BIOORGANIC CHEMISTRY **24,** 81–94 (1996)
ARTICLE NO. 0009

Synthesis and Chiral Separation of Some Antitumor Agents

SATENDRA SINGH, KAREN L. MEYER,[1] AND ROBERT A. MAGARIAN[2]

Department of Medicinal Chemistry and Pharmaceutics, College of Pharmacy, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190

Received June 19, 1995

Four Z-isomers of 1,1-dichloro-2,2,3-triarylcyclopropane (DTACs), designed as potent antitumor agents, were synthesized from their appropriately substituted ethenes, which were prepared from the Grignard reaction followed by the dehydration of their intermediate carbinols. The stereospecific addition of dichlorocarbene to the ethenes followed by fractional crystallization afforded (*Z*)-1,1-dichloro-2-(4-benzyloxyphenyl)-2-(4-methoxyphenyl)-3-phenylcyclopropane and (*Z*)-1,1-dichloro-2,3-diphenyl-2-(4-methoxyphenyl)cyclopropane. Displacement of the bromo group from the ethoxy side chain intermediates with dimethylamine gave the desired basic side chain compounds, (*Z*)-1,1-dichloro-2,3-diphenyl-2-[4-(2-dimethylaminoethoxy)phenyl]cyclopropane and (Z)-1,1-dichloro-2-[4-(2-dimethylaminoethoxy)phenyl]-2-(4-methoxyphenyl)-3-phenylcyclopropane. While both E- and Z-stereoisomers of the DTACs were isolated using fractional crystallization, only the Z-compounds were resolved on a chiral stationary phase consisting of amylose tris-3,5-dimethylphenyl carbamate coated on silica gel. Complete resolution of the E-compounds was not observed with this system.

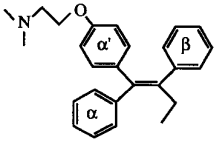
INTRODUCTION

Breast cancer is one of the most common and serious diseases affecting women. Current estimates indicate that one in eight American women who reach age 95 will develop breast cancer (*1*). It is generally believed that estrogen can directly stimulate the growth of breast cancer, therefore, its inhibition with antiestrogens at the estrogen receptor (ER) can provide a particularly useful strategy for the treatment of hormone-dependent breast tumors in postmenopausal females and possibly in premenopausal women and can act as a chemosuppressant in women at high risk of developing breast cancer. Nonsteroidal antiestrogens which exhibit potent antitumor effects represent a major advance in the management of breast cancer. The representative of this class is tamoxifen, (*Z*)-1-[*p*-(2-dimethylaminoethoxy)phenyl]-1,2-diphenylbut-1-ene (Chart **1**), the only antiestrogen clinically available for the adjunctive treatment of primary breast cancer in postmenopausal women (*2*, *3*). Its principal action is ascribed to its competition with natural hormone estradiol (Chart **1**) for binding to the estrogen receptor protein, thereby reducing the ability of estradiol to stimulate nuclear transcription and consequent cell growth

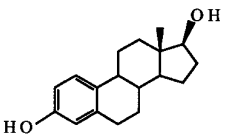
[1] Present address: Department of Botany and Microbiology, University of Oklahoma, Norman, OK 73019.

[2] To whom correspondence should be addressed.

