Structure–Activity Studies of 2,3,4,4a,5,9b-Hexahydroindeno[1,2-*c*]pyridines as Antispermatogenic Agents for Male Contraception[1]

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Analogs of (4a*RS*,5*SR*,9b*RS*)-2-ethyl-2,3,4,4a,5,9b-hexahydro-7-methyl-5-*p*-tolyl-1*H*-indeno[1,2-*c*]pyridine (Sandoz 20-438, **10a**; R^1 = ethyl, R^2 = R^3 = methyl, R^4 = H) have been synthesized and tested in mice for their ability to reduce testes weight and disrupt spermatogenesis. The activity was strongly dependent on stereoisomerism and chirality, consistent with a mechanism of action involving interaction with a specific macromolecule. It was affected by changes in the nitrogen substituent and most strikingly by changes in the *p*-substituent of the 5-aryl ring. A hydrogen, fluorine, hydroxy, or methoxy substituent led to loss of activity, whereas methyl (Sandoz 20-438, **10a**), carboxylate (RTI-4587-054, **10k**; R^1 = ethyl, R^2 = methyl, R^3 = COOH, R^4 = H), ester (RTI-4587-056, **12b**; R^1 = ethyl, R^2 = methyl, R^3 = COOMe, R^4 = H), formyl (RTI-4587-030, **12i**; R^1 = ethyl, R^2 = methyl, R^3 = CHO, R^4 = H), or hydroxymethyl (RTI-4587-055, **12g**; R^1 = ethyl, R^2 = methyl, R^3 = CH2OH, R^4 = H) groups resulted in antispermatogenic compounds. Methyl ester **12b** was an effective antifertility agent, without apparent effects on mating, when given orally to male mice at 7–15 mg/kg daily for 35 days. Further evaluation of these compounds as male contraceptive agents and probes for study of spermatogenesis appears warranted.

A safe and effective nonhormonal male contraceptive drug would provide a new dimension in fertility control. An ideal contraceptive for the male would effectively and reversibly arrest the production of spermatozoa or block their fertilizing capacity without affecting hormonal status, libido, or accessory sex organs and their functions. The induction of azoospermia or severe oligospermia is currently possible by using hormonal steroids or gonadotrophic hormone-releasing hormone (GnRH) agonists/antagonists. Supplementary androgen is used with the GnRH analogs to overcome the problem of diminished libido and secondary sex characteristics.[2]

The goal of an effective antispermatogenic agent can be approached by seeking a lead in the biology of compounds primarily designed for effects unrelated to contraception but which have unexpectedly revealed antifertility effects on routine biological evaluation. Structure–activity relationship (SAR) studies based on such compounds can not only lead to clinically useful products but in conjunction with biological studies can help to identify steps in the reproductive process which are susceptible to attack and thus open up new areas for study.

The hexahydroindenopyridine derivative (4a*RS*,5*SR*,-9b*RS*)-2-ethyl-2,3,4,4a,5,9b-hexahydro-7-methyl-5-*p*-tolyl-1*H*-indeno[1,2-*c*]pyridine (Sandoz 20-438, **10a**) shown in Scheme **1** was synthesized by Ebenother, Bastian, and Gradient over 20 years ago.[3] Serotonin antagonism and analgesic activities were stated to be associated with this type of compound.[3] In the course of toxicity studies, Sandoz 20-438 showed strong antispermatogenic activity in dogs. Similar effects were found in mice and rats. The compound shows no overt toxicity nor effects on libido,[4,5] nor does it have genetic toxicity, since it had no dominant lethal effects and no mutagenic potential in a variety of assays.[6] A single oral dose of 30 mg/kg to rats markedly reduces the weight of the testes within 24 h. Degenerative changes in the seminiferous tubules are observed. Spermatids become pycnotic, occasionally forming multinucleated associations. Sertoli cells remain in the control range.[4] Serum LH and FSH in chronically treated rats are slightly increased, indicating that the compound does not act by gonadotrophin suppression. Leydig cell function is not affected since there is no reduction in the weight of the seminal vesicles. Chronic administration to dogs induces complete inhibition of spermatogenesis with no signs of general toxicity. Erection capability and ejaculation are not inhibited, although the sperm number falls to zero. Drug withdrawal is followed by return of sperm number and histological appearance to normal within 8–11 weeks.[5]

No recent studies have been reported on developing this very interesting structural lead. Thus, almost no published information on SAR was available prior to our current work. Augstein et al.,[7] however, briefly mentioned that the analogous 4a,9a-*cis*-H9,H9a-*cis*-9-phenyl-2,3,4,4a,9,9a-hexahydro-1*H*-indeno[2,1-*c*]pyridine causes "testicular damage" in rats at doses in excess of 25 mg/kg/day given for 12 weeks. On the basis of these reports, compound **10a** was selected as the lead compound in our search for new contraceptive agents for the male.

Chemistry

The synthetic route to indenopyridine analogs (Scheme **1**) followed the general patent procedures of Ebenother et al.[3] with some modifications. Although the *all-trans* isomer of Sandoz 20-438 (compound **3** of Scheme **1**) was

