18 and 19 were also prepared. As would be expected from the parent N^6-substituted NECA analogue,[7] 19 showed nearly as high a selectivity ratio as 17, with less than a 2-fold loss of affinity, but 18, unlike in the parent series,[4,22] was somewhat less A_2 selective than 17, and showed a 10-fold loss in binding affinity.

In summary, we have identified a series of N^6-substituted adenosine derivatives that show substantial selectivity for the A_2 receptor. Development of a detailed model of the N^6 binding region of the A_2 receptor allowed us to refine the series, producing several agonists with 3–6 nM affinity and 20–40-fold binding selectivity for the A_2 receptor. This potency and selectivity should make these compounds very useful probes for adenosine pharmacology.

Acknowledgment. We thank Dr. C. Humblet for assistance in establishing the A_2 model and G. Lu and J. Fergus for assistance with pharmacological studies.

(22) Ukena, D.; Bohme, E.; Schwabe, U. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1984, *327*, 36.

† Department of Chemistry.
‡ Department of Pharmacology.

Alexander J. Bridges,*,† Robert F. Bruns‡
Daniel F. Ortwine,† Steven R. Priebe†
Deedee L. Szotek,† Bharat K. Trivedi†
Parke-Davis Pharmaceutical Research Division
Warner-Lambert Company
2800 Plymouth Road
Ann Arbor, Michigan 48105
Received February 12, 1988

Articles

Nonisomerizable Analogues of (Z)- and (E)-4-Hydroxytamoxifen. Synthesis and Endocrinological Properties of Substituted Diphenylbenzocycloheptenes

Raymond McCague,*† Guy Leclercq,‡ and V. Craig Jordan§

Drug Development Section, Cancer Research Campaign Laboratory, Institute of Cancer Research, Sutton, Surrey, SM2 5NG, U.K., Laboratoire de Cancerologie Mammaire, Service de Medecine, Institut Jules Bordet, 1000 Bruxelles, Belgium, and Department of Human Oncology, University of Wisconsin Clinical Cancer Center, University of Wisconsin, Madison, Wisconsin 53792. *Received June 25, 1987*

Substituted 8,9-diphenyl-6,7-dihydro-5*H*-benzocycloheptenes 6–8, which are ring-fused analogues of (*Z*)-*trans*-4-hydroxytamoxifen, (*E*)-*cis*-tamoxifen, and (*E*)-*cis*-4-hydroxytamoxifen, were synthesized from 7-methoxy-1-benzosuberone. The hydroxy compounds 6 and 8 were individually prepared via a common synthetic intermediate from which either the perfluoro-*p*-tolyl or the methyl ether functions could be cleaved specifically. Compounds were assayed for binding affinity to estrogen receptors in cytosol and in MCF-7 whole cells and for growth inhibition of MCF-7 cells in vitro and rat uteri in vivo. The endocrinological properties of the cyclic analogues 5–7 paralleled those of the corresponding derivatives of tamoxifen although in the MCF-7 assay 6 was slightly less effective than 4-hydroxytamoxifen at 10^{-6} and 10^{-7} M. The compound 8 analogous to *cis*-4-hydroxytamoxifen antagonized the growth stimulation by estradiol of MCF-7 cell or rat uterus growth, and it is therefore an antiestrogen, but its potency was somewhat less, both as an antiestrogen and an estrogen, than reported for *cis*-4-hydroxytamoxifen attributable to modification of the biochemical properties of the latter by isomerization to the more potent trans isomer. Curiously, in the absence of estradiol, compound 8 stimulated MCF-7 cell growth at low concentration (10^{-8} M) but inhibited growth at higher concentration. In contrast, compound 7, which lacked the hydroxy function, was a full estrogen in the rat uterine growth assay. These compounds should be ideal for further structure–activity studies of triarylethylene-based antiestrogens without complications caused by isomerization.

(*Z*)-*trans*-4-Hydroxytamoxifen (1-[4-[2-(dimethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-2-phenyl-1-butene) (2) is an important metabolite of the nonsteroidal antiestrogen tamoxifen (1) in patients undergoing treatment for advanced breast cancer.[1] It has attracted considerable interest since it has a much greater antiestrogenic potency in vitro than the parent drug.[2,3] However, although the pure isomers of 4-hydroxytamoxifen can be prepared,[4] they undergo a facile isomerization to give a mixture of isomers,[5,6] a process that has been shown to occur during cell culture experiments;[7,8] for instance, when MCF-7 cells are grown in a medium containing (*E*)-*cis*-4-hydroxytamoxifen, it is the trans isomer that preferentially accumulates in the cells.[8] (Trans and cis are used in this paper to refer to the relative positions of the aryl ring bearing the basic side chain and alkyl function on the

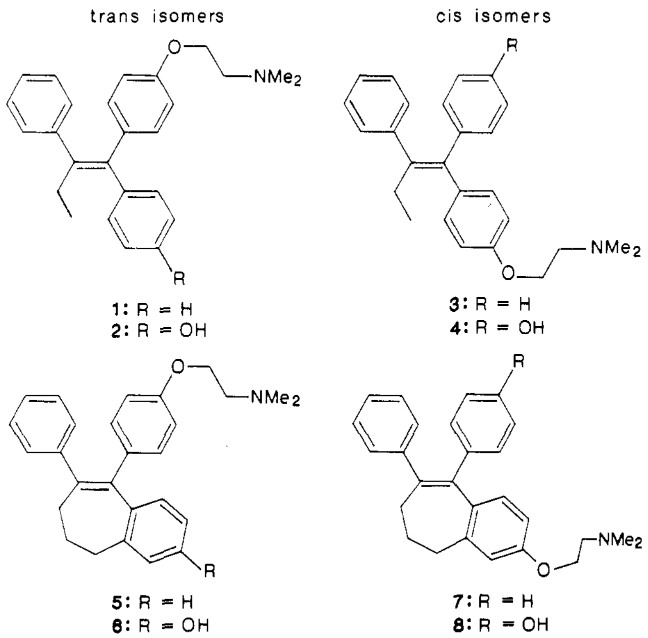
† Institute of Cancer Research.
‡ Institut Jules Bordet.
§ University of Wisconsin.
* Address correspondence to Dr. R. McCague, Cancer Research Campaign Laboratory, Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, SM2 5NG, England.