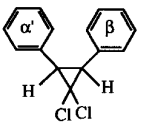
0045-2068/96 $18.00



1; Tamoxifen



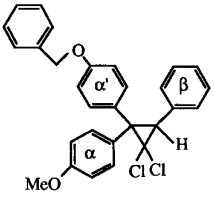
2; Estradiol



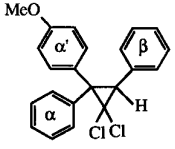
3; Analog II

CHART 1

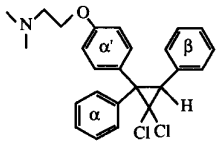
(*2*). Tamoxifen, however, has partial agonist activity which can lead to thromboembolitic events and secondary endometrial tumors, and virtually all patients with metastatic disease develop tamoxifen resistance (*4–6*). Because of the partial agonist activity of tamoxifen, researchers have been synthesizing both nonsteroidal and steroidal antiestrogens in an attempt to find pure antiestrogens (those devoid of any uterotropic activity) (*7–12*). The reasoning behind the search for pure antiestrogens is based on the recognition that antiestrogens generally possess partial estrogenic activity and complete blockade of estrogen action cannot be achieved by agents like tamoxifen. Novel pure antiestrogens should be more effective than partial agonists in reducing the mitogenic action of estrogen on the growth of breast tumors and complete ablation of hormonal-dependent tumor growth is very desirable since it could provide a more rapid and longer-lasting remission. In addition, pure antiestrogens could prove more effective in patients who experience relapse during tamoxifen therapy, serving as second-line treatment, and could dem-



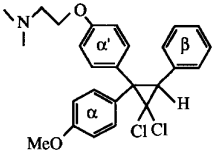
4



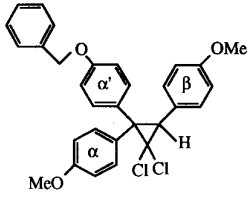
5



6



7



8

CHART 2

onstrate a greater efficacy in first-line treatment of advanced breast cancer (*11*, *13*, *14*).

The purpose of this earlier research was to search for antiestrogens that were devoid of estrogen agonist activity. Efforts in this area have led to several interesting experimental compounds (*15–23*). Reports from our laboratory (*15*, *16*) and others (*20*, *23*) have demonstrated that the introduction of a dichlorocyclopropyl or dihydroclopropyl moiety in place of the olefinic bridge in estrogenic stilbenes greatly reduces or abolishes their estrogenic action. One compound, analog II (Chart **1**), has antiestrogenic properties without estrogen agonist activity in the mouse and is comparable in activity to tamoxifen against the hormone-dependent 7,12-dimethyl benz[a]anthracene-induced rat mammary tumor model (*24*, *25*). Structure–activity relationship studies of some of the analog II derivatives (*15–17*, *26*) led us to design more effective cyclopropyl antiestrogens. Starting with analog II as the lead compound, we synthesized a series *Z*-1,1-dichloro-2,2,3-triarylcyclopropanes (Z-DTACs, **4–8**) by introducing a third phenyl ring and a polar substituent in analog II so that the compounds possess the structural features of both analog II and the clinically useful Z-triarylethylenes (Chart **2**). These Z-diastereomers were found to be antiestrogenic without any estrogenic activity in the mouse and inhibited the growth of ER positive MCF-7 human breast cancer cells in culture (*19*). As stereoisomers often exhibit distinct bioactivities, separating the enantiomers was necessary to fully evaluate the antitumor activity of the Z-DTACs. Unfortunately the synthetic methods used to prepare these compounds did not yield substantial amounts of the pure diastereomers required for further testing. In addition, unlike tamoxifen, the DTAC stereoisomers consist of enantiomeric pairs. In this study, we report a modified and improved synthetic method for the Z-DTACs, and the chiral separation of Z-isomers (**4–7**) into their (+) and (−) enantiomers for biological evaluation. The E-diastereomers of compounds **6** and **7** were also obtained for testing.

EXPERIMENTAL

Materials and Methods

Unless otherwise stated, all the starting materials were obtained from Aldrich Chemical Co. (Milwaukee, WI) and were used without further purification. Aqueous dimethylamine was obtained from Fluka Chemical Corp. (Ronkonkoma, NY). HPLC grade solvents, which were purchased from Fisher Scientific (Houston, TX) were filtered through a 0.45-μm nylon-66 membrane filter (Ranin Instrument Co., Inc., Woburn, MA) and manually mixed by volume prior to use. Anhydrous THF was freshly distilled from CaH_2.

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Elemental analysis was performed by Midwest Microlab Ltd. (Indianapolis, IN). ^{1}H NMR spectra were recorded on a Varian EM-360A or XL-300 spectrometer. Ultraviolet-visible spectra were recorded on a Shimadzu UV 160U spectrophotometer. Optical rotations were determined with a Rudolph Autopol III autonomic polarimeter.

