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NOVEL AROMATASE INHIBITORS

A. S. BHATNAGAR,[1]* A. HÄUSLER,[1] K. SCHIEWECK,[1] L. J. BROWNE,[2]
R. BOWMAN[2] and R. E. STEELE[2]

Research Department, Pharmaceuticals Division [1]CIBA-GEIGY Ltd, CH-4002 Basel, Switzerland and [2]CIBA-GEIGY Corporation, Summit, NJ 07901, U.S.A.

Summary—Aminoglutethimide (AG), an inhibitor of the aromatase enzyme, inhibits the biosynthesis of estrogens and displays well-documented anti-tumor efficacy in breast-cancer. However, this efficacy is accompanied by a relative lack of specificity in inhibiting aromatase and moderate tolerability. We report on two new non-steroidal aromatase inhibitors (CGS 16949A and CGS 18320B) which are more potent, selective and efficacious in their inhibition of aromatase than AG. Both compounds inhibit aromatase more potently *in vitro* and *in vivo* (over 400 and 1000 times respectively) than AG. They are both more selective in their inhibition of aromatase with CGS 18320B showing an improved selectively over CGS 16949A. When administered to adult female rats, both compounds elicit responses in serum hormones similar to those seen after ovariectomy. The duration of action of CGS 18320B, however, appears to be longer than that of CGS 16949A. CGS 18320B and CGS 16949A cause almost complete regression of DMBA-induced mammary tumors in adult female rats and almost completely suppress the appearance of new tumors. Thus CGS 16949A and CGS 18320B represent significant advances in the search for novel aromatase inhibitors which are more potent, selective and efficacious than aminoglutethimide.

INTRODUCTION

Estrogen deprivation is well established as effective therapy in the treatment of estrogen-dependent breast cancer. Aminoglutethimide (AG), an inhibitor of the aromatase enzyme, has been clinically used in the treatment of breast cancer in postmenopausal women and its anti-tumor efficacy has been well-documented (for reviews see Refs [1, 2]). However, AG is a relatively non-selective inhibitor of the aromatase enzyme in that it also inhibits other enzymes involved in steroid biosynthesis at concentrations similar to those at which it inhibits aromatase [3, 4]. This, coupled with its moderate tolerability, spurred the search for aromatase inhibitors which were much more potent and selective in their action as inhibitors of estrogen biosynthesis than AG and were also better tolerated.

Inhibitors of aromatase can be broadly divided into two major chemical classes. Inhibitors whose structures are steroidal and thus function as substrate mimics for the enzyme and compounds which are non-steroidal in structure and most probably interact with the cytochrome $P\text{-}450_{Arom}$. An example of a commercially available steroidal aromatase inhibitor is testolactone and that of a non-steroidal inhibitor is aminoglutethimide (Orimeten®/Cytadren®).

In this review we describe the progress which has been made in the search for novel aromatase inhibitors in terms of their potency and selectivity *in vitro* and their efficacy *in vivo* in non-tumor bearing animals (endocrine efficacy) and in animals bearing DMBA-induced mammary carcinomas (anti-tumor efficacy). We report predominantly on two new non-steroidal aromatase inhibitors, CGS 16949A {4-(5,6,7,8-tetrahydroimidazo[1,5a]-pyridin-5-yl)benzonitrile monohydrochloride} and CGS 18320B {bis-(*p*-cyanophenyl)-imidazo-1-yl-methane hemisuccinate} and briefly on the steroidal aromatase inhibitor CGP 32349 (4-hydroxyandrostendione). The latter has been reviewed in this volume by Brodie and coworkers [5]. The chemical structures of these three new aromatase inhibitors along with the reference compounds aminoglutethimide are shown in Fig. 1.

POTENCY OF AROMATASE INHIBITION *IN VITRO*

The potency with which AG, CGS 16949A, CGS 18320B and CGP 32349 inhibit microsomal

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*To whom correspondence should be addressed: Dr Ajay S. Bhatnagar, c/o CIBA-GEIGY Ltd, K-125.802, CH-4002 Basel, Switzerland.