(1) Daniel, C. P.; Gaskell, S. J.; Bishop, H.; Nicholson, R. I. *J. Endocrinol.* 1979, *83*, 401.
(2) Nicholson, R. I.; Syne, J. S.; Daniel, C. P.; Griffiths, K. *Eur. J. Cancer.* 1979, *15*, 317.
(3) Jordan, V. C.; Collins, M. M.; Rowsby, L.; Prestwich, G. *J. Endocrinol.* 1977, *75*, 305.
(4) McCague, R. *J. Chem. Res., Synop.* 1986, 58; *J. Chem. Res., Miniprint* 1986, 771.
(5) Robertson, D. W.; Katzenellenbogen, J. A. *J. Org. Chem.* 1982, *47*, 287.
(6) Ruenitz, P. C.; Bagley, J. R.; Moklar, C. M. *J. Med. Chem.* 1982, *25*, 1056.
(7) Katzenellenbogen, B. S.; Norman, M. J.; Eckert, R. L.; Peltz, S. W.; Mangel, W. F. *Cancer. Res.* 1984, *44*, 112.
(8) Katzenellenbogen, J. A.; Carlson, K. E.; Katzenellenbogen, B. S. *J. Steroid Biochem.* 1985, *22*, 589.

0022-2623/88/1831-1285$01.50/0

olefinic bond. In the case of tamoxifen and 4-hydroxytamoxifen, trans is *Z* and cis is *E*.) This feature makes the interpretation of structure–activity studies of these compounds unreliable. In particular, it has been difficult to prove that the antiestrogenicity observed for *cis*-4-hydroxytamoxifen (4)[7,9] is not merely a consequence of its isomerization to a mixture containing the more potent trans isomer, especially when it is considered that *cis*-tamoxifen (3) is fully estrogenic.[9,10] Additionally, the isomerization of 2 will increase the complexity of the metabolism profile of tamoxifen.

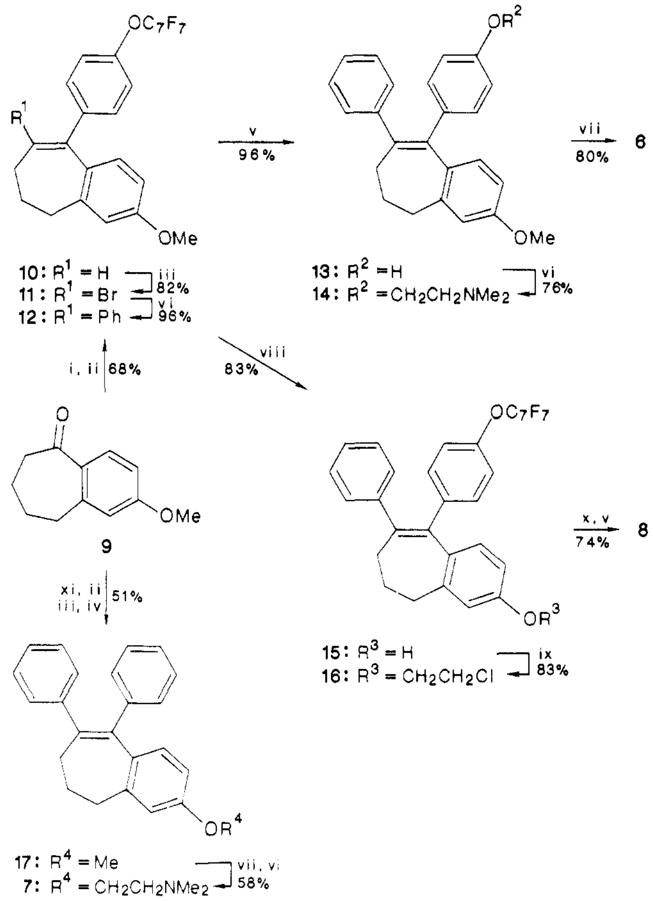
We have recently reported the synthesis of the ring-fused analogue 5 of tamoxifen and have shown by X-ray crystallography that the seven-membered ring provides the compound with stereochemical features very close to those of tamoxifen and also that its biological activity is similar to that of tamoxifen.[11] This paper reports the synthesis of the 4-hydroxylated derivative (6) of 5 and also its isomer (8) corresponding to *cis*-4-hydroxytamoxifen (4). For completeness, the cyclic analogue 7 corresponding to *cis*-tamoxifen has also been prepared. The biological properties of these compounds have been compared in vitro by examining their binding affinity to estrogen receptors and the growth inhibition of the MCF-7 human mammary carcinoma cell line and additionally in vivo by examining their effect on rat uterine weight.



Results and Discussion

Synthesis. The synthesis of the target compounds 6 and 8 makes use of the perfluorotolyl group for protection of the phenolic function as is illustrated in Scheme I. The starting methoxybenzosuberone 9 was conveniently prepared from *m*-anisaldehyde in three steps by the published procedure.[12] In order to prepare both 6 and 8 efficiently from 9, the methyl ether function was retained as a protected phenol while the diarylbenzocycloheptene structure was constructed. Reaction of 9 with the Grignard reagent

Scheme I[a]



[a] Reagents: (i) 4-$C_7F_7OC_6H_4MgBr$, Et_2O, 30 °C; (ii) HCl, EtOH, 80 °C, (iii) $C_5NH_5 \cdot HBr_3$, CH_2Cl_2; (iv) PhZnCl, $Pd(PPh_3)_4$, THF, 60 °C; (v) NaOMe, Me_2NCHO, 30 °C; (vi) $Me_2NCH_2CH_2Cl \cdot HCl$, NaH, Me_2NCHO, 60 °C; (vii) $C_5NH_5 \cdot HCl$, 200 °C; (viii) HBr, HOAc, 100 °C; (ix) $ClCH_2CH_2Cl$, NaOH (aqueous), $Bu_4N^+HSO_4^-$, 80 °C; (x) Me_2NH, EtOH, 80 °C; (xi) PhMgBr, Et_2O, 20 °C.

formed from heptafluoro-*p*-tolyl 4-bromophenyl ether[13] and acid-catalyzed dehydration of the resulting tertiary alcohol gave the olefin 10. Introduction of the phenyl group at the 8-position by the method reported in the synthesis of 5[11] gave compound 12, which could be manipulated to give either 6 or 8 since either the methyl ether or the perfluorotolyl ether function in 12 could be cleaved to the phenol in the presence of the other function.