HPLC Conditions

A Beckman Gold HPLC system equipped with dual Model 110 (A and B) pumps (only one pump was used) and 320SX controller, a 210A loop injector (20- and 500-μl loops were used), a 163 variable wavelength uv detector, and an Analog Interface Module 406 was used. Chiralpak AD columns, either analytical (250 $\times$ 4.6 mm, i.d.) or semipreparative (250 $\times$ 10 mm, i.d.) (Diacel Chemical Industries, CA) were used for chiral separation. These columns consisted of amylose tris-3,5-dimethylphenyl carbamate as chiral stationary phase coated on a 10-μm silica gel. A short nitrile guard column (5 μm, 10 $\times$ 3 mm, i.d.) (Regis Chemical Company, IL) was used with both columns. The columns were maintained at ambient temperature throughout the separation. Dead time was estimated with 1,3,5-tri-*t*-butylbenzene (*27*).

Three solvent systems were used at a flow rate of 1 ml/min for analytical analysis. Solvent system A consisted of hexane : ethanol (9 : 1; v/v), B consisted of hexane : 2-propanol (9 : 1; v/v), and C consisted of hexane : 2-propanol : dimethylamine (9 : 1 : 0.1; v/v). A volume of 5 μl containing 1 mg/ml of compound in the same solvent mixture was injected into the HPLC system. For semipreparative separation the same solvent system (A, B, and C) were used at 2.4 ml/min flow rate. A volume of 100–500 μl containing 6–7 mg/ml of compound dissolved in either hexane : 2-propanol (2 : 1 or 3 : 2) or hexane : chloroform (4 : 1) was injected.

Synthesis

Benzyl-4-benzyloxyphenyl ketone **(*10*).** The method of Iyer and Gopalachari (*28*) was used to prepare ketone **10.** A white solid crystallized from ethanol/benzene yielded white needles (7.1 g, 81%), mp 134–136°C (lit. (*28*) 132°C). ^{1}H NMR ($CDCl_3$; 60 MHz): δ 4.24 (s, 2H, $ArCH_2O$), 5.18 (s, 2H, CH_2CO), 7.04 (d, 2H, Ar), 7.28–7.42 (overlapping s, 10H, ArH), 8.07 (d, 2H, ArH).

(*Z/E*)-*1*-(*4-Benzyloxyphenyl*)-*1*-(*4-methoxyphenyl*)-*2-phenyl ethene* **(*12*).** A solution of Grignard reagent was generated from 4-bromoanisole (**11;** 7.5 ml, 59.6 mmol) and magnesium turnings (1.1 g, 43.7 mmol) in 280 ml of anhydrous THF which was refluxed for 2 h. A solution of **10** (12.0 g, 39.7 mmol) in THF (60 ml) was added and a gentle reflux was maintained for 16 h. The mixture was allowed to cool to room temperature and a saturated solution of NH_4Cl (12 ml) was added. The mixture was transferred to a separatory funnel and ether (15 ml) and water (5 ml) were added. The aqueous layer was removed and the ether layer was extracted with water (3 $\times$ 10 ml). The aqueous layers were combined and extracted with ether (3 $\times$ 15 ml). The combined ether layers were dried over anhydrous $MgSO_4$ and filtered, and the solvent was removed under reduced pressure. Without further purification, the intermediate carbinol was dehydrated with 2N H_2SO_4 (30 ml) in 95% ethanol (150 ml) at room temperature for 1 h. The mixture was then transferred to a separatory funnel, extracted with ether (3 $\times$ 15 ml), and the combined ether layers were washed first with a saturated aqueous solution of sodium bicarbonate followed by water until neutral. The organic layer was dried (anhydrous $MgSO_4$), filtered, concentrated, and purified over silica gel (pet ether/CH_2Cl_2, 6 : 1 to 1 : 1) to yield 9.2 g (63%) of an oil. Fractional crystallization from pet ether/acetone gave

