Synthesis and Biological Evaluation of *gem*-Dichlorocyclopropyl and Cyclopropyl Analogs of Stilbene Congeners as Potential Antiestrogens

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Abstract □ A series of *gem*-dichlorocyclopropyl and cyclopropyl analogs of stilbene congeners was synthesized and examined for estrogenic and antiestrogenic activity using the uterotropic assay in the immature mouse. The relative receptor affinity *in vitro* was determined by measuring [^{3}H]estradiol displacement from the rat uterine cytosol receptor. The 11 test compounds synthesized in this study did not produce estrogenic or antiestrogenic activity at the dosage levels used (1–25 μg), but did produce a significant displacement of [^{3}H]estradiol in the rat uterine receptor binding assay with analog XVIII possessing the greatest binding affinity and compound XI the lowest affinity. Structure–affinity relationships of this series were established from the receptor binding assay and comparisons between these analogs and a previously reported series are summarized.

Keyphrases □ *gem*-Dichlorocyclopropyl analogs—synthesis, biological evaluation, cyclopropyl analogs, stilbene congeners, potential antiestrogens □ Stilbene—congeners, potential antiestrogens, synthesis, biological evaluation of *gem*-dichlorocyclopropyl and cyclopropyl analogs □ Antiestrogens—synthesis, biological evaluation of *gem*-dichlorocyclopropyl and cyclopropyl analogs of stilbene congeners □ Biological evaluation—synthesis, *gem*-dichlorocyclopropyl and cyclopropyl analogs of stilbene congeners, potential antiestrogens □ Cyclopropyl analogs—synthesis and biological evaluation, stilbene congeners, potential antiestrogens

In a previous paper (1)[1], a series of cyclopropyl analogs (I–X) of stilbene and stilbenediol was reported as a novel class of nonsteroidal estrogens and antiestrogens. The interesting pharmacological properties of these analogs prompted the investigation of a new series of related compounds. The preparation of some related cyclopropyl analogs (XI–XXI), their estrogenic, antiestrogenic, and receptor binding activities are reported here. The receptor binding effects of this new series were compared to the first series (1) to examine more fully the total structure–affinity relationships with the hope of providing additional insight into the steric requirements of the ligand that binds to the estrogenic receptor. This relationship between the ligand–receptor binding and biological response is of great interest, because elucidation of this relationship would help to clarify the mechanism of action of both estrogenic and antiestrogenic compounds at the cellular level.

EXPERIMENTAL[2]

Preparation of Starting Olefins—These compounds were not available and were prepared by previously reported procedures (2–12).

General Method for the Preparation of *gem*-Dichlorocyclopropyl Analogs XI–XVI (Table I) (13–15)—A molar ratio (1:10) of triethylbenzylammonium chloride to the starting olefin was dissolved in excess chloroform (10 times the molar concentration of olefin) contained in a three-neck flask fitted with an air condenser and dropping funnel. The