Removal of the perfluorotolyl function by sodium methoxide and then dimethylaminoethylation gave 14. Interestingly, the basic side chain was unaffected while the methyl ether function was cleaved by pyridine hydrochloride to give 6. The basic side chain would have been protonated under these demethylation conditions, which presumably inhibits its dealkylation.

The methyl ether function in 12 could be selectively cleaved with hydrobromic acid or pyridine hydrochloride. The product 15 was not dimethylaminoethylated directly because the perfluorotolyl group is not compatible with the strongly basic conditions preferred for this particular alkylation. The chosen procedure was chloroethylation by dichloroethane under phase-transfer conditions and then treatment with dimethylamine. Final cleavage of the perfluorotolyl function with sodium methoxide gave 8. Compound 7 was simply prepared as also shown in Scheme I.

(9) Jordan, V. C.; Haldemann, B.; Allen, K. E. *Endocrinology (Baltimore)* **1981**, *108*, 1353.
(10) Harper, M. J. K.; Walpole, A. L. *Nature (London)* **1966**, *212*, 87.
(11) McCague, R.; Kuroda, R.; Leclercq, G.; Stoessel, S. *J. Med. Chem.* **1986**, *29*, 2053.
(12) Hicks, M. G.; Jones, G.; Sheikh, H. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2297.
(13) Jarman, M.; McCague, R. *J. Chem. Res., Synop.* **1985**, 114; *J. Chem. Res., Miniprint* **1985**, 1301.

Table I. Effects of Compounds 6 and 8 on MCF-7 Cell Growth

		opt density (mean ± SD)[a]	
compd	concn, M	compd alone[c]	compd + 10^{-8} M estradiol
6	control	0.417 ± 0.021 (100)	0.727 ± 0.097 (174)
	10^{-8}	0.126 ± 0.026 (30)	0.520 ± 0.056 (125)
	10^{-7}	0.066 ± 0.033 (16)	0.147 ± 0.048 (35)
	10^{-6}	0.083 ± 0.036 (20)	0.068 ± 0.035 (16)
8	control	0.337 ± 0.130 (100)	0.707 ± 0.056 (210)
	10^{-8}	0.440 ± 0.030 (130)	0.659 ± 0.083 (196)
	10^{-7}	0.328 ± 0.034 (97)	0.566 ± 0.096 (168)
	10^{-6}	0.162 ± 0.079 (48)	0.363 ± 0.097 (108)

[a] Each value corresponds to the mean of optical density measurements from four separate samples of culture. One-way analysis of the variance showed that both compounds inhibited the growth of MCF-7 cells (6, $p < 0.001$; 8, $p < 0.01$). The Newman Keuls test showed that 6 is effective at all concentrations and suppressed the stimulatory effect of estradiol ($p < 0.01$); 8 was effective at 10^{-6} M and suppressed the stimulatory effect of estradiol at the same concentration ($p < 0.01$). [b] Percentage of control value. [c] Effect in a routine control experiment of 4-hydroxytamoxifen with an identical experimental protocol: control 0.453 ± 0.014 (100); 10^{-8} M, 0.218 ± 0.061 (48); 10^{-7} M, 0.171 ± 0.032 (38); 10^{-6} M, 0.067 ± 0.020 (15).

Relative Binding Affinity (RBA) for Estrogen Receptors. In a cytosol preparation, the cyclic compounds **5** and **7** gave the same values of RBA as the corresponding trans and cis isomers of tamoxifen (1.0 and 0.1, respectively). As for 4-hydroxy derivatives of tamoxifen, the hydroxylated cyclic compounds had significantly increased binding affinity when compared with their nonhydroxylated counterparts (trans isomer **6**, RBA = 30; cis isomer **8**, RBA = 5; cf. *trans*-4-hydroxytamoxifen (**2**), RBA = 100; *cis*-4-hydroxytamoxifen (**4**), RBA = 2). The lower RBA of compound **6** than that of *trans*-4-hydroxytamoxifen may be due to the extra substitution in the aromatic ring that bears the hydroxyl group. Curiously, however, 2-methyl-4-hydroxytamoxifen, in which the same pattern of ring substitution is present, maintained the same RBA as 4-hydroxytamoxifen.[14]

Previously reported studies on triphenylethylene antiestrogens have shown that the relative binding affinity to estrogen receptors in MCF-7 whole cells is always lower than the value in the cytosol assay, a property thought to be related to the poor ability of these compounds to activate the estrogen receptor.[15] Similarly, compounds **6** and **8** gave reduced values (trans isomer **6**, RBA = 1.5; cis isomer **8**, RBA = 0.15) in this whole-cell assay comparable to those obtained for *trans*- and *cis*-4-hydroxytamoxifen (2.9 and 0.4, respectively).

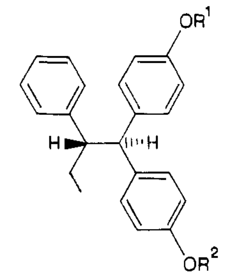
Effect on MCF-7 Cell Growth. Table I gives data for the antiproliferative effects of the hydroxylated compounds **6** and **8** on the growth of the MCF-7 human mammary tumor cell line in vitro. The trans isomer **6** was a potent antiestrogen that gave a marked inhibition of the rate of growth at 10^{-8} M and was capable at this concentration of blocking much of the growth stimulation caused by 10^{-8} M estradiol. The extent of the antitumor effect is much greater than that of the nonhydroxylated cyclic analogue **5**[11] or tamoxifen, being essentially the same as observed for *trans*-4-hydroxytamoxifen, and similar to that observed for the 4-hydroxydihydrotamoxifen (**18**), which can also be considered as a nonisomerizable equivalent of 4-hydroxytamoxifen.[16] Thus, although compound **6** had a lower RBA than did 4-hydroxytamoxifen, the antitumor activity was not impaired.

(14) Foster, A. B.; Jarman, M.; Leung, O.-T.; McCague, R.; Leclercq, G.; Devleeschower, N. *J. Med. Chem.* **1985**, *28*, 1491.
(15) Stoessel, S.; Leclercq, G. *J. Steroid Biochem.* **1986**, *25*, 677.
(16) McCague, R.; Leclercq, G. *J. Med. Chem.* **1987**, *30*, 1761.

Table II. Effect of Compounds 5 and 6 on the Stimulation of Rat Uterine Growth by Estradiol

compd	daily dose	uterine wet weights, mg ± SEM[a]
control		44 ± 4
estradiol benzoate (EB)	0.2 μg	108 ± 5
5	12.5 μg + EB 0.2 μg	92 ± 5
	25 μg + EB 0.2 μg	75 ± 4[b]
	50 μg + EB 0.2 μg	77 ± 5[b]
6	5 μg + EB 0.2 μg	75 ± 2[b]
	10 μg + EB 0.2 μg	72 ± 2[b]
	20 μg + EB 0.2 μg	71 ± 3[b]

[a] Each result is the average of weight measurements from eight rats. [b] Significantly different (Student's *t* test) than EB alone ($p < 0.01$).



18: $R^1 = CH_2CH_2NMe_2$; $R^2 = H$
19: $R^1 = H$; $R^2 = CH_2CH_2NMe_2$

The cis isomer **8** caused a significant stimulation of growth at 10^{-8} M, indicating an estrogenic activity. However, at higher concentrations growth was inhibited, indicating that a counteracting antiestrogen effect is then dominant. The antiestrogenicity of **8** is further apparent by its ability to suppress the growth stimulation by 10^{-8} M estradiol. A similar profile of growth agonism/antagonism had been observed for *cis*-4-hydroxydihydrotamoxifen (**19**).[16]

Effect on Rat Uterine Weight. Table II gives data in vivo for the effects of the trans seven-membered ring analogue **5** and its hydroxylated derivative **6** on the growth of the immature rat uterus. Both of these compounds suppress the uterine growth stimulation by estradiol, although **6** is slightly the more potent, presumably as a consequence of its higher affinity to the estrogen receptor. 4-Hydroxytamoxifen has been shown similarly to be more potent than tamoxifen in this assay.[3] At the highest concentration used, neither **5** nor **6** was able to reduce the uterine growth rate to the control level, owing to their intrinsic estrogenicity, a feature consistently seen for triarylethylene antiestrogens.

The *cis*-hydroxybenzocycloheptene **8** was studied in the most detail because of its parallel with *cis*-4-hydroxytamoxifen for which there is uncertainty in the interpretations of its observed activity. The biological properties of the isomers of 4-hydroxytamoxifen in the immature rat uterine weight assay have been described previously.[9] Compound **2** is a potent inhibitor of estradiol action whereas **4** is much less active. The daily doses of compounds **2** and **4** that completely inhibit estradiol benzoate action to the level observed with the compounds alone are 1.25 and 20 μg daily, respectively.[9] The ability of **8** to suppress the growth stimulation by estradiol is much lower; 100 μg of **8** daily inhibited the effects of estradiol. Nevertheless, these results demonstrate that the action of the fixed ring derivative **8** is an antiestrogen.

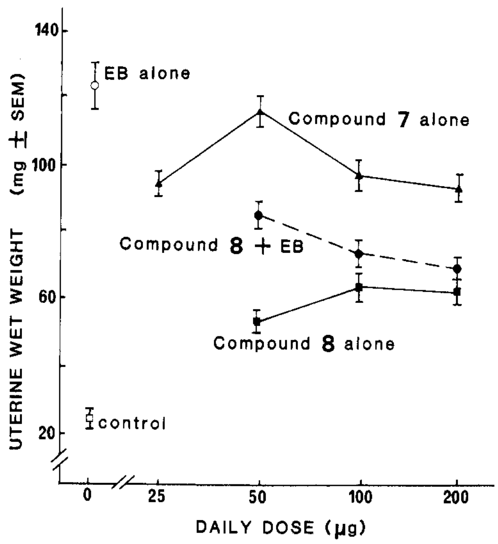


Figure 1. Comparison of the effects of compounds 7 and 8 on rat uterine growth showing the effect of hydroxylation in the cis isomer 8. In a preliminary experiment with compound 8, a daily dose of 10 μg produced an immature rat uterine weight of 47 ± 3 mg ($N = 8$) alone but did not affect the increase in uterine weight produced by estradiol benzoate.

In the absence of estradiol, 8 exerts a stimulation of uterine growth but at a level no greater than is reported for tamoxifen.[9] Compared with *cis*-4-hydroxytamoxifen, the cyclic analogue 8 is less estrogenic since a 10-fold greater quantity[9] is required to give a similar stimulation of uterine growth. Thus uterine weights of 60 mg were obtained in rats treated with 10 μg of *cis*-4-hydroxytamoxifen but with 100 μg of 8. Again this result is explained by isomerization of the *cis*-4-hydroxytamoxifen since the trans isomer is more potent as an estrogen as well as an antiestrogen, about 1 μg of *trans*-4-hydroxytamoxifen being all that is required to produce a 60-mg uterine weight.[9] On the other hand, compound 7, which lacks the 4-hydroxyl group, is a full estrogen and a 50 μg/day dose has virtually the same effect as a dose of estradiol benzoate of 0.2 μg/day. This activity is similar to that reported for *cis*-tamoxifen 3.[9] These influences of the cis seven-membered ring compounds are illustrated graphically in Figure 1.

Conclusions

Compounds 5–8 have the advantage over the corresponding series of triarylbutenes such as tamoxifen that their syntheses are readily accomplished without any stereochemical ambiguity, and consequently there is no need to separate isomers either of the product or a precursor. Also, because of their nonisomerizability, any need to verify the isomer composition prior to cell culture experiments, such as is suggested for easily isomerizable antiestrogens,[8] is avoided.

The good correspondence of the endocrinological properties of 5, 7, and 6 with those established for *trans*- and *cis*-tamoxifen and *trans*-4-hydroxytamoxifen, respectively, is consistent with the close correlation of stereochemistry between the triarylbutenes and the seven-membered ring compounds, particularly in relation to the orientations of the phenyl rings as determined by X-ray crystallography of 5.[11] In addition, the activity of the ring-fused analogue 8 of *cis*-4-hydroxytamoxifen is very similar to that of the 4-hydroxydihydrotamoxifen **19**,[16] a result that supports our claim that **19** can be considered a nonisomerizable equivalent of *cis*-4-hydroxytamoxifen.[16] Since compounds 8 and **19** differ from *cis*-4-hydroxytamoxifen in a completely different way, this is strong evidence that the properties observed for 8, i.e., a combination of weak antiestrogenic and weak estrogenic properties comparable to those of tamoxifen, are those that *cis*-4-hydroxytamoxifen would have given if it did not isomerize. [These conclusions are also supported by data obtained in a prolactin synthesis assay using rat pituitary gland cells in culture (Jordan, V.C.; Koch, R.; Langan, S.; McCague, R., *Endocrinology*, in press).] This result validates the previous assertion that *cis*-4-hydroxytamoxifen is an antiestrogen, but it is noteworthy that the extent of antiestrogenicity for 8 is somewhat less than recorded for *cis*-4-hydroxytamoxifen, consistent with the idea that isomerization of the latter modifies its observed biological properties. Nevertheless the hypothesis[9] is still valid that in binding to the estrogen receptor the phenolic group orients the molecule and that it is the resultant position of the (dimethylamino)ethoxy side that determines whether antiestrogenic activity is present. A consequence of these studies is that isomerization of *trans*–4–hydroxytamoxifen cannot be considered a mechanism allowing formation of an estrogen agonist that would oppose the action of the parent drug. However, dealkylation of the side chain[17] to give a strongly estrogenic bis-(phenol)[18] might do so.

The results provide further evidence of the compromising estrogenic and antiestrogenic properties that are a feature of triarylethylene-based antiestrogens. This unwanted estrogenicity has been circumvented in a series of antiestrogenic 7α-substituted estradiol derivatives recently reported by Wakeling and Bowler.[19] The irregular dose–response curves obtained in the MCF-7 cell growth assay, whereby estrogenic action of 8 is observed only at 10^{-8} M and whereby the dose–response curve of 6 is rather flat, is supportive of literature evidence of the allosteric nature of the estrogen receptor protein.[20] For the purpose of studying such behavior of the estrogen receptor and as tools for probing the estrogen receptor binding surface through structure–activity studies, the compounds described should be ideal since ambiguities in data interpretation caused by isomerization are avoided and since good comparisons with the abundant literature on the biological properties of tamoxifen are still possible. Such studies could provide valuable information in the continuing search for improved antiestrogens for the treatment of estrogen-dependent breast cancer.

Experimental Section

Chemical Methods. General Procedures. ^1H NMR spectra (250 MHz) (internal Me_4Si) were obtained by courtesy of the University of London Intercollegiate Research Service. Mass spectra (electron impact, 70 eV) were obtained with a VG 7070H spectrometer and a VG 2235 data system. Chromatography refers to column chromatography on silica gel (Merck 15111) with the solvent indicated applied at a positive pressure of 0.5 atm. Tetrahydrofuran (THF) was dried by distillation from potassium benzophenone; dimethylformamide (DMF) was dried by distillation at 20 mmHg from CaH_2. Commercially available anhydrous Et_2O was used without further purification. Other solvents were distilled before use.

3-Methoxy-6,7-dihydro-9-[4-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]phenyl]-5*H*-benzocycloheptene (10). To a stirred solution of 4-bromophenyl 2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenyl ether[13] (25.8 g, 66.4 mmol) in anhydrous Et_2O (100 mL) containing Mg (2.34 g, 97 mmol) was added a solution of $BrCH_2CH_2Br$ (5.8 g, 31 mmol) in Et_2O (20 mL)

(17) Mauvais-Jarvis, P.; Baudot, N.; Castaigne, D.; Banzet. P.; Kuttenn, F. *Cancer Res.* **1986**, *46*, 1521.
(18) Robertson, D. W.; Katzenellenbogen, J. A.; Long, D. J.; Rorke, E. A.; Katzenellenbogen, B. S. *J. Steroid Biochem.* **1982**, *16*, 1.
(19) Wakeling, A. E.; Bowler, J. *J. Endocrinol.* **1987**, *112*, R7.
(20) Sasson, S.; Notides, A. C. *J. Biol. Chem.* **1983**, *258*, 8118.

dropwise over 1 h. To the resulting solution of Grignard reagent was added a solution of 2-methoxy-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-5-one[10] (7-methoxy-1-benzosuberone, **9**) (8.41 g, 44.2 mmol). The mixture was stirred overnight at 22 °C and then poured into dilute HCl(aq) (200 mL), and the product was extracted with Et_2O (2 × 100 mL). The combined Et_2O solutions were concentrated, and the crude tertiary alcohol was dissolved in EtOH (200 mL). Concentrated HCl(aq) (50 mL) was added, and the mixture was heated under reflux for 2 h, poured into water (400 mL), and extracted with Et_2O (2 × 200 mL). The combined Et_2O solutions were concentrated. Chromatography of the residue (5% CH_2Cl_2 in petroleum ether, bp 40–60 °C) gave **10** (12.27 g, 68%): mp 84–85 °C (from petroleum ether, bp 60–80 °C); NMR ($CDCl_3$) δ_H 1.98 (q, J = 7 Hz, 2, H-7), 2.17 (quint, J = 7 Hz, 2, H-6), 2.63 (t, J = 6.9 Hz, 2, H-5), 3.84 (s, 3, OMe), 6.34 (t, J = 7.3 Hz, 1, H-8), 6.74 (dd, J = 2.7, 8.5 Hz, 1, H-2), 6.83 (d, J = 2.7 Hz, 1, H-4), 6.91 (d, J = 8.5 Hz, 1, H-1), 6.92 (d, J = 8.8 Hz, 2, Ar *H* ortho to OC_7F_7), 7.25 (d, J = 8.8 Hz, 2, Ar *H* meta to OC_7F_7). Anal. ($C_{25}H_{17}F_7O_2$) C, H, F.

3-Methoxy-6,7-dihydro-8-bromo-9-[4-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]phenyl]-5*H*-benzocycloheptene (11). To a stirred solution of **10** (12.27 g, 25.7 mmol) in CH_2Cl_2 (50 mL) was added pyridine hydrobromide perbromide (9.0 g, 28.1 mmol). After 3 h, the solution was washed with dilute HCl (1 M, 50 mL), to which Na_2SO_3 (0.2 g) had been added, and then with H_2O (50 mL), dried with Na_2SO_4, and concentrated. Recrystallization of the residue from petroleum ether (bp 80–100 °C) gave **11** (11.65 g, 82%), mp 115–117 °C. Anal. ($C_{25}H_{16}BrF_7O_2$) C, H, Br, F.

3-Methoxy-6,7-dihydro-8-phenyl-9-[4-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]phenyl]-5*H*-benzocycloheptene (12). A solution of PhZnCl was prepared by the addition of PhLi (34.6 mL of a 1.8 M solution of cyclohexane–Et_2O, 70:30; 62.3 mmol) to $ZnCl_2$ (8.49 g, 62.3 mmol) in THF (80 mL) under N_2 at 0 °C. To this solution was added a solution of **11** (11.65 g, 20.7 mmol) in THF (20 mL) and $Pd(PPh_3)_4$ (200 mg, 0.17 mmol). The resulting mixture was heated under reflux for 2 h and then poured into H_2O (400 mL), and the product was extracted with Et_2O (2 × 200 mL). The extracts were concentrated. Chromatography of the residue (10% CH_2Cl_2 in petroleum ether, bp 40–60 °C) gave **12** (11.08 g, 96%): mp 148–149 °C (from petroleum ether, bp 80–100 °C); NMR ($CDCl_3$) δ_H 2.17 (quint, J = 7 Hz, 2, H-6), 2.40 (t, J = 6.9 Hz, 2, H-7), 2.78 (t, J = 7.0 Hz, 2, H-5), 3.83 (s, 3, OMe), 6.70 (d, J = 8.8 Hz, 2, Ar *H* ortho to OC_7F_7), 6.71 (d, J = 8.6 Hz, 1, H-1), 6.61–6.70 (m, 2, H-2 and H-4), 6.88 (d, J = 8.8 Hz, 2, Ar *H* meta to OC_7F_7), 7.05–7.25 (m, 5, Ph). Anal. ($C_{31}H_{21}F_7O_2$) C, H, F.

3-Methoxy-6,7-dihydro-8-phenyl-9-(4-hydroxyphenyl)-5*H*-benzocycloheptene (13). A solution of **12** (2.60 g) in DMF (20 mL) was treated with NaOMe (3.5 g), and the mixture was stirred at 35 °C for 2 h and then poured into saturated aqueous $NaHCO_3$ (80 mL). The product was extracted with Et_2O (2 × 60 mL). The extracts were concentrated, and the residue was dissolved in petroleum ether (bp 40–60 °C). Phenol **13** precipitated (1.53 g, 96%) and was recrystallized from petroleum ether (bp 80–100 °C), mp 175–177 °C. Anal. ($C_{24}H_{22}O_2$) C, H.

3-Methoxy-6,7-dihydro-8-phenyl-9-[4-[2-(dimethylamino)ethoxy]phenyl]-5*H*-benzocycloheptene (14). A solution of the phenol **13** (1.312 g, 3.8 mmol) in DMF (50 mL) was treated with NaH (1.3 g, 54 mmol) at room temperature under N_2. The yellow stirred mixture was heated to 60 °C, and $Me_2NCH_2CH_2Cl{\cdot}HCl$ (1.3 g, 9.0 mmol) was added in portions over 10 min. After 30 min, the yellow color had discharged, the mixture was cooled, and excess NaH was destroyed by the addition of 2-propanol (2 mL). The mixture was then poured into H_2O (100 mL) and extracted with Et_2O (2 × 50 mL). The extracts were washed with H_2O (100 mL), dried with Na_2SO_4, and concentrated. Recrystallization of the residue from petroleum ether (bp 80–100 °C) gave **14** (1.20 g, 76%), mp 123–125 °C (from petroleum ether, bp 80–100 °C). Anal. ($C_{28}H_{31}NO_2$) C, H, N.

6,7-Dihydro-8-phenyl-9-[4-[2-(dimethylamino)ethoxy]phenyl]-5*H*-benzocyclohepten-3-ol (6). A mixture of **14** (777.5 mg, 1.88 mmol) and pyridine hydrochloride (1.0 g, 8.65 mmol) was heated at 200 °C for 2 h. The hot mixture was then poured into aqueous Na_2CO_3 (5% w/v; 40 mL) and extracted with EtOAc (3 × 50 mL). The extracts were washed with H_2O (100 mL) and concentrated. Chromatography of the residue (10:1 Et_2O–NEt_3) gave **6** (597 mg, 80%): mp 208–210 °C (from MeOH); MS, m/z 399 ($M^{\bullet+}$, 8%), 72 ($Me_2NCH_2CH_2^+$, 25), 58 ($Me_2NCH_2^+$, 100); NMR (Me_2SO-d_6) δ_H 2.0–2.1 (m, 2, H-6), 2.17 (s, 6, NMe_2), 2.2–2.3 (m, 2, H-7), 2.56 (t, J = 5.8 Hz, 2, OCH_2CH_2N), 2.65–2.75 (br t, J = 7 Hz, 2, H-5), 3.92 (t, J = 5.8 Hz, 2, OCH_2CH_2N), 6.55 (sharp m, 2, H-1 + H-2), 6.63 (d, J = 8.8 Hz, 2, Ar *H* ortho to $OCH_2CH_2NMe_2$), 6.70 (m, 1, H-4), 6.72 (d, J 8.8 Hz, 2, Ar *H* meta to $OCH_2CH_2NMe_2$), 7.0–7.2 (m, 5, Ph). Anal. ($C_{27}H_{29}NO_2$) C, H, N.

6,7-Dihydro-8-phenyl-9-[4-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]phenyl]-5*H*-benzocyclohepten-3-ol (15). A suspension of **12** (1.709 g) in a solution of HBr in AcOH (48% w/v; 20 mL) was heated under reflux. After 5 h, the solution was poured into H_2O (100 mL), and the resulting mixture was basified with aqueous NaOH (3 M; ca. 150 mL required). Then saturated aqueous $NaHCO_3$ (50 mL) was added, and the product was extracted with Et_2O (2 × 60 mL). The combined extracts were washed with H_2O (100 mL), dried with Na_2SO_4, and concentrated. The residue was recrystallized from petroleum ether (bp 80–100 °C) to give **15** (1.38 g, 83%): mp 194–196 °C (from petroleum ether, bp 80–100 °C); MS, m/z 544 (M^+, 100). Anal. ($C_{30}H_{19}F_7O_2$) C, H, F.

3-(2-Chloroethoxy)-6,7-dihydro-8-phenyl-9-[4-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]phenyl]-5*H*-benzocycloheptene (16). A solution of **15** (303.6 mg) in $ClCH_2CH_2Cl$ (12 mL) containing n-$Bu_4N^+HSO_4^-$ (100 mg) and aqueous NaOH (1.5 M; 12 mL) was heated under reflux for 1 h, and then the organic layer was concentrated. Chromatography of the residue (1:5 CH_2Cl_2–petroleum ether, bp 40–60 °C) gave **16** (280 mg, 83%), mp 147–148 °C (from petroleum ether, bp 80–100 °C). Anal. ($C_{32}H_{22}ClF_7O_2$) C, H.

3-[2-(Dimethylamino)ethoxy]-6,7-dihydro-8-phenyl-9-(4-hydroxyphenyl)-5*H*-benzocycloheptene (8). A solution of **16** (198 mg) in ethanolic Me_2NH (33% w/v; 15 mL) was heated under reflux for 20 h. The mixture was then concentrated to dryness, and the residual oil was dissolved in DMF (5 mL). NaOMe (1.0 g) was added, and the mixture was stirred at 40 °C for 1 h and then partitioned between Et_2O (2 × 25 mL) and saturated aqueous $NaHCO_3$ (25 mL). The combined Et_2O solutions were concentrated, and the residue was recrystallized from methanol to give **8** (96.6 mg, 74%): mp 196–198 °C; NMR (250 MHz Me_2SO-d_6) δ_H 2.05 (m, 2, H-6), 2.22 (s, 6, NMe_2), 2.25 (m, 2, H-7), 2.62 (t, J = 5.7 Hz, 2, OCH_2CH_2N), 2.73 (br t, J = 6 Hz, 2, H-5), 4.05 (t, J = 5.7 Hz, 2, OCH_2CH_2N), 6.46 (d, J = 8.5 Hz, 2, Ar *H* ortho to OH), 6.62 (d, J = 8.5 Hz, 2, Ar *H* meta to OH), 6.57 (d, J = 8.3 Hz, 1, H-1), 6.72 (dd, J = 2.2, 8.3 Hz, 1, H-2), 6.88 (d, J = 2.2 Hz, 1, H-4), 7.0–7.2 (m, 5, 8-Ph), 9.24 (s, 1, OH). Anal. ($C_{27}H_{29}NO_2$).

3-Methoxy-8,9-diphenyl-6,7-dihydro-5*H*-benzocycloheptene (17). A solution of **9** (1.59 g, 8.36 mmol) in dry THF (5 mL) was added to a solution of PhMgBr [prepared in the usual manner from PhBr (2.63 g, 16.8 mmol) and Mg (400 mg, 16.8 mmol) in Et_2O (15 mL)]. After 20 h, the mixture was partitioned between dilute HCl (1 N; 30 mL) and Et_2O (30 mL). The Et_2O solution was concentrated, the residue was dissolved in EtOH (50 mL), concentrated HCl (30 mL) was added, and the mixture was heated under reflux for 4 h. The cooled mixture was then poured into H_2O (100 mL) and extracted with Et_2O (2 × 60 mL). The extracts were concentrated. Chromatography of the residue, eluting with 5% CH_2Cl_2 in petroleum ether (bp 60–80 °C), gave 3-methoxy-9-phenyl-6,7-dihydro-5*H*-benzocycloheptene as an oil (1.53 g, 73%). Incorporation of a phenyl group into the 8-position by the method described above for the conversion **10** → **12** proceeded in 70% yield, overall yield for **9** → **17** was 51%. The product **17** had mp 149–151 °C (from petroleum ether bp 80–100 °C): NMR ($CDCl_3$) δ_H 2.18 (quint, J = 7 Hz, 2, H-6), 2.41 (t, J = 7 Hz, 2, H-7), 2.80 (t, J = 7 Hz, 2, H-7), 3.83 (s, 3, OMe), 6.68 (dd, J = 2.7, 8.6 Hz, 1, H-2), 6.81 (m, 2, H-1 and H-4), 6.88–6.92 and 7.03–7.25 (m, 10, 2 Ph). Anal. ($C_{24}H_{22}O$) C, H.

3-[2-(Dimethylamino)ethoxy]-8,9-diphenyl-6,7-dihydro-5*H*-benzocycloheptene (7). A mixture of **17** (985 mg) and pyridine hydrochloride (1.7 g) was heated at 200 °C for 2 h and then cooled, and the resulting solid was broken up under Et_2O (100 mL) and dilute HCl (1 N; 100 mL) until it had dissolved. The Et_2O solution was dried (Na_2SO_4) and concentrated, and the residue was dissolved in petroleum ether, bp 60–80 °C. The crude phenol that precipitated was coollected by filtration and dissolved in DMF (30 mL), which was stirred under N_2 at 25 °C. NaH (0.7 g) was added, the mixture was warmed to 65 °C, and $Me_2NCH_2CH_2Cl\cdot HCl$ (0.75 g) was added in small portions over 30 min. After a further 30 min, the yellow color of the phenolate anion had discharged. The mixture was cooled in an ice bath, excess NaH was destroyed by the addition of 2-propanol (1 mL), and then the mixture was partitioned between Et_2O (100 mL) and H_2O (100 mL). The Et_2O solution was dried (Na_2SO_4) and concentrated. The residue was crystallized from petroleum ether, bp 80–100 °C to give crystals of **7** (681 mg, 58%): mp 125–126 °C; NMR ($CDCl_3$) δ_H 2.17 (quint, J = 7 Hz, 2, H-6), 2.35 (s, 6, NMe_2), 2.40 (t, J = 7 Hz, 2, H-7), 2.74 (t, J = 5.8 Hz, 2, CH_2N), 2.79 (t, J = 7 Hz, 2, H-5), 4.09 (t, J = 5.8 Hz, 2, OCH_2), 6.70 (dd, J = 2.6, 8.5 Hz, 1, H-2), 6.78 (d, J = 8.5 Hz, 1, H-1), 6.85 (d, J = 2.6 Hz, 1, H-4), 6.88–6.93 and 7.04–7.26 (m, 10, 2 Ph). Anal. ($C_{27}H_{29}NO$) C, H, N.

Binding-Affinity Studies.[15,21,22] Calf uterine cytosol was incubated at 18 °C for 30 min with 5×10^{-9} M [^{3}H]estradiol in the absence and presence of increasing amounts (10^{-9}–10^{-5} M) of the cyclic tamoxifen analogue (**5–8**) or unlabeled estradiol (control). Unbound compounds were then removed by dextran-coated charcoal, and the amounts of estrogen receptor bound [^{3}H]estradiol were measured. The relative concentrations of estradiol and cyclic tamoxifen analogues required to achieve 50% inhibition of [^{3}H]estradiol binding is the RBA; i.e., RBA = ([I_{50}] of estradiol/[I_{50}] of test compound) × 100. This procedure gives values of the same order of magnitude with cytosol from rat immature uterus, human breast tumors, or MCF-7 cells.

An MCF-7 whole cell assay was additionally carried out on the hydroxylated compounds **6** and **8**. MCF-7 cells were incubated at 37 °C for 50 min with 10^{-9} M [^{3}H]estradiol in the absence or presence of increasing amounts (10^{-10}–10^{-5} M) of **6**, **8**, or unlabeled estradiol (control). Bound compounds were then extracted with ethanol, and the amounts of estrogen receptor bound [^{3}H]estradiol were measured. The RBA values were calculated as for the cytosol assay.

Effect of the Cyclic Hydroxytamoxifen Analogues 6 and 8 on MCF-7 Cell Growth.[23] MCF-7 cells were plated at a density of 5000 cells/mL in 96-multiwell dishes. After 24 h of culture, compounds **6** and **8** were added to the culture dishes according to the protocol described previously. Estradiol was also added to evaluate its extent of antagonism of growth inhibition of compounds **6** and **8**. Final concentrations were as follows: estradiol, 10^{-8} M; compounds **6** and **8**, 10^{-8}, 10^{-7}, and 10^{-6} M. After 5 days of culture, the monolayer was fixed with 90% ethanol and colored with hematoxylin. The intensity of the coloration giving a measure of the number of cells was determined with a multiscan spectrophotometer at 540 nm (Flow Laboratories Inc).

Determination of the Effect of Compounds on Rat Uterine Growth. Immature female Sprague–Dawley rats were injected subcutaneously daily with solutions of compounds (Table II) in 0.1 mL of peanut oil for 3 days and then sacrificed on day 4. Uteri were removed, excess liquid was removed by blotting, and the uteri were weighed.

Acknowledgment. This investigation was supported by grants from the Cancer Research Campaign and Medical Research Council (to R.M.), from the Caisse Generale d'Epargne et de Retraite de Belgique (to G.L.), and from the National Institute of Health (Grant R01-CA 32713), Bethesda, MD (to V.C.J.).

Registry No. **6**, 113748-77-9; **7**, 113748-78-0; **8**, 113748-79-1; **9**, 6500-65-8; **10**, 113748-80-4; **11**, 113748-81-5; **12**, 113748-82-6; **13**, 109610-82-4; **14**, 113748-83-7; **15**, 113748-84-8; **16**, 113748-85-9; **17**, 113748-86-0; **17** (phenol), 113748-88-2; PhLi, 591-51-5; $Me_2NCH_2CH_2Cl\cdot HCl$, 4584-46-7; $ClCH_2CH_2Cl$, 107-06-2; PhMgBr, 100-58-3; Me_2NH, 124-40-3; 4-bromophenyl 2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenyl ether, 97631-87-3; 3-methoxy-9-phenyl-6,7-dihydro-5*H*-benzocycloheptene, 113748-87-1.

(21) Leclercq, G.; Deboel, M.-C.; Heuson, J.-C. *Int. J. Cancer.* **1976**, *18*, 750.
(22) Olea-Serrano, N.; Devleeschouwer, N.; Leclercq, G.; Heuson, J.-C. *Eur. J. Cancer Clin. Oncol.* **1985**, *21*, 965.
(23) Madeddu, L.; Roy, F.; Leclercq, G. *Anticancer Res.* **1986**, *6*, 11.

Electrophysiologic and Antiarrhythmic Activities of 4-Amino-*N*-[2-(diethylamino)ethyl]-3,5-dimethylbenzamide, a Sterically Hindered Procainamide Analogue

David W. Robertson,* E. E. Beedle, Harve Wilson, C. John Parli, Jeffrey K. Smallwood, and Mitchell I. Steinberg

Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, Indiana 46285. *Received November 12, 1987*

Procainamide is a widely used antiarrhythmic that is fraught with therapeutic limitations such as a short half-life, production of autoimmune antibodies and a lupus-like syndrome, and complex pharmacokinetics. We synthesized the congeners of procainamide possessing one or two methyl substituents ortho to the 4-amino moiety (compounds **4** and **5**, respectively), in order to sterically encumber the 4-amino substituent and prevent or diminish the rate of metabolic N-acetylation. Moreover, we anticipated that this structural alteration might eliminate the autoimmune toxicities associated with procainamide. Like procainamide, the two methylated analogues significantly reduced the rate of rise and amplitude of the action potential when studied in isolated canine Purkinje fibers. Whereas procainamide caused no significant change in action potential duration (APD), both methylated congeners significantly reduced APD at 70% and 95% repolarization. Moreover, the dimethylated congener was significantly more efficacious than procainamide in reducing ERP (effective refractory period) and increasing the ERP/APD_{70}. The ability of these compounds to block ouabain-induced arrhythmias was studied in anesthetized dogs. Addition of two methyl groups ortho to the amine produced an increase in potency: The conversion doses for procainamide and the monomethyl and dimethyl congeners were 19.0, 18.3, and 14.3 mg/kg, respectively, following iv administration. After iv administration to rats, procainamide was extensively metabolized to *N*-acetylprocainamide and displayed a half-life of 0.4 h. In contrast, dimethylprocainamide was *not* metabolized by N-acetylation, had a half-life of 1.4 h, and provided greater peak plasma concentrations. Thus, addition of methyl substituents ortho to the 4-amino group of procainamide alters the electrophysiological characteristics of the compound, increases its potency against ouabain-induced arrhythmias in vivo, increases its plasma half-life, and prevents N-acetylation.

Procainamide (**1**; Chart I) is a widely used antiarrhythmic drug that is sometimes effective in the management of ventricular premature depolarizations, life-threatening paroxysmal ventricular tachycardia, atrial

0022-2623/88/1831-1290$01.50/0