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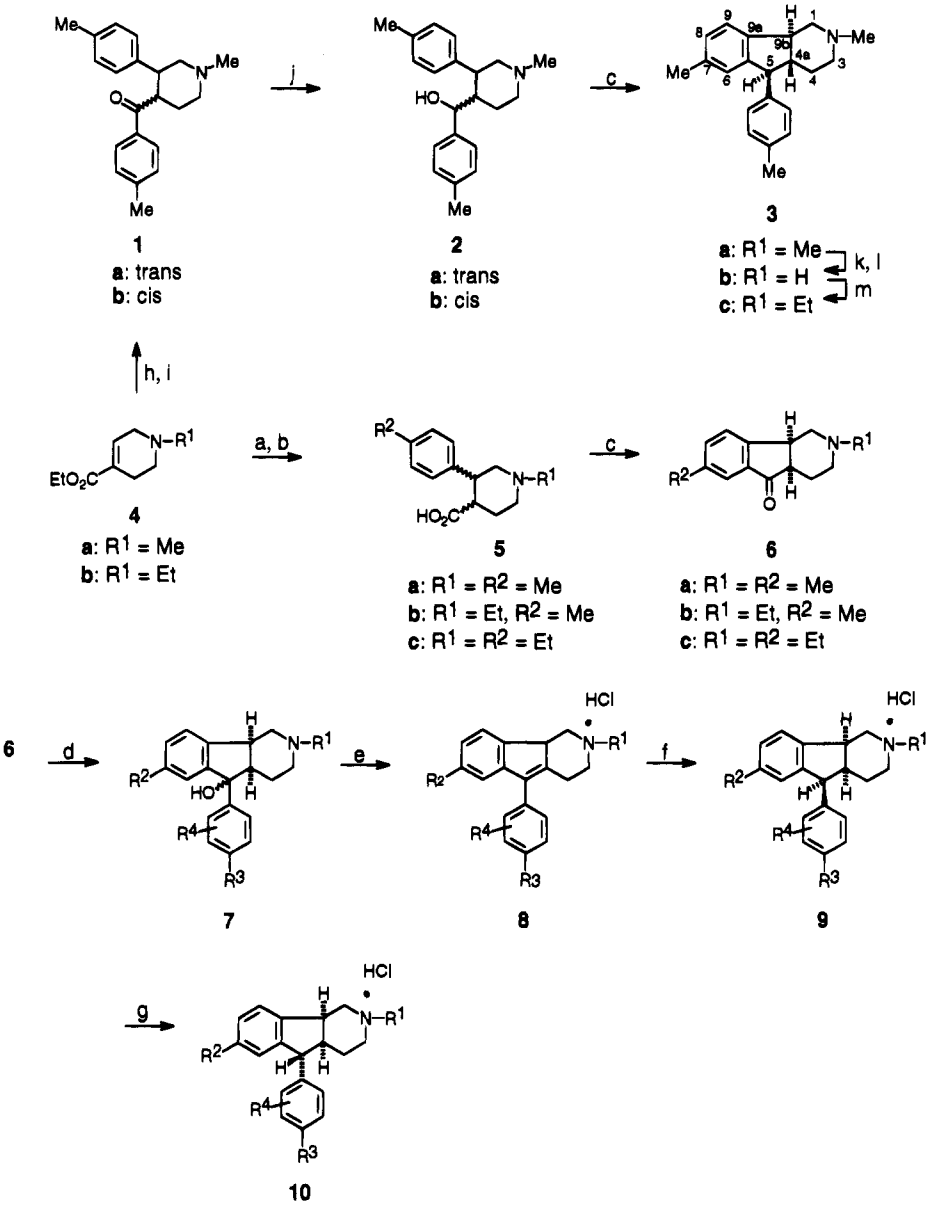
Scheme 1[a]



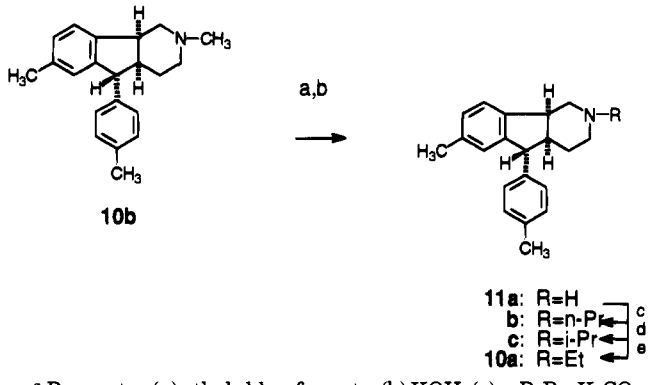
[a] Reagents: (a) p-$R_2C_6H_4MgBr$, −10 °C, Et_2O; (b) 18% HCl, reflux; (c) PPA; (d) ArLi or ArMgBr; (e) 5% HCl, MeOH, reflux; (f) $PdCl_2$, $NaBH_4$, H_2 (3 atm), 50 °C, EtOH/H_2O; (g) 25% (w/v) KOH, n-butanol, reflux; (h) p-MeC_6H_4MgBr, THF, reflux; (i) separate diastereomers; (j) $NaBH_4$, EtOH; (k) ethyl chloroformate; (l) KOH; (m) EtI, K_2CO_3. Compounds were racemates. Relative stereochemistry only is shown.

made on a small scale from the *N*-ethyl compound **4b**, large scale synthesis began with the commercially available *N*-methyl compound **4a**. Following the route shown in Scheme **1**, reaction of **4a** with 2 mol of *p*-tolylmagnesium bromide gave predominantly the *trans* isomer **1** along with a small amount of *cis* isomer. Sodium borohydride reduction of *trans*-**1** gave an alcohol. On the basis of the relatively sharp melting point and NMR spectrum, the isolated material was probably a single diastereoisomer, but absence of the other isomer was not established. Polyphosphoric acid cyclization yielded hexahydroindenopyridine **3a**. The base **3a** was N-demethylated to the nor compound **3b**, which was ethylated to yield **3c**. Attempted epimerization of **3c** at the 5-position with refluxing KOH in *n*-butanol (conditions which epimerize the *all-cis* compound **9**) was unsuccessful, due presumably to the thermodynamic stability of the *trans*-4a,5 system of **3c**.

The synthesis of the *all-cis* compounds **9** and the 4a,9b-*cis*,4a,5-*trans*-compounds **10** began with reaction of ethyl isonicotinate with iodoethane followed by reduction with $NaBH_4$ to give the tetrahydropyridine **4b**.[8] This compound or the commercially available methyl analog **4a** was treated with arylmagnesium bromide and then hydrolyzed to give **5** (Scheme **1**) which was cyclized with polyphosphoric acid to yield the ketone intermediate **6**. The reaction of ketone **6** with arylmagnesium bromide or aryllithium followed by dehydration gave olefin **8**. Reduction of the olefin with $H_2/PdCl_2/NaBH_4$[9] resulted in the *all-cis* compound **9**. Epimerization of **9** at the C-5-position by refluxing in *n*-butanol containing KOH gave the 4a,9b-*cis*,4a,5-*trans* compound **10**. Use of the appropriate aryl Grignard or aryllithium reagents gave the analogs shown. The *N*-propyl and *N*-isopropyl analogs (**11c,d**) were obtained by the alkylation of the corresponding secondary amine **11a** (Scheme 2) obtained by N-demethylation of **10b**. The N-ethyl analogs could also be made in this manner.