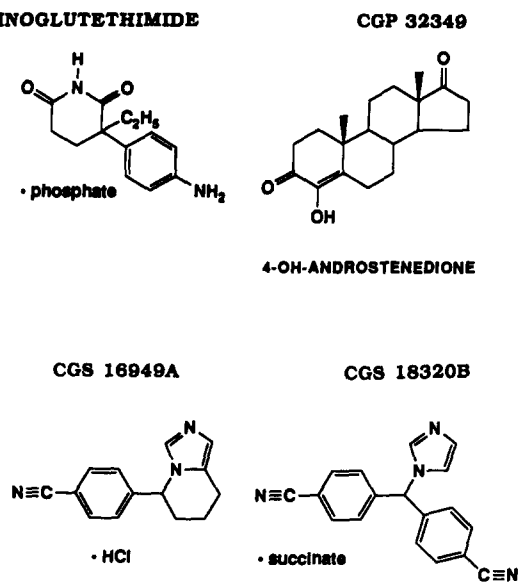

Fig. 1. Chemical structures of one steroidal and three non-steroidal aromatase inhibitors.

aromatase obtained from human placenta and rat ovary is shown in Table 1. The methodology for these *in vitro* assays has been described in detail previously [6]. CGS 18320B is clearly the most potent of these four inhibitors with an IC_{50} of 3 nM. Thus it is over 700 times as potent as AG, 20 times as potent as CGP 32349 and has about the same potency as CGS 16949A in the standard assay for aromatase obtained from human placenta. Other than AG, these inhibitors show competitive inhibition of the aromatase enzyme as evidenced from the generation of Lineweaver–Burk plots.

SELECTIVITY OF AROMATASE INHIBITION *IN VITRO*

We have recently reported on the methodology developed by us to assess selectivity of aromatase inhibition [3, 4]. These *in vitro* methods involve the measurement of LH-stimulated progesterone and estrogen production in hamster ovarian tissue [3] and ACTH-stimulated production of corticosterone and aldosterone in rat adrenal tissue [4]. Inhibition curves are generated using various concentrations of each inhibitor. The IC_{50}s with which each of the four inhibitors inhibits estrogen, progesterone, corticosterone and aldosterone production is shown in Table 2.

It is evident that of the four inhibitors, AG is the least selective inhibitor. AG inhibits the production of all 4 steroid hormones at similar concentrations, although the production of estrogen is inhibited most potently of the four.

CGS 16949A inhibits progesterone and corticosterone production at concentrations 5,000 and 3,000 times higher, respectively, than those needed to inhibit estrogen. However, aldosterone is inhibited at concentrations which are 30 times higher those needed for estrogen inhibition. This inhibition of aldosterone by CGS 16949A has also been documented *in vivo* in male rats by measuring circulating serum aldosterone concentrations [4].

CGS 18320B is about as effective as CGS 16949A in inhibiting estrogen production and also as selective terms of progesterone and corticosterone production. However, CGS 18320B has improved selectivity over CGS 16949A in terms of aldosterone inhibition in that it only inhibits aldosterone at concentrations which are 100 times higher than those needed for the inhibition of estrogen.

Of all of the four inhibitors, CGP 32349 shows the best selectivity profile. Although it is 15–30 times less potent in inhibiting estrogen than CGS 18320B and CGS 16949A, it does not significantly inhibit progesterone, corticosterone

Table 1. Concentrations for 50% inhibition (IC_{50}) of aromatase from human placenta and rat ovary by the four aromatase inhibitors. For experimental details see Ref. [6]

	IC_{50} (μM) for inhibition of			
	CGS 18320B	CGS 16949A	Aminoglutethimide	CGP 32349
Human placental aromatase	0.003	0.005	1.9	0.062
Rat ovarian aromatase	0.003	0.005	0.84	—
Type of inhibition	Competitive	Competitive	Mixed	Competitive

Table 2. Selectivity of aromatase inhibition by the four aromatase inhibitors. Concentrations for 50% inhibition (IC_{50}) of the production of estrogen and progesterone in hamster ovarian tissue and of the production of corticosterone and aldosterone in rat adrenal tissue *in vitro*. For experimental details see Refs [3, 4]

	IC_{50} (μM) for inhibition of			
	CGS 18320B	CGS 16949A	Aminoglutethimide	CGP 32349
Estrogen production	0.06	0.03	13.0	0.88
Progesterone production	>300.0	160.0	60.0	≥330.0
Corticosterone production	110.0	100.0	50.0	>330.0
Aldosterone production	6.1	1.0	110.0	>330.0

Table 3. Inhibition of aromatase *in vivo* by the three non-steroidal aromatase inhibitors. For experimental details see Refs [6, 7]

	ED_{50} (mg/kg, p.o.)		
	CGS 18320B	CGS 16949A	Aminoglutethimide
Inhibition of ovarian estrogen content	0.004	0.03	10.0
Inhibition of uterine hypertrophy	0.01	0.03	30.0

or aldosterone production at the highest concentrations tested. In our assay system, the concentration of 330 μM represents the limit of solubility of CGP 32349 in the aqueous incubation medium.

ENDOCRINE EFFICACY *IN VIVO*

We have reported previously on two methods developed by us to assess aromatase inhibition *in vivo* [6, 7]. The first method [6] measures the ovarian content of estrogen after treatment with the aromatase substrate androstenedione and an aromatase inhibitor. Inhibition of ovarian estrogen content serves as an index of the efficacy of the aromatase inhibitor *in vivo*. The second method [7] measures inhibition of the uterine weight increase resulting after treatment of immature female rats with a standard dose of androstenedione. This uterine hypertrophy is caused by the estrogen produced when androstenedione is aromatized in the ovary. Thus inhibition of this androstenedione-induced uterine hypertrophy is a further index of the efficacy of an aromatase *in vivo*.

The results obtained when the three non-steroidal aromatase inhibitors are administered orally in both of these models are tabulated in Table 3. CGS 16949A and CGS 18320B are both from about 300- to over 1000-fold more potent than AG in inhibiting aromatase *in vivo* in both models. CGP 32349 did not inhibit aromatase *in vivo* when doses up to 100 mg/kg were administered orally.

Administration of aromatase inhibitors to adult, cyclic female animals should result in attenuation of estrogen biosynthesis and, at best, should mimic the sequelae of surgical castration in terms of serum hormones and estrogen-dependent target tissues. We were able to show that CGS 16949A, when administered orally once-daily for 14 days, caused a significant and dose-dependent fall in serum estradiol and a significant rise in serum luteinizing hormone (LH) concentrations [7]. At the maximum daily oral dose of 3 mg/kg used in these experiments, serum estradiol and LH concentrations measured at autopsy, 4 h after the last dose, were similar to those seen 14 days after ovariectomy. Although uterine weight was significantly suppressed after treatment with CGS 16949A, it was only suppressed by about 20% of control values. Pituitary weight was significantly suppressed, but there was no effect on adrenal, thyroid or liver weights.

Using the same experimental protocol as was used in the studies with CGS 16949A [7], CGS 18320B was administered to adult cyclic female animals at a dose of 1 mg/kg. The results of this study are shown in Table 4. Serum estradiol concentrations were significantly reduced and serum LH was significantly elevated. Uterine weight was significantly suppressed to about 40% of control values. Pituitary and adrenal weights were also significantly suppressed. There was no effect on ovarian, thyroid or liver weights.

When one compares the results obtained with CGS 16949A and CGS 18320B, it is apparent, that CGS 18320B at a lower daily dose is more effective than CGS 16949A in reducing uterine weight which is a direct consequence of the reduction of circulating estradiol. Serum estradiol levels, however, are nearly equally and maximally reduced by both inhibitors. This apparent contradiction could be explained if

Table 4. Effects of a 14-day treatment with CGS 18320B (1 mg/kg once daily p.o.) on organ weights and serum hormone concentrations in adult female rats. Experimental details were identical to those reported for CGS 16949A [7]

	Relative organ weights (mg/100 g body wt)					
Treatment	Uterus	Ovary	Pituitary	Adrenal	Thyroid	Liver
Control	145 ± 18	45.5 ± 2.3	4.9 ± 0.3	29.3 ± 1.5	6.2 ± 0.8	4428 ± 96
CGS 18320B (1 mg/kg, p.o.)	84 ± 15**	51.9 ± 1.6	3.9 ± 0.2**	23.4 ± 1.1**	6.1 ± 0.8	4284 ± 56

	Serum hormone concentrations	
Treatment	Estradiol (pg/ml)	LH (ng/ml)
Control	32.8 ± 8.3	20.7 ± 2.3
CGS 18320B	12.6 ± 0.9**	144.0 ± 20.0**

**$2P < 0.01$ (Dunnett's t-test).

CGS 16949A had a shorter duration of action than CGS 18320B. Thus, the duration of action of each inhibitor was investigated after a single oral dose to animals whose serum estradiol levels were raised by pre-treatment with pregnant mare's serum gonadotropin (PMSG) [8]. It was determined that a single oral dose of 0.1 mg/kg CGS 16949A had a duration of action lasting about 14 h whereas CGS 18320B (0.03 mg/kg) still suppressed serum estradiol maximally after 24 h.

Thus in adult cyclic female animals, CGS 18320B is more efficacious in reducing uterine weight than CGS 16949A although both inhibitors are equally effective in reducing serum estradiol and raising serum LH levels. This higher efficacy is probably related to the longer duration of action of CGS 18320B as compared with CGS 16949A.

ANTI-TUMOR EFFICACY *IN VIVO*

The animal model used for these studies was the classical DMBA-induced mammary carcinoma in adult female rats. Details of the method have been reported previously [9].

Using the DMBA-induced mammary carcinoma model in rats, results with both CGS 16949A and AG have been reported previously [9]. It was found that CGS 16949A not only markedly and dose-dependently caused regression of palpable mammary tumors but also almost totally suppressed the appearance of new tumors during the treatment period. The ED_{50} of the anti-tumor effect of CGS 16949A was estimated to be between 0.1–0.5 mg/kg/day. AG, under the same conditions, is only marginally effective in reducing tumor growth at its maximally tolerated dose of 100 mg/kg/day.

The results obtained with CGS 18320B, using the same experimental conditions as those reported for CGS 16949A [9], are shown in Fig. 2 and Table 5. Just as with CGS 16949A, CGS 18320B also causes marked and dose-dependent regression of DMBA-induced mammary tumors as seen in Fig. 2. The ED_{50} for this effect is estimated to be about 0.05 mg/kg/day. As can be seen from Table 5, CGS 18320B also caused almost complete suppression of the appearance of new tumors. If the response of the tumors is based on the sum of the complete and partial regressions (CR + PR), then at the

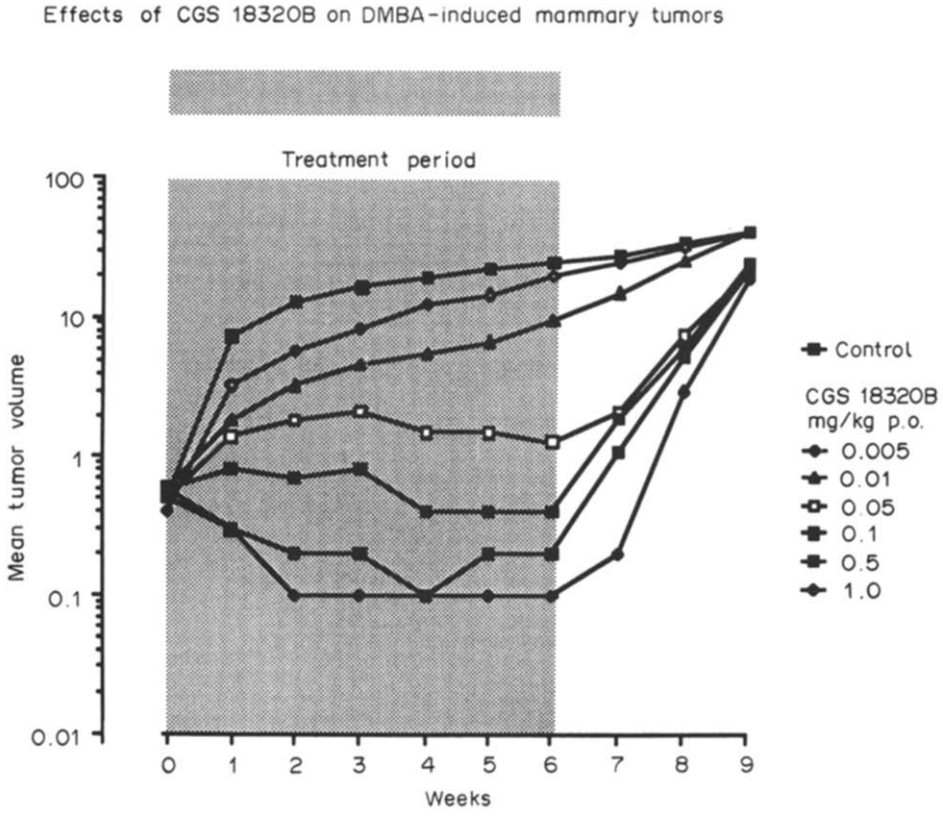

Fig. 2. Dose dependency of the effects of CGS 18320B on the mean tumor volume of DMBA-induced mammary tumors in female Sprague–Dawley rats. Experimental details are identical to those reported with CGS 16949A [9].

Table 5. Effects of continuous oral treatment with CGS 18320B on regression of established and suppression of new DMBA-induced mammary carcinomas in female Sprague–Dawley rats at the end of 42 days of treatment. Experimental conditions were identical to those reported for CGS 16949A [9].

Treatment	No. of tumors[1]	Response[2] (CR + PR)%	SD%	P%	New tumors
Control	27	0	11	89	67
CGS 18320B (p.o. × 42)					
0.005 mg/kg	25	0	16	84	66
0.01 mg/kg	26	4	15	81	53
0.05 mg/kg	26	85	11	4	7
0.1 mg/kg	22	68	23	9	8
0.5 mg/kg	25	80	12	8	2
1.0 mg/kg	28	96	4	0	1

CR = complete regression: tumors not palpable; PR = partial regression: decrease of 50–99% in tumor size; SD = stable disease: no change in tumor size; P = progression: increase of > 10% in tumor size. [1]At start of treatment; [2]at end of treatment.

highest dose of 1.0 mg/kg/day, CGS 18320B causes almost complete regression of tumors existing at the start of the experiment and almost totally prevents the appearance of new tumors.

Thus in the DMBA-induced mammary carcinoma model, CGS 18320B and CGS 16949A are highly effective in causing the regression of tumors. At all doses tested, both CGS 16949A and CGS 18320B were well tolerated over the duration of the treatment period. In terms of anti-tumor efficacy and tolerability, both CGS 16949A and CGS 18320B are far superior to aminoglutethimide in this tumor model.

CONCLUSIONS

The development of novel aromatase inhibitors for use in the treatment of estrogen-dependent breast cancer has been reviewed here in terms of potency, selectivity and efficacy of aromatase inhibition. The reference starting point was aminoglutethimide which, although clinically effective, is not specific in its inhibition of aromatase within its therapeutically effective dose-range and whose tolerability is moderate. The new non-steroidal inhibitors described here are orders of magnitude more potent in inhibiting aromatase than AG. Although their selectivity profiles are vastly improved compared to AG, threading-the-needle of complete selectivity has yet to be achieved. However, report of a new non-steroidal compound [10] which has an improved selectivity profile over the inhibitors mentioned here and which is in the early stages of clinical testing is encouraging.

The striking endocrine and anti-tumor efficacy of CGS 16949A and CGS 18320B in animals coupled with the enhancements in potency and selectivity represent significant advances in the search for aromatase inhibitors which are more potent, selective, efficacious and better tolerated than aminoglutethimide.

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