki, K.; Wang, Z. *Synlett* **2005**, 1656.
(18) Katritzky, A. R.; Le, K. N. B.; Khelasvili, L.; Mohapatra, P. P. *J. Org. Chem.* **2006**, *71*, 9861.
(19) Short, J. H.; Biermacher, U.; Dunnigan, D. A.; Lambert, G. F.; Martin, D. L.; Nordeen, C. W.; Wright, H. B. *J. Med. Chem.* **1965**, *8*, 223.
(20) Herbert, R. B.; Jackson, F. B.; Nicolson, I. T. *J. Chem. Soc., Perkin Trans. 1* **1984**, 825.
(21) Katritzky, A. R.; Shobana, N.; Pernak, J.; Afridi, A. S.; Fan, W. Q. *Tetrahedron* **1992**, *48*, 7817.
(22) Kumaraswamy, S.; Selvi, R. S.; Kumara Swammy, K. C. *Synthesis* **1997**, 207.
(23) (a) Olofson, R. A.; Martz, J. T.; Senet, J.-P.; Piteau, M.; Malfroot, T. *J. Org. Chem.* **1984**, *49*, 2081. The same procedure was applied to tamoxifen, see: (b) da Costa, G. G.; McDaniel-Hamilton, L. P.; Heflich, R. H.; Marques, M. M.; Beland, F. A. *Carcinogenesis* **2001**, *22*, 1307.