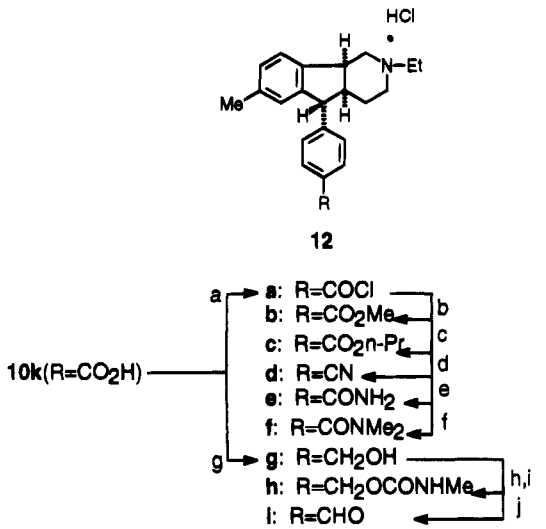
Reaction of the tricyclic ketone **6b** (R^1 = Et, R^2 = Me) with the dilithium reagent obtained from p-bromoben-

Scheme 2[a]



[a] Reagents: (a) ethyl chloroformate; (b) KOH; (c) *n*-PrBr, K_2CO_3; (d) *i*-PrBr, K_2CO_3; (e) EtI, K_2CO_3. Compounds were racemates. Relative stereochemistry only is shown.

Scheme 3[a]



[a] Reagents: (a) $SOCl_2$; (b) MeOH; (c) *n*-PrOH; (d) $SO_2(NH_2)_2$, sulfolane; (e) concentrated NH_4OH; (f) 40% $NHMe_2$ (aq); (g) B_2H_6, THF; (h) 20% phosgene in toluene; (i) 40% $MeNH_2$ (aq); (j) Dess–Martin periodinane, CH_2Cl_2. Compounds were racemates. Relative stereochemistry only is shown.

zoic acid and 2 equiv of *n*-butyllithium led to the olefin **8k** (R^1 = Et, R^2 = Me, R^3 = COOH) which was catalytically reduced and then epimerized to the carboxylic acid **10k**. As shown in Scheme 3, **10k** was reduced with diborane to the hydroxymethyl analog **12g** which was then oxidized by periodinane to the aldehyde **12i**. Further analogs were synthesized by derivatization of aldehyde **12i** or alcohol **12g** or by reactions of the intermediate acid chloride **12a**. The nitrile **12d** was formed in good yield by the acid-catalyzed decomposition of an intermediate *N*-acylsulfonamide.

The synthesis of B-seco analog **13** (Figure 1) was accomplished by N-ethylation and hydrolysis of ethyl isonipecotate to the corresponding *N*-ethyl-4-piperidinecarboxylic acid. Reaction with the lithio derivative obtained from *m*-bromotoluene and *n*-butyllithium gave the corresponding ketone, which upon reaction with *p*-tolylmagnesium bromide followed by dehydration gave olefin **14**. Hydrogenation of **14** by the procedure of Canas-Rodriguez[9] gave a complex mixture of overreduced products. However, hydrogenation under milder conditions (10% Pd/C, 50 °C) gave **13** in very good yield. Compounds **16a,b** were synthesized by the route shown in Scheme 1 for **2a,b**, beginning with **4b**. The synthesis of the indeno[2,1-*c*]pyridine analog **17** (Figure 1) began with arecoline hydrochloride but was otherwise completely analogous to the synthesis of Sandoz 20-438 from *N*-methyl compound **4a**.

Structural Verification by NMR Studies. The stereochemistry of the compounds was established by NMR techniques. A 2-D-COSY spectrum of Sandoz 20-438 allowed an almost complete assignment of individual protons on the hexahydropyridine ring. However, there was some ambiguity in the assignments of 4-eq-H (2.6 ppm) and 3-ax-H (2.9 ppm). The application of a 2-D-HETCOR spectrum resolved this problem and also provided information on C-13 chemical shifts which permitted assignment of carbon atoms of **10a** except for those of the aromatic groups. The chemical shift assignments of protons and carbons are summarized in Tables 1 and 2, respectively.

Identification of the 5-hydrogen in the NMR spectrum helped to confirm the stereochemistry of the isomers **3c**, **9a**, and **10a**. For the compound with the 4a,5 hydrogens *cis* to one another (**9a**), the 5-H appeared at 4.50 ppm with a coupling constant of 8.7 Hz. In its *trans* isomer (**10a**), the position shifted to 4.12 ppm with J = 10.6 Hz. For the *all-trans* isomer **3c**, the position of the 5-H moved to 4.20 ppm but the J-value remained at 11.0 Hz. The *all-cis* configuration of **9** is consistent with addition of hydrogen to olefin **8** from the 9b-H face. The *trans*-4a,9b stereochemistry of **3** was derived from the *trans* stereochemistry of intermediate **1** ($J_{3,4}$ = 11.0 Hz).

Resolution of Racemates. Racemic Sandoz 20-438 was resolved by formation of diastereoisomeric salts with the enantiomers of 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (BNPPA). The optical purity of the enantiomers was established by chromatography on a chiral (cellulose carbamate on silica gel) column. The 5-(*p*-carbomethoxyphenyl) racemate **12b** was resolved by diastereoisomeric salt formation with (*R*)- and (*S*)-mandelic acid. Optical purity of the resulting (*d*)-**12b** and (*l*)-**12b** was established by chiral chromatography. Hydrolysis of the individual enantiomers yielded the enantiomers (*d*)-**10k** and (*l*)-**10k** of the corresponding 5-(*p*-carboxyphenyl) racemate **10k**. The *d*- and *l*-designations are based on rotation at the sodium D line in chloroform for **12b** enantiomers and methanol for the **10k** enantiomers. Possibly as a result of the solvent difference, optical rotation does not reflect stereochemical equivalence. Thus, hydrolysis of (*d*)-**12b** yielded (*l*)-**10k** and (*l*)-**12b** yielded (*d*)-**10k**.