TABLE 1
Characterization of Z/E Isomers

Comp. No.	Isomer	M.p. (°C)	^{1}H NMR data[a,b]					
			Cyclopropyl H	OCH_3	$OCH_2C_6H_5$	OCH_2CH_2	OCH_2CH_2	NCH_3
4	Z	153–156	3.53	3.79	5.02			
	E	134–136	3.53	3.78	5.03			
5	Z	128–130	3.53	3.75				
	E	116–120	3.53	3.76				
24	Z	92–96	3.54			4.23	3.63	
	E	113–115	3.52			4.24	3.59	
25	Z	128–129	3.50	3.76		4.22	3.60	
	E	114–120	3.52	3.79		4.25	3.62	
6	Z	111–112	3.52			4.02	2.69	2.31
	E	112–114	3.53			4.05	2.75	2.34
7	Z	120	3.49	3.76		4.01	2.69	2.31
	E	108–111	3.52	3.77		4.04	2.72	2.33

[a] ^{1}H NMR spectra were run on a Varian XL-300 spectrometer with TMS as internal standard in $CDCl_3$. The chemical shifts are given in ppm (δ).
[b] Compared to the ^{1}H NMR of the X-ray structure of the (Z)-triarylcyclopropanes (*37, 38*).

E-isomer (2.21 g, 14%) mp 114–115.5°C. ^{1}H NMR ($CDCl_3$; 300 MHz): δ 3.85 (s, 3H, OCH_3), 5.10 (s, 2H $ArCH_2O$), 6.85 (s, 1H, vinyl H), 6.85–7.00 (m, 4H, ArH), 7.02–7.20 (m, 7H, ArH), 7.23–7.32 (m, 2H, ArH), 7.32–7.52 (m, 5H, ArH).

The Z-isomer was obtained from mother liquor by fractional crystallization from pet ether/acetone (**12;** 3.41 g, 22%) mp 60–61°C. ^{1}H NMR ($CDCl_3$; 300 MHz): δ 3.86 (s, 3H, OCH_3), 5.10 (s, 2H, $ArCH_2O$), 6.86 (s, 1H, vinyl H), 6.87–7.00 (m, 4H, ArH), 7.05–7.20 (m, 7H, ArH), 7.27–7.31 (m, 2H, ArH), 7.35–7.52 (m, 5H, ArH).

(Z)-1,1-Dichloro-2-(4-benzyloxyphenyl)-2-(4-methoxyphenyl)-3-phenylcyclopropane **(*4*).** The Z-ethane of **12** (3.2 g, 8.2 mmol) was stirred with 50% aq NaOH solution (13 ml), benzyltriethylammonium chloride (TEBA, 0.3 g, 1.3 mmol) and chloroform (40 ml) for 72 h at room temperature. The organic and aqueous layers were separated and the aqeous layer was extracted three times with 25 ml of dichloromethane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was partially purified over silica gel (pet ether/benzene, 1:1) and recrystallized from pet ether/ethyl acetate to afford 1.9 g (49%) of the Z isomer as a white solid. The E-isomer of **4** was obtained in a similar manner from *E*-1-(4-benzyloxyphenyl)-1-(4-methoxyphenyl)-2-phenyl ethene in 41% yield after being recrystallized from pet ether/ethanol. ^{1}H NMR data are shown in Table 1.

(Z/E)-1-(4-Methoxyphenyl)-1,2-diphenyl ethene **(*16*).** Method A: A solution of 4-bromoanisole (**11:** 15.3 ml, 122 mmol) in THF (60 ml) was slowly added to a flame-dried flask containing magnesium turnings (2.69 g, 112 mmol) and THF (65 ml). The Grignard reaction was facilitated by iodine crystals and heat. The mixture was heated and refluxed for 2 h. Deoxybenzoin (**13;** 20.0 g, 102 mmol) in THF (65

ml) was then added and a gentle reflux was maintained for 5.5 h, cooled to room temperature, and treated with a saturated aqueous NH_4Cl solution (20 ml) for 30 min, then filtered and the filtrate extracted with ether (3 × 25 ml). Removal of solvent afforded an oil (8.0 g), which was dissolved in benzene (150 ml) and heated to reflux for 2.5 h in the presence of p-TSA (1.5 g). The mixture was transferred to a separatory funnel and extracted two times with 10 ml of saturated sodium bicarbonate solution. The aqueous extracts were combined and washed with benzene (3 × 20 ml). The benzene layers were combined, dried (anhydrous $MgSO_4$), filtered, concentrated under vacuum, and the resulting oil was purified over silica gel (pet ether/CH_2Cl_2, 1 : 0 to 7 : 1) to afford 23.0 g (79%) of the Z/E ethene mixture. ^{1}H NMR ($CDCl_3$; 60 MHz): δ 3.83 (s, 3H, OCH_3), 6.70–7.40 (m, 15H, ArH and vinyl H).

Method B: A solution of benzyl magnesium chloride (**15;** 2 M solution, 32 ml, 64 mmol) in THF was added dropwise to the reaction flask contianing 4-methoxybenzophenone (**14;** 12.0 g, 5 mol) and THF (45 ml). The reaction was warmed slowly and heated to reflux, which was maintained overnight. The reaction mixture was cooled and a saturated NH_4Cl solution (25 ml) was added. The mixture was transferred to a separatory funnel and other (50 ml) and water (25 ml) were added. The aqueous layer was separated and the ether layer was extracted with water (3 × 25 ml). The combined aqueous layers were extracted with ether (3 × 50 ml). The combined ether layers were dried (anhydrous $MgSO_4$), filtered, and concentrated under vacuum. The intermediate carbinol (18.5 g) was obtained and dehydrated with 1.4 g p-TSA in 90 ml benzene for 3.5 h at reflux. The mixture was cooled and the benzene layer was extracted with water until free of acid, dried (anhydrious $MgSO_4$), and concentraetd under vacuum. The residual oil was purified over silica gel (pet ether/CH_2Cl_2; 4 : 1), which afforded 8.91 g (56%) of a clear oil.

(*Z*)-*1,1-Dichloro-2,3-diphenyl-2-*(*4-methoxyphenyl*) *cyclopropane* **(*5*).** Ethene (**16;** 4.49 g, 15.7 mmol), 50% aq NaOH (25 ml), chloroform (40 ml), and TEBA (0.30 g, 1.3 mmol) were mixed together and stirred at room temperature for 72 h. The two layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 ml). The combined organic layers were dried (anhydrous $MgSO_4$), filtered, and concentrated under vacuum to afford 5.9 g crude product, which was purified over silica gel (pet ether/CH_2Cl_2, 100 : 1 to 4 : 1). Recrystallization of the white solid from pet ether followed by pet ether/acetone gave the Z-isomer as white crystals (0.69 g, 12%). The E-isomer (2.2 g) was obtained from the mother liquor. Recrystallization of the *E*-1,1-dichloro-2,3-diphenyl-2-(4-methoxyphenyl) cyclopropane from pet ether and then from pet ether/acetone gave 0.49 g (8.5%) of white crystals (see Table 1 for ^{1}H NMR data of Z- and E-isomers).

4-(*2-Bromoethoxy*)*benzophenone* **(*20*).** A mixture of 4-hydroxybenzophenone (**17;** 40.0 g, 202 mmol) and 1,2-dibromoethane (140 ml) was added to a flask containing 10% aqueous NaOH solution (140 ml) and TEBA (4.8 g, 20 mmol). The mixture was refluxed for 48 h and allowed to cool to room temperature at which time two layers separated. The aqueous layer was extracted four times with 100 ml of CH_2Cl_2 and the combined organic layers were washed free of base. Removal of the solvent and the recrystallization of the brown solid from 95% EtOH yielded 35.5 g (57%) of a light tan solid, mp 68–70.5°C (lit. (*29*) 62°C and lit. (*30*) 77°C).

^{1}H NMR ($CDCl_3$; 60 MHz): δ 3.68 (t, J = 6.3Hz, 2H, CH_2Br), 4.39 (t, J = 6.3Hz, 2H, CH_2O), 6.98 (d, 2H, ArH), 7.39–7.89 (m, 7H, ArH).