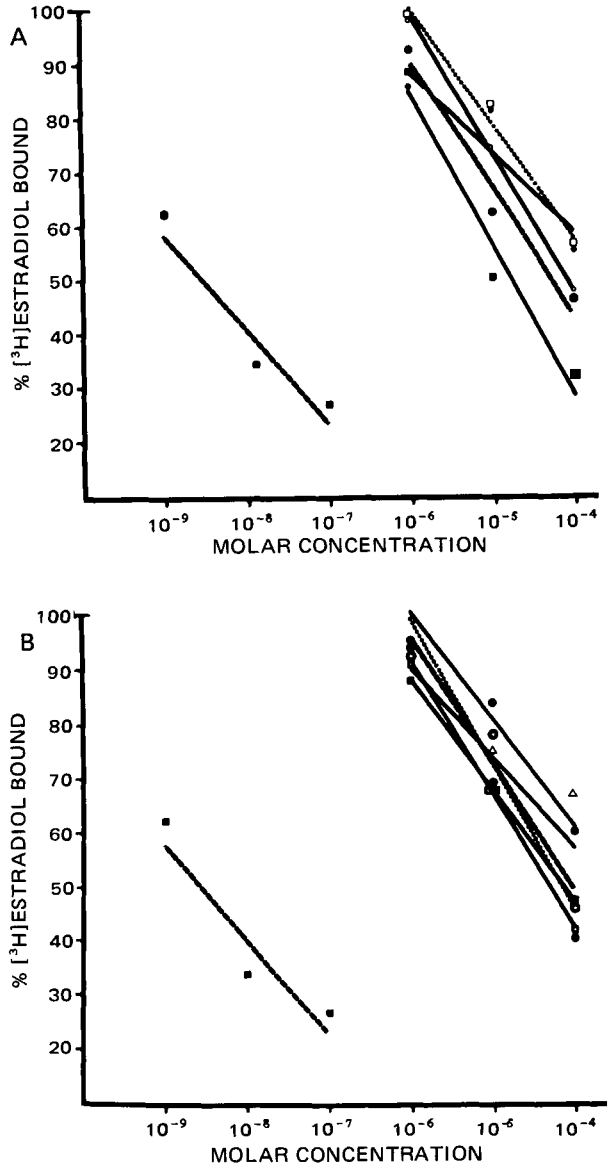

Figure 1—*Uterine receptor binding activity of the cyclopropyl analogs XI–XXI. The receptor binding data is presented in two parts (A and B) due to the overlap in binding between these compounds. Each point represents the mean of two determinations. Key: (A) (●) XI; (○) XIII; (⊙) XV; (⊡) XVIII; (□) XIX. (B) (▣) XII; (◉) XIV; (◘) XVII; (◉) XVI; (⊙) XX; (△) XXI. (A, B) (■) 17β-estradiol.*

[1] This is the third paper in a series. See Refs. 1 and 14 for previous papers.

[2] Melting points were determined on a Thomas-Hoover capillary melting point apparatus. Neither melting points nor boiling points are corrected. The elemental analyses were determined by Midwest Microlab, Inc., Indianapolis, Ind. IR spectra were determined with a Beckman IR20A spectrophotometer using polystyrene film as a standard to ascertain reproducibility. The PMR spectroscopic analyses were recorded in a Varian T-60 spectrometer using deuterochloroform as a solvent, and chemical shifts are reported relative to the internal standards tetramethylsilane. Analytical samples had compatible IR and PMR spectra.

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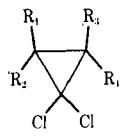
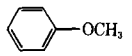
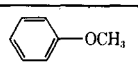
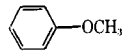
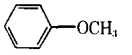
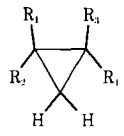
Table I—*gem*-Dichlorocyclopropyl Analogs of Stilbene Congeners

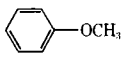
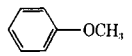
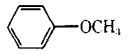
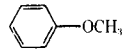


Compound No.	R_1	R_2	R_3	R_4	Formula	mp°	Yield, %	Analysis, % Calc.	Found
XI	H			CH_3	$C_{18}H_{18}O_2Cl_2$	99–100[a]	77	C 64.10 H 5.37 Cl 21.02	63.93 5.28 21.31
XII	CH_3			CH_3	$C_{19}H_{20}O_2Cl_2$	112[b]	48	C 64.96 H 5.74 Cl 20.19	64.97 5.69 20.10
XIII	H			CH_3	$C_{16}H_{14}Cl_2$	71–71.5[b]	57	C 69.33 H 5.09 Cl 25.58	69.32 5.21 25.73
XIV	CH_3			CH_3	$C_{17}H_{16}Cl_2$	142–143[c]	60	C 70.11 H 5.54 Cl 24.35	70.38 5.79 24.19
XV		H		CH_3	$C_{16}H_{14}Cl_2$	74.5–75.5[b]	62	C 69.33 H 5.09 Cl 25.58	69.58 5.15 25.51
XVI		CH_3		CH_3	$C_{17}H_{16}Cl_2$	109.5–110.5[b]	37	C 70.11 H 5.54 Cl 24.35	69.95 5.63 24.56

[a] Recrystallized from methanol. [b] Purified by sublimation. [c] Recrystallized from *n*-propyl alcohol.

Table II—Cyclopropyl Analogs of Stilbene Congeners



Compound No.	R_1	R_2	R_3	R_4	Formula	mp or bp°	Yield, %	Analysis, % Calc.	Found
XVII	H			CH_3	$C_{18}H_{20}O_2$	156–159/0.01 mm[a]	61	C 80.56 H 7.51	80.85 7.72
XVIII	CH_3			CH_3	$C_{19}H_{22}O_2$	105–106°[b]	68	C 80.81 H 7.86	81.10 7.62
IX	H			CH_3	$C_{16}H_{16}$	96–99/0.05 mm[a]	55	C 92.26 H 7.74	91.88 7.74
XX	CH_3			CH_3	$C_{17}H_{18}$	116.5–117.5[c]	80	C 91.84 H 8.16	91.58 7.92
XXI		H		CH_3	$C_{16}H_{16}$	97–99/0.05 mm[a]	69	C 92.26 H 7.74	91.29 8.13

[a] Distillation. [b] Purified by sublimation. [c] Recrystallized from methanol.

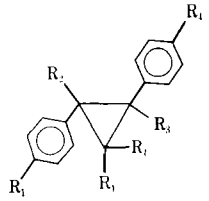
flask was cooled in an ice water bath and a 33–50% aqueous sodium hydroxide solution (sodium hydroxide–chloroform, 2:1) was added dropwise while the mixture was magnetically stirred. After the addition was completed, the ice bath was removed and stirring continued for 6–120 hr. The dark mixture was diluted with excess water and the aqueous layer was separated and extracted three times with chloroform. The chloroform extracts were combined, washed three times with water, dried over anhydrous magnesium sulfate, and filtered to remove the drying agent. The chloroform was removed under reduced pressure, yielding a dark liquid or solid which was purified as described in Table I. PMR ($CDCl_3$): XI, 7.13 (q, 8H), 3.80 (s, 6H), 3.05 (s, 1H), 1.40 (s, 3H); XII, 7.13 (q, 8H), 3.82 (s, 6H), 1.40 (s, 6H); XIII, 7.37 (s, 10H), 3.17 (s, 1H), 1.43 (s, 3H); XIV, 7.32 (s, 10H), 1.40 (s, 6H); XV, 7.13 (m, 10H), 2.85 (s, 1H), 1.80 (s, 3H); XVI, 7.33 (m, 10H), 1.77 (s, 6H).

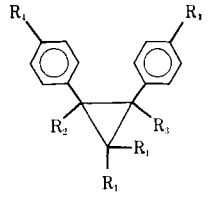
General Method for the Preparation of Cyclopropyl Analogs XVII–XXI (Table II)—The method of Gassman and Pape (16) was modified. *gem*-Dichloro intermediates XI–XVI and tetrahydrofuran were added to a 100-ml flask fitted with a reflux condenser. This solution was stirred by means of a magnetic stirrer and sodium metal was cut in small pieces and added to the solution followed by *tert*-butyl alcohol. The mixture was stirred and heated at reflux for 12 hr. The unreacted sodium was consumed with methanol and then water was added. The aqueous layer was separated and extracted twice with ether. The ether extracts were combined, dried over anhydrous magnesium sulfate, filtered, and the solvent removed under reduced pressure yielding a solid or liquid which was purified as described in Table II. PMR ($CDCl_3$): XVII, δ 7.04 (m, 8H), 3.70 (s, 6H), 2.23 (m, 3H), 1.18 (m, 3H); XVIII, δ 7.10 (q, 8H), 3.80 (s, 6H), 1.10 (s, 2H), 1.00 (s, 6H); XIX, δ 7.41 (m, 10H), 2.33 (m, 1H), 1.29 (m, 2H), 1.03 (s, 3H); XX, δ 7.35 (s, 10H), 1.18 (s, 2H), 1.03 (s, 6H); XXI, δ 7.16 (m, 10H), 2.60 (m, 2H), 1.93 (m, 1H), 1.36 (m, 3H).

Uterotropic Assay for Estrogenic and Antiestrogenic Activity—The assay for estrogenic activity was a modification (1) of the original uterotropic method of Rubin *et al.* (17) using Swiss-Webster mice. Each cyclopropyl analog was examined over a dosage range of 1–25 μg (total dose).

The uterotropic assay also was used to evaluate the antiestrogenic activity of the test compounds which did not produce an estrogenic response in the previous assay. The antiestrogenic assay was conducted as described for estrogenic activity, except that each animal in the cyclopropyl analog treatment groups received a standard stimulating dose of estradiol (0.04 μg). The test compounds and estradiol were administered separately at different injection sites to minimize possible physical interaction or reduced absorption of either compound. Antiestrogenic activity was measured as a decrease in estradiol-stimulated uterotropic response in groups which received both the test compound and estradiol as compared to a group that received estradiol alone.

Table III—Receptor Binding Assay



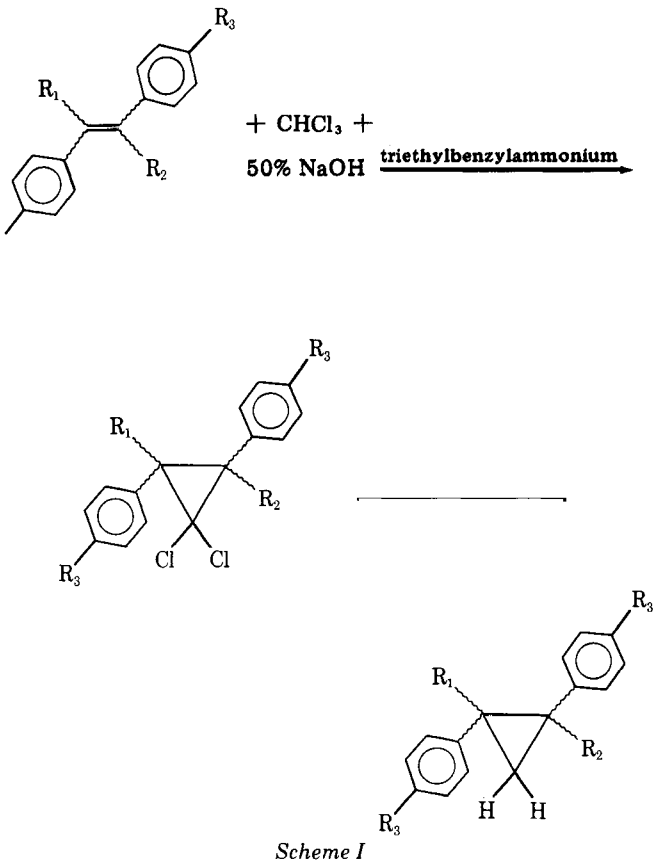


Compound No.	Configuration	R_1	R_2	R_3	R_4	Relative Binding Activity[a] (Estradiol Response, %)
Estradiol						100
XI	*trans*	Cl	CH_3	H	O—CH_3	8.3×10^{-5}
XII	*trans*	Cl	CH_3	CH_3	O—CH_3	5.9×10^{-4}
XIII	*trans*	Cl	CH_3	H	H	5.3×10^{-4}
XIV	*trans*	Cl	CH_3	CH_3	H	5.1×10^{-4}
XV	*cis*	Cl	CH_3	H	H	6.3×10^{-4}
XVI	*cis*	Cl	CH_3	CH_3	H	5.5×10^{-4}
XVII	*trans*	H	CH_3	H	O—CH_3	7.3×10^{-4}
XVIII	*trans*	H	CH_3	CH_3	O—CH_3	2.2×10^{-3}
XIX	*trans*	H	CH_3	H	H	1.5×10^{-4}
XX	*trans*	H	CH_3	CH_3	H	1.1×10^{-4}
XXI	*cis*	H	CH_3	H	H	8.0×10^{-4}

[a] $\frac{\text{Concentration of estradiol that displaced 50\% [}^3\text{H]estradiol}}{\text{Concentration of analog that displaced 50\% [}^3\text{H]estradiol}} \times 100$.

Receptor Binding Assay—A modification (1) of the competitive receptor binding assay method of Korenman (18) was used in this study. Uteri from mature (250 g) female Sprague-Dawley rats were used to prepare the cytosol receptor agent for this assay. Each test compound was assayed at three concentrations over a range of 10^{-4} to 10^{-6} *M* for the cyclopropyl analogs and 10^{-7} to 10^{-9} *M* for the estradiol standard. The test compounds were dissolved in ethanol, and in all cases the final concentration of ethanol was <2% of the total incubation volume.

The [^{3}H]estradiol displacement curve for each test compound was determined by linear regression analysis and plotted graphically. The relative receptor binding activity of each analog was determined using the ratio (concentration of unlabeled estradiol producing 50% displacement of [^{3}H]estradiol/concentration of cyclopropyl analog producing 50% displacement of [^{3}H]estradiol) × 100. Parallelism between the curve produced by each of the analogs and the unlabeled estradiol standard (an index of assay displacement specificity) was determined according to the method of Bliss (19).



Scheme I

RESULTS AND DISCUSSION

Chemistry—Scheme I illustrates the method of Dehmlow and Schonefeld (13) used for the synthesis of the *gem*-dichloropropanes (Table I) in this study. This is a phase transfer reaction catalyzed by triethylbenzylammonium chloride using chloroform and concentrated aqueous sodium hydroxide solution (33%) to generate a dichlorocarbene. The carbene adds stereospecifically across the double bond in the starting olefins, thus, generating the desired *gem*-dichlorocyclopropyl intermediate (Table I). This reaction was easily carried out and provided yields between 50 and 70%; however, analog XVI was obtained in only 37% yield, and XI and XII yielded dark mixtures before purification. The method of Gassman and Pape (16) was modified to remove the *gem*-dichloro groups from the cyclopropyl ring, and is illustrated in Scheme I. This modified reaction involved the use of the dichloro intermediate, sodium metal, and *tert*-butyl alcohol in refluxing tetrahydrofuran. The dehalogenated cyclopropyl analogs (Table II) were isolated in 60–80% yields.

Pharmacology—All cyclopropyl analogs prepared were tested for estrogenic and antiestrogenic activity in a concentration range of 1–25 μg (total dose) using the immature mouse uterotropic assay (17). None of the test compounds produced an estrogenic response (increase in uterine weight) or an antiestrogenic response (antagonism of estradiol-induced increase in uterine weight) within the dosage range used in this assay system.

Each test compound produced a significant displacement of [^{3}H]estradiol in the rat uterine receptor binding assay (Table III, Figs. 1A and 1B) with analog XVIII displaying the greatest binding affinity and compound XI the lowest. However, the binding affinity of most of the test compounds was similar and in the order of 10^{-3} to 10^{-5}% of 17β-estradiol on a molar basis. Accordingly, receptor binding curves were illustrated in two separate figures due to the significant overlap in analog-induced [^{3}H]estradiol displacement activity.

Since substituents play an important role in the receptor recognition of substrates, it was of interest to look at the nature of the substituent and its contribution to the binding processes. Consequently, the structure–affinity relationships exhibited by this substituted *cis*- and *trans*-diphenylcyclopropane series (Table III) are discussed briefly.

Structural modifications restricted to monomethyl (R_2) and dimethyl (R_2 and R_3) substitutions in *gem*-dichloro-*cis*- and *trans*-2,3-diphenylcyclopropanes (XIII–XVI) did not alter binding activity. The additional methyl group (R_3) in dimethyl substitution does appear, however, to increase activity when in combination with *p*-methoxyphenyl substituents (XII *versus* XI and XVIII *versus* XVII). *gem*-Dichloro substitution (R_1 = Cl) in monomethyl *trans*-diphenylcyclopropanes (XIX *versus*

XIII) and dimethyl analogs (XX *versus* XIV) generally produced a small increase in binding affinity. *p*-Methoxy groups increased activity in the dehalogenated cyclopropyl analogs (XIX *versus* XVII and XX *versus* XVIII) with the greatest receptor binding affinity found in compound XVIII. The *cis*-isomer XXI displayed greater receptor affinity than the *trans*-isomer XIX, but there were no apparent differences in the *gem*-dichloro analogs (XV *versus* XIII and XVI *versus* XIV).

When the receptor binding activities of these analogs were compared to the present compounds (1), it was found that the monomethyl and dimethyl substituents at R_2 and R_3 (Table III) in the hydrophobic cyclopropyl skeleton led to a reduction in receptor binding affinity of the derivatives, while diethyl substitution increased receptor binding ability.

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ACKNOWLEDGMENTS

Abstracted in part from a thesis submitted by John F. Stobaugh to the University of Oklahoma in partial fulfillment of the Master of Science degree requirement.

Moment Analysis for the Separation of Mean *In Vivo* Disintegration, Dissolution, Absorption, and Disposition Time of Ampicillin Products

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Received September 28, 1981, from the *Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, Japan.* Accepted for publication December 18, 1981.

Abstract □ The *in vivo* disintegration, dissolution, absorption, and disposition processes of ampicillin products are separated by means of moment analysis. This method is model-independent, that is, any specific model is not assumed. The mean residence time (MRT), mean absorption time (MAT), mean dissolution time (MDT), and mean disintegration time (MDIT) are calculated for several dosage forms of ampicillin. The fraction of dose absorbed (F) is also separated into several fractions corresponding to these *in vivo* processes. Bioavailability and bioequivalence are discussed in terms of the zero and first moments. The flip-flop behavior of ampicillin is proved by the fact that the MRT following intravenous injection is less than the MAT of any oral dosage form. Absorption of released ampicillin is proved to be a rate-determining step, since the MRT of released ampicillin in the GI tract is the greatest of all MRT corresponding to the *in vivo* processes. Moment analysis is compared with classical compartment theory, and a new component concept is introduced.

Keyphrases □ Ampicillin—moment analysis, *in vivo* disintegration, dissolution, absorption, disposition time □ Disintegration—ampicillin, moment analysis, *in vivo* dissolution, absorption, disposition time □ Dissolution—ampicillin, moment analysis, *in vivo* disintegration, absorption, disposition time □ Absorption—ampicillin, moment analysis, *in vivo* disintegration, dissolution, disposition time

In recent years moment analysis has been developed in the pharmacokinetic field as a method to comprehend drug behavior in the body, that is, absorption, distribution, metabolism, and excretion (1–10)[1]. Since statistical moments are characteristic of the shape of the statistical distribution curves such as plasma concentration–time curve or urinary excretion rate–time curve, they are only dependent on the observed time course data and are independent of the pharmacokinetic compartment model. Zero moment represents the area under the plasma concentration–time curve (AUC) or the total amount of drug excreted in urine, which is widely used as a model-independent parameter. The first moment, which is defined as the mean residence time (MRT), gives significant information with respect to kinetic features of the process which a drug undergoes in the GI tract and the body (1).

The absorption of a drug from its oral preparation involves a process too complex to be described by a simple mathematical equation. Therefore, a model-independent approach has been undertaken to evaluate the absorption rate (1–3, 11–13). These methods are based on deconvolution. The mean absorption time (MAT) is the useful index of the rate of bioavailability (1–3). The *in vivo* drug absorption involves disintegration and dissolution steps

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