Biology

One difficulty in identifying a male contraceptive agent which affects spermatogenesis is the long dosing time often required to observe an effect on fertility. This time factor and the accompanying necessity for significant quantities of compound for dosing impede the testing and slow down the screening of compounds. Therefore, for screening purposes, we utilized direct observation of the testis in order to find early effects which should be related to antifertility. The present series of compounds has a readily observable effect on both testis weight and also the spermatogenic index (SI). This index[10] was used to assign a semiquantitative estimate of the sperm producing ability of the testes, based on the histologic appearance of the spermatogenic cells in the seminiferous tubules throughout one or more

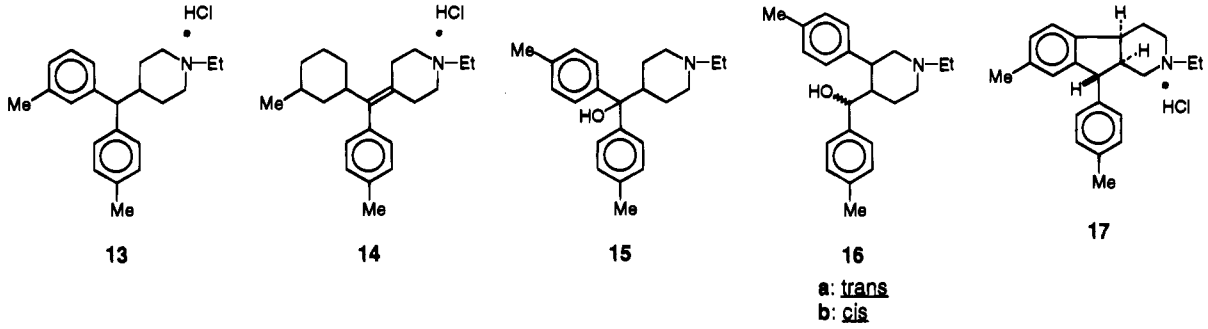


Figure 1. Miscellaneous analogs tested.

Table 1. ^{1}H NMR of Sandoz 20-438 (HCl Salt)[a]

H	chemical shift (ppm)
NCH_2CH_3	1.5 (t)
4 ax	1.87 (bfd)
aromatic (CH_3)	2.22 (s)
aromatic (CH_3)	2.38 (s)
1 ax	2.41 (m)
4 eq	2.60 (m)
4a	2.68 (m)
3 ax	2.95 (m)
NCH_2CH_3	3.10 (q)
3 eq	3.49 (m)
1 eq	3.51 (m)
9b	4.0 (m)
5	4.12 (d)
aromatic H	6.9–7.7

[a] Data were obtained at 500 MHz in $CDCl_3$ (solvent).

Table 2. ^{13}C NMR of Sandoz 20-438 (HCl Salt)[a]

carbon	chemical shift (ppm)
β (CH_3)	9.09
aromatic (CH_3)	21.05
aromatic (CH_3)	21.05
4	21.30
9b	40.02
4a	45.88
3	48.00
5	50.61
α (CH_2)	52.42
1	53.45
aromatic	123.77–145.81

[a] Data were obtained at 500 MHz in $CDCl_3$ (solvent).

cross-sections of the testes. Each testis was rated for its spermatogenic potential on a scale of 0–6. If seminiferous tubules at various stages of the spermatogenic cycle were not affected in the same manner, an average SI was assigned to the testicular cross-section.

The numerical index and the criteria for rating were as follows: 0—no cells or only Sertoli cells present; 1—Sertoli cells and spermatogonia present; 2—Spermatogonia and spermatocytes present; 3—Spermatogonia, spermatocytes, and round spermatids present in normal numbers (early; steps 1–9 for mice, steps 1–9 for rats) with fewer than 5 late spermatids/tubule; 4—Spermatogonia, spermatocytes, and early spermatids with 5–25 late spermatids present/tubule; 5—all cell types present and 50–75 late spermatids present/tubule; or 6—all cell types present and more than 100 late spermatids/tubule.

Results and Discussion

Selection of Screening Protocol. Effects were observed in the testes of mice as early as 6–12 h after treatment with a single 30 mg/kg oral dose of Sandoz 20-438. Chromolysis was observed in nuclei of round (early step) spermatids, and relatively few elongated spermatids were present. The next step was the development of multinucleated cells, followed by vacuolation and pycnotic cells. Later effects were atrophy of seminiferous tubules, with only Sertoli and Leydig cells remaining.[11] By 3 days (72 h) after dosing, both SI and testes weight were significantly affected, and the effect was more intense by day 14 (Table 3). The effects were dose-related, being observed to a lesser extent at a single 10 mg/kg dose (Table 3). Multiple daily dosing was even more effective (data not shown). However, from the data of Table 3, it was concluded that sacrifice 72 h after a single dose would be suitable for comparing analog activity.

Comparison of Effects of Analogs on Testis Weight and Spermatogenic Index. Table 4 compares the effects of 40 analogs tested. Each assay tested 5 mice/dose. A single dose was administered on day 0, and the mice were killed 72 h later on day 3. Each assay contained a vehicle control and a positive control of 30 mg/kg **10a**.

For the oral route, mean testes weights for controls averaged 212.4 ± 17.9 mg (SD) and the mean SI averaged 5.71 ± 0.30 for a total of 14 separate studies. The positive control values averaged 159.2 ± 14.6 mg for testes weights and 2.86 ± 0.56 for SI. For the subcutaneous route, mean testes weights for controls averaged 231.6 ± 17.5 mg and the SI averaged 5.92 ± 0.14 in three separate studies. The positive control values averaged 192.2 ± 2.2 mg for testes weights and 3.70 ± 0.14 for SI. For convenience in comparing between separate assays, both testes weight and SI are expressed in Tables 4 and 5 as percent inhibition, calculated as [(control value − test value)/control value] × 100. Statistical significance was tested in each assay based on comparison of the actual control and test weights or SI for each test dose.