4-(2-Bromoethoxy)-4'-methoxybenzophenone **(*21*).** 4-Methoxybenzoic acid (**18;** 13.0 g, 85.5 mmol) and β-bromophenetole (**19;** 13 g, 65 mmol) were added to a flask equipped with a mechanical stirrer containing pyrophosphoric acid (PPA; 140 g). The reaction mixture was warmed to 100°C and stirred vigorously for 4 h. It was then cooled, ice/water was added, and the mixture was filtered. The filtrate was extracted with ether. The solvent was removed under vacuum, and residue was dissolved in $CHCl_3$ and extracted with 10% aqueous NaOH solution. Removal of solvent and recrystallization from MeOH afforded 15.7 g (72%) solid, mp 110–111°C (lit. (*31*) 111.5–113°C). ^{1}H NMR ($CDCl_3$; 60 MHz): δ 3.67 (t, 2H, J = 6.3Hz, CH_2Br), 3.91 (s, 3H, OCH_3), 4.39 (t, 2H, J = 6.3Hz, CH_2O), 6.96 (b, 4H, ArH), 7.85 (b, 4H, ArH).

(*Z/E*)*-1-*[*4-*(*2-Bromoethoxy*)*phenyl*]*-1,2-diphenyl ethene* **(*22*).** To a solution of ketone (**20;** 17.5 g, 57 mmol) in dry THF (50 ml) was added benzyl magnesium chloride in THF (2 M solution, 30 ml, 60 mmol), which was diluted with additional THF (20 ml). The resulting reaction mixture was refluxed overnight. The reaction mixture was cooled and a saturated solution of NH_4Cl (25 ml) was added. The product was extracted four times with 100 ml of ether which was washed with water until neutral (4 × 50 ml). Removal of the solvent afforded the intermediate carbinol, which was dehydrated using p-TSA (1.15 g) by heating to reflux for 4 h in benzene (90 ml). The reaction mixture was cooled and the organic layer was extracted with water until neutral (3 × 50 ml). Removal of solvent yielded an oil which was purified over silica gel (pet ether/CH_2Cl_2, 4:1) to afford a white solid 16.6 g (77%) of a mixture of Z/E-ethene, mp 92–94°C. ^{1}H NMR ($CDCl_3$; 300 MHz): δ 3.67 (t, 2H, J = 6.3 Hz, CH_2Br), 4.32 (t, 2H, J = 6.3Hz, CH_2O), 6.87 (s, 1H, vinyl H), 6.90–7.36 (m, 14 H, ArH).

(*Z/E*)*-1-*[*4-*(*2-Bromoethoxy*)*phenyl*]*-1-*(*4-methoxyphenyl*)*-2-phenyl ethene* **(*23*).** Ethene **23** was prepared as described above using the ketone (**21;** 5.50 g, 16.4 mmol) and benzyl chloride (2 M solution, 10.7 ml, 21.4 mmol) in THF. The reaction mixture was heated to reflux which continued overnight. The reaction mixture was cooled and ether (35 ml) and water (15 ml) were added. The two layers were separated and the aqueous layer was extracted with ether (3 × 35 ml). The combined ether layers were dried (anhydrous $MgSO_4$), filtered, and concentrated to obtain 7.78 g crude carbinol, which was dehydrated with p-TSA (0.6 g) in benzene (70 ml) while refluxing overnight. The benzene layer was washed with water (3 × 30 ml) until free of acid and stripped off under vacuum. Purification of the product by column chromatography over silica gel (pet ether/CH_2Cl_2, 4:1) yielded 5.20 g (78%) oil. ^{1}H NMR ($CDCl_3$; 300 MHz): δ 3.67, 3.69 (t, J = 6.6 Hz, 2H, CH_3Br), 3.86, 3.87 (s, 3H, OCH_3), 4.33, 4.34 (t, J = 6.6Hz, 2H, CH_2O), 6.89, 6.90 (s, 1H, vinyl H), 6.85–7.52 (m, 13H, ArH).

(*Z/E*)*-1,1-Dichloro-2,3-diphenyl-2-*[*4-*(*2-bromoethoxy*)*phenyl*]*cycloproane* **(*24*).** Ethene (**22;** 5.0 g, 13.2 mmol), TEBA (0.5 g,2.2 mmol), 50% aqueous NaOH solution (30 ml), and chloroform (55 ml) were stirred at room temperature for 95 h. The two layers were separated and the aqueous layer was extracted with CH_2Cl_2 (4 × 25 ml). The combined organic layers were dried and concentrated. After purification