From these data clear structure–activity relationships emerge. A high degree of stereoselectivity was established by finding that the diastereoisomer of **10a** with all hydrogens *cis* (**9a**) was ineffective at 90 mg/kg as was the diastereoisomeric *trans, trans* compound **3c**. Further indication of the importance of stereochemistry is the lack of activity, at 90 mg/kg, of the compound in which the 4a,5-position contains a double bond (**8a**), thus flattening the molecule from the configuration of **10**. Resolution of Sandoz 20-438 and testing of the individual optical isomers demonstrated pronounced enantioselectivity. The (−)-enantiomer [(*l*)-**10a**] was found to be inactive at 90 mg/kg, whereas the (+)-enantiomer [(*d*)-**10a**] was strongly active at 3–10 mg/kg.

Table 3. Effect of a Single Oral Dose of Sandoz 20-438 (**10a**) on Testicular Weight, Spermatogenic Index, and Body Weight Change as a Function of Dose and Time after Dosing[a]

dose	time after dosing (Da)	change in body weight (g)	testes wt (mg)		spermatogenic index
			right	left	
vehicle control	1		113.2 ± 7.3	105.4 ± 7.1	5.6 ± 0.24
	3		124.2 ± 6.0	115.8 ± 5.7	5.6 ± 0.24
	14	3.14	114.8 ± 6.4	111.2 ± 6.3	5.9 ± 0.10
3	1		104.9 ± 5.3	96.9 ± 4.9	6.0 ± 0.00
	3		112.6 ± 8.4	106.1 ± 8.5	5.8 ± 0.20
	14	3.62	126.5 ± 9.3	119.3 ± 7.1	6.0 ± 0.00
10	1		88.2 ± 4.4	88.5 ± 2.9	4.0 ± 0.16[b]
	3		112.3 ± 5.6	107.8 ± 5.6	5.1 ± 0.37
	14	3.18	104.8 ± 5.8	101.6 ± 6.6	3.5 ± 0.00[b]
30	1		93.9 ± 2.2	92.8 ± 3.4	4.4 ± 0.10[b]
	3		81.0 ± 3.5[b]	78.2 ± 3.7[b]	3.0 ± 0.00[b]
	14	3.22	49.4 ± 2.7[b]	47.1 ± 2.6[b]	1.7 ± 0.12[b]

[a] The vehicle was acetate buffer, pH 5. Animals were dosed with 10 mL/kg of the appropriate concentration to yield the required dose in mg/kg. Data are given as mean ± SEM ($n = 5$). [b] $p < 0.05$, compared to control.

The substituent on the nitrogen had a pronounced effect on activity. A hydrogen substituent (**11a**) resulted in a compound inactive at 90 mg/kg. The *N*-methyl analog (**10b**) was only slightly less potent than the ethyl compound, but an *n*-propyl substituent (**10e**) resulted in a marked decrease in potency. An isopropyl substituent on the nitrogen (**11c**) also resulted in a compound less potent than the ethyl analog. Changing the position of the nitrogen to the indeno[2,1-*c*]pyridine structure (**17**) abolished activity at 30 mg/kg.

Even more striking was the effect on activity of changes at the *p*-position of the 5-phenyl substituent. Compound **10h** in which the *p*-substituent was hydrogen showed no significant reduction in testes weight or SI at a dose of 90 mg/kg. The *p*-fluoro (**10e**), *p*-hydroxy (**10o**), and *p*-methoxy (**10f**) analogs were inactive at this dose, as was compound **10g** in which both the *p*-methyl and 7-methyl groups were replaced by hydrogen. Compounds which possess an *o*-methyl (**10c**) or a *m*-methyl (**10d**) substituent but no *p*-methyl group were inactive at 90 mg/kg. If the *p*-methyl substituent was retained but the 7-methyl was replaced by hydrogen (**10i**) or ethyl (**10l**), some activity was retained. The presence of both *o*- and *p*-methyl groups (**10j**) reduced activity.

This apparent requirement for the *p*-methyl group suggested that either its influence on binding to an active site is quite strong or it is metabolized to give an active compound. We tested this latter hypothesis by synthesizing and testing possible oxidative metabolites [*p*-CH_2OH (**12g**), *p*-CHO (**12i**), and *p*-COOH (**10k**)]. These compounds inhibited spermatogenesis; in particular the *p*-COOH compound (**10k**) and its methyl (**12b**) and propyl (**12c**) esters were potent inhibitors. Although our hypothesis led to the synthesis of active compounds and the data suggest that the carboxylic acid is the active substance, further testing of the hypothesis is needed.

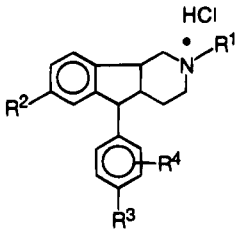
The carboxylate analogs also exhibited marked enantioselectivity, with (*d*)-**10k** and (*l*)-**12b** showing significant activity at 3 mg/kg, whereas their enantiomers were inactive at 90 mg/kg, a minimum 30-fold difference. [Note that (*d*)-**10k** and (*l*)-**12b** have the same absolute stereochemistry, as discussed above.]

In order to further characterize the *p*-substituent and determine whether other polar substituents at that position would result in active compounds, some derivatives of the active compounds were prepared. The *p*-carboxamide (**12e**) and *p*-*N*,*N*-dimethylcarboxamide (**12f**) were inactive at 30 mg/kg (highest dose tested), as was the nitrile (**12d**). Also the *N*-methylcarbamate (**12h**) of alcohol **12g** was inactive at that dose. It appears that only a carboxyl substituent or one which can be readily metabolized to a free carboxyl group (including the methyl group) resulted in potent antispermatogenic activity.

None of the simpler analogs of Sandoz 20-438 tested appeared to be antispermatogenic. These included the B-seco analogs **13** and **14** as well as the other flexible analogs **15** and **16a**,**b**. The lack of observed activity of the isomeric indeno[2,1-*c*]pyridine derivative **17** is somewhat unexpected, in view of the statement of Augstein et al. that an *all-cis*-hexahydroindeno[2,1-*c*]pyridine analog lacking the *N*-ethyl and aromatic methyl substitutents had an effect on the testes of rats.[6] However the cumulative dose used was quite high (in excess of 25 mg/kg/day for 12 weeks) compared with the single doses used here in mice. Overall the diastereoselectivity, enantioselectivity, and fairly specific substituent requirements are consistent with the interaction of the active compounds with a specific macromolecule such as a receptor, enzyme, or binding protein.

Effect of Route of Administration. Selected compounds were also tested for effects after subcutaneous (sc) administration. Often parenteral administration, which reduces the first pass metabolism effect, may result in greater potency than oral administration. However, as shown in Table 5, the sc route resulted in significantly lower potency with Sandoz 20-438, its *d*-enantiomer, the *p*-formyl, *p*-carboxyl, and *p*-hydroxymethyl compounds, and esters of the *p*-carboxyl compound. However some of the compounds exhibited a marked sedative effect when administered by the sc route. In particular, mice dosed sc with Sandoz 20-438 at 30 mg/kg and its *d*-enantiomer at 10 mg/kg were quite lethargic up to 3–6 h after dosing. A less intense sedative effect lasted for 15–60 min after an oral dose of 30 mg/kg Sandoz 20-438 or 10 mg/kg its *d*-enantiomer. By 24 h after dosing, all the animals appeared to be normal. The sedative effect was also structure-related, with the *l*-enantiomer being inactive. Furthermore it was sensitive to the *p*-substituent, with sedation occurring for up to 3 h after an sc 30 mg/kg dose of the *p*-hydroxymethyl compound and lesser to very little sedation occurring with the *p*-carboxyl compound and its esters. These observations suggest that sedation is favored by a lipophilic *p*-substituent and that Sandoz

Table 4. Comparison of Effects of Hexahydroindenopyridine Analogs on Testes Weight and Spermatogenic Index 3 Days after a Single Oral Dose[a]



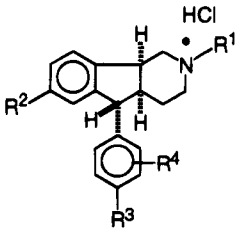
compd	R^1	R^2	R^3	R^4	4a,5/4a,9 relationship of hydrogens	dosage (mg/kg)	testes weight (% reduction from control)	spermatogenic index (% reduction from control)
3c	Et	Me	Me	H	*trans/trans*	90	−18	0
8a	Et	Me	Me	H	$\Delta^{4a,5}$	90	5	−9
9a	Et	Me	Me	H	*cis/cis*	90	2	−9
9b	Me	Me	Me	H	*cis/cis*	30	12	7
10a	Et	Me	Me	H	*trans/cis*	3	10	5
						10	10	31[b]
						30	24[b]	50[b,c]
(*d*)-**10a**	Et	Me	Me	H	*trans/cis*	1	11	7
						3	13	31[b,d]
						10	25[b]	59[b,e]
						30	43[b]	62[b]
(*l*)-**10a**	Et	Me	Me	H	*trans/cis*	30	10	9
						90	4	−9
10b	Me	Me	Me	H	*trans/cis*	3	8	−3
						10	7	26[b]
						30	18	38[b]
10c	Et	Me	H	*o*-Me	*trans/cis*	90	3	−9
10d	Et	Me	H	*m*-Me	*trans/cis*	90	−11	−9
10e	Et	Me	F	H	*trans/cis*	90	5	−5
10f	Et	Me	OMe	H	*trans/cis*	3	−7	2
						10	−17	−6
						90	5	−5
10g	Et	H	H	H	*trans/cis*	90	−2	2
10h	Et	Me	H	H	*trans/cis*	90	−8	−9
10i						10	5	0
						30	3	20[b]
	Et	H	Me	H	*trans/cis*	90	20	35[b]
10j	Et	Me	Me	Me(2′-)	*trans/cis*	10	1	0
						30	6	3
						90	11	30[b]
10k	Et	Me	COOH	H	*trans/cis*	3	13	2
						10	24	36[b]
						30	28[b]	51[b,f]
(*d*)-**10k**	Et	Me	COOH	H	*trans/cis*	3	−5	21[b]
						10	26[b]	40[b]
						30	16	67[b]
						90	27[b]	70[b]
(*l*)-**10k**	Et	Me	COOH	H	*trans/cis*	90	−7	0
10l	Et	Et	Me	H	*trans/cis*	3	3	−2
						10	2	7
						30	26[b]	33[b,d]
10m	Et	Et	COOMe	H	*trans/cis*	3	−18	4
						10	0	−4
						30	8	23[b]
10o	Et	Me	OH	H	*trans/cis*	30	−1	−5
11a	H	Me	Me	H	*trans/cis*	90	−3	−5
11b	*n*-Pr	Me	Me	H	*trans/cis*	30	11	0
11c	*i*-Pr	Me	Me	H	*trans/cis*	10	7	10
						30	15	28[b]
						90	23	45[b]
12b	Et	Me	COOMe	H	*trans/cis*	3	5	5
						10	17	27[b]
						30	32[b]	57[b,g]
(*d*)-**12b**	Et	Me	COOMe	H	*trans/cis*	90	3	−5
(*l*)-**12b**	Et	Me	COOMe	H	*trans/cis*	3	11	28[b]
						10	26[b]	47[b]
						30	30[b]	61[b]
						90	34[b]	68[b]
12c	Et	Me	COO-*n*-Pr	H	*trans/cis*	3	11	2
						10	3	33[b]
						30	31[b]	56[b,d]
12d	Et	Me	CN	H	*trans/cis*	30	13	−4
12e	Et	Me	$CONH_2$	H	*trans/cis*	30	−7	−2
12f	Et	Me	$CONMe_2$	H	*trans/cis*	30	−3	3
12g	Et	Me	CH_2OH	H	*trans/cis*	3	4	−5

Table 4 (Continued)

compd	R^1	R^2	R^3	R^4	4a,5/4a,9 relationship of hydrogens	dosage (mg/kg)	testes weight (% reduction from control)	spermatogenic index (% reduction from control)
						10	13	20[b]
						30	23[b]	57[b,d]
12h	Et	Me	$CH_2OCONHMe$	H	*trans/cis*	30	7	8
12i	Et	Me	CHO		*trans/cis*	3	7	0
						10	6	2
						30	7	31[b,d]
13	*g*					3	2	2
						30	9	−5[d]
14	*g*					90	−5	0
15	*g*					90	−21	0
16a	*g*					90	−9	0
16b	*g*					90	**0**	0
17	*g*					30	0	2

[a] Five mice were used per dose. Vehicle A was 0.15 M acetate buffer, pH 5, and it was used for compounds **3c−9b**, (*d*)-**10a**, (*l*)-**10a**, **10a−j**, **11a−c**, and **14−17**. Vehicle B was ethanol, Tween-20, water (3:7:90, v/v/v), and it was used for compounds (*d*)-**10a**, **10a**,**k−m**, **12b−13**. Vehicle C was 1% Tween-20 in water, and it was used for compounds **10k**,**o** (*d*)-**10k**, (*l*)-**10k**, **12b**, (*d*)-**12b**, and (*l*)-**12b**. No vehicle differences were found. Animals were dosed with 10 mL/kg of the appropriate concentration to yield the desired dose in mg/kg. A single dose was administered on day 0, and the mice were killed 72 h later on day 3. Each assay contained a vehicle control and a positive control of 30 mg/kg **10a**. For convenience in comparing between separate assays, both testes weight and the spermatogenic index are expressed as percent inhibition, calculated as [(control value − test value)/control value] × 100. [b] $p < 0.05$ (Dunnett's one-tailed *t*-test) versus control. Statistical significance was tested in each assay based on comparison of the control values for testes weights or SI with those for each test dose. [c] Mean of 12 assays. [d] Mean of two assays. [e] Mean of five assays. [f] Mean of four assays. [g] See Figure 1 for structures.

Table 5. Comparison of Effects of Hexahydroindenopyridine Analogs on Testes Weight and Spermatogenic Index 3 Days after a Single Subcutaneous Dose[a]



compd	R^1	R^2	R^3	R^4	dosage (mg/kg)	testes weight (% reduction from control)	spermatogenic index (% reduction from control)
10a	Et	Me	Me	H	5	5	0
					10	−3	10
					30	14	39[b−d]
(*d*)-**10a**	Et	Me	Me	H	10	12	41[b−d]
(*l*)-**10a**	Et	Me	Me	H	10	−11	0
10k	Et	Me	COOH	H	3	5	8
					10	−3	27
					30	17	35[b−d]
12b	Et	Me	COOMe	H	3	10	10
					10	1	28
					30	19	46[b,c]
12c	Et	Me	COO-*n*-Pr	H	3	7	−1
					10	10	13
					30	17	43[b,c]
12g	Et	Me	CH_2OH	H	30	6	40[b,c]
12i	Et	Me	CHO	H	30	−4	30[b]

[a] Five mice were used per dose. Vehicle B (Table 4, footnote a) was used. Animals were dosed with 10 mL/kg of the appropriate concentration to yield the required dose in mg/kg. A single dose was administered on day 0, and the mice were killed 72 h later on day 3. Each assay contained a vehicle control and a positive control of 30 mg/kg **10a**. For convenience in comparing between separate assays, both testes weight and the spermatogenic index are expressed as percent inhibition, calculated as [(control value − test value)/control value] × 100. [b] $p<0.05$ (Dunnett's one-tailed *t*-test) versus control. Statistical significance was tested in each assay based on comparison of the actual control and testes weights or SI for each test dose. [c] ANOVA detected a significant difference ($p < 0.05$) between the oral (gavage) and subcutaneous routes in a side by side comparison. [d] Mean of two assays.

20-438 upon oral administration undergoes metabolism that reduces the sedation.

Antifertility Effects. The fertility of male mice was determined after they were dosed for 35 days with methyl ester **12b**. Results are shown in Table 6. A 7.5 mg/kg daily dose reduced the number of litters to 10% of controls, and a 15 mg/kg dose completely prevented pregnancy. These results corresponded with the decrease in SI and testis weight. In spite of the lack of conception, mating occurred as shown by the observation of equivalent numbers of vaginal copulatory plugs in the control and treated animals.

Conclusions

In conclusion, a structure−activity study of (4a*RS*,5*SR*,9b*RS*)-2-ethyl-2,3,4,4a,5,9b-hexahydroindeno[1,2-*c*]pyridines has shown that certain of these compounds exhibit potent antispermatogenic and antifertility activity in mice. This activity is strongly dependent on stereoisomerism, chirality, and specific substitution

Table 6. Testicular Weights, Spermatogenic Index, and Fertility of Male CD-1 Mice Treated with RTI-4587-056 (12b)[a]

testes compd	dosage (mg/kg)	testes wt total (mg)[b]	spermatogenic index	pregnancy index[e]	no. of litters[f]	vaginal plugs found
control	0.0	245.42 ± 16.30	6.00 ± 0.00	18/18 (100%)	18	13
12b	3.75	219.08 ± 18.90[c]	5.50 ± 0.50[c]	16/16 (100%)	16	11
12b	7.5	114.70 ± 7.00[d]	3.00 ± 0.52[d]	2/20 (10%)	2	14
12b	15.0	108.94 ± 11.01[d]	1.60 ± 0.10[d]	0/18 (0%)	0	14

[a] Male mice (8–10/group) were treated daily (orally by gavage) for 35 days. The vehicle was 1% Tween-20 in water. Animals were dosed with 10 mL/kg of the appropriate concentration to yield the required dose in mg/kg. One-half of the animals in each group were sacrificed 7 days after the last day of treatment for determination of testes weights and spermatogenic index. [b] Numbers are means ± standard error of the mean ($n = 5$). [c] $n = 4$. [d] Significantly lower than controls (Dunnett's one-tailed t-test, $p < 0.05$). [e] Each male was housed with two females for 1 week beginning 1 day after the last dose. This index is based on a potential of 100%. The denominator is the number of females; the numerator is the number of litters born. [f] Number of litters per treatment group.

patterns and substituents. These data suggest that the compounds exert their observed activity by interacting with a specific site in a macromolecule such as a receptor, an enzyme, or a binding protein. The activity of the 5-(*p*-carboxyphenyl) analog and the lack of activity of 5-(*p*-substituted-phenyl) analogs which cannot undergo metabolism to a 5-(*p*-carboxyphenyl) compound are consistent with (but not proof of) a hypothesis that Sandoz 20-438 is metabolically activated. The potent oral activity is encouraging for the development of these compounds as male contraceptive agents in humans, as well as for control of fertility in domestic, feral, and wild animals. In addition, the specificity of action is such that compounds such as RTI-4587-054 (**10k**) should be useful in mechanistic investigations of spermatogenesis.

Experimental Section

Physical properties of the compounds and analytical results are given in Table 7.

Synthesis of 1-Alkyl-3-arylisonipecotic Acids 5. These compounds were prepared following a reported procedure.[3]

Synthesis of (4a*RS*,9b*RS*)-2-Alkyl-2,3,4,4a,5,9b-hexahydro-1*H*-indeno[1,2-*c*]pyridin-5-ones 6. These compounds were prepared from the respective isonipecotic acid and polyphosphoric acid using a reported procedure.[3]

Synthesis of 5-Aryl-2,3,4,9b-tetrahydro-1*H*-indeno[1,2-*c*]pyridine Hydrochlorides 8a–m. General Procedure. Method A (for 8a–j,l). To a solution of the requisite aryllithium or Grignard reagent in ether (2 equiv; obtained from commercial sources or from the corresponding aryl bromide and lithium or magnesium metal) at −78 °C was added the appropriate ketone **6** (1 equiv) as a solution in ether dropwise. After the addition, the mixture was stirred at either 0 °C, room temperature, or reflux for 2–4 h (see below), the reaction quenched with saturated NH_4Cl, and the mixture extracted with chloroform. The combined chloroform extracts were dried (Na_4SO_4), filtered, and concentrated under reduced pressure to afford crude alcohol **7**. Dehydration was effected by refluxing **7** in 5% methanolic hydrogen chloride and monitoring the progress of the reaction by TLC (ca. 1 h). The product was concentrated under reduced pressure and purified by column chromatography (SiO_2, 0–5% MeOH in $CHCl_3$) and/or recrystallization to give the desired olefin **8**. Upon isolation of the product olefin from column chromatography, conversion back into the hydrochloride was carried out with either methanolic or ethereal hydrogen chloride.

Method B (for 8k,m). To a mechanically stirred solution of *p*-bromobenzoic acid (1.5 equiv) in THF at −78 °C was added *n*-butyllithium (3 equiv of a 2.5 M solution in hexane) dropwise such that the reaction temperature never exceeded −70 °C. After the resulting thick, light yellow mixture was stirred for an additional 1.5 h, the appropriate tricyclic ketone (**6b** or **6c**; 1 equiv) was added as a solution in THF dropwise over a 20–30 min period and stirring was continued for 2.5 h at −78 °C. The mixture was poured into ice-cold 1 M HCl and extracted with ether. The acidic aqueous layer was heated to drive off residual THF, refluxed at ca. 100 °C for 4 h, and then concentrated under reduced pressure to give a solid. This solid was recrystallized from EtOAc–MeOH to give the desired olefin.

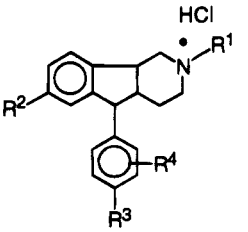
General Procedure for the Synthesis of (4a*RS*,5*SR*,9b*RS*)-2,3,4,4a,5,9b-Hexahydro-1*H*-indeno[1,2-*c*]pyridine Hydrochlorides 10a–m. To a stirring suspension of $PdCl_2$ (15 mol %) in a 50% ethanol–water mixture containing NaCl (15 mol %) was added $NaBH_4$ (30 mol %) at room temperature followed by the requisite olefin **8** and enough concentrated HCl to adjust the pH to 0. The resulting mixture was shaken on a Parr apparatus at 50 °C under a hydrogen atmosphere (50 psi) until TLC indicated complete consumption of starting material. The solution was filtered through Celite and concentrated under reduced pressure to afford the crude product **9** which was used without further purification for the next step. To a solution of KOH in *n*-butanol (20%, w/v) was added **9** (1 g/20 mL of solvent), and the resulting mixture was heated at reflux until TLC indicated complete or nearly complete consumption of starting material. For the synthesis of **10c,j**, *n*-hexanol was used instead of *n*-butanol. Workup for all compounds but **10k,m** was as follows: the dark brown reaction mixture was cooled to room temperature, poured into ice-cold water, and extracted three times with ether. The combined ether extracts were washed successively with water and brine, dried (Na_2SO_4), and concentrated under reduced pressure to afford the crude product which was purified by column chromatography (SiO_2, 0–5% MeOH in $CHCl_3$). The hydrochloride salt was synthesized from the free base using methanolic or ethereal hydrogen chloride. For compounds **10k,m**, workup consisted of cooling the dark brown reaction mixture to 5°C and acidification to pH 1 with ice-cold 18% HCl. The products were isolated from the byproduct KCl by concentrating the acidic mixture to dryness under reduced pressure, redissolving the desired material in absolute ethanol, filtering away the KCl, and concentrating the filtrate under reduced pressure. Purification was accomplished by recrystallizing from EtOAc–MeOH.

(4a*RS*,5*SR*,9b*RS*)-2-Ethyl-2,3,4,4a,5,9b-hexahydro-7-methyl-5-(4-hydroxyphenyl)-1*H*-indeno[1,2-*c*]pyridine Hydrochloride (10o). A solution of **10f** (556 mg, 1.56 mmol) in 48% HBr (8 mL) and just enough glacial acetic acid to form a homogeneous mixture (ca. 4 mL) was refluxed for 5 h. The mixture was cooled and concentrated *in vacuo* to a solid which was converted to the free base by use of silica gel chromatography and chloroform–methanol–concentrated NH_4OH (80:18:2, v/v/v) as eluent, converted to the HCl salt by use of 3% HCl in MeOH, and recrystallized from EtOAc–MeOH. Yield = 350 mg (65%).

(4a*RS*,5*SR*,9b*SR*)-2-Ethyl-7-methyl-1,3,4,4a,5,9b-hexahydro-5-(4-methylphenyl)-1*H*-indeno[1,2-*c*]pyridine Hydrochloride (3c). This compound was prepared from **4a** by Scheme 1 by a patent procedure.[3]

(4a*RS*,5*SR*,9b*RS*)-2,3,4,4a,5,9b-Hexahydro-7-methyl-5-(4-methylphenyl)-1*H*-indeno[1,2-*c*]pyridine Hydrochlo-

Table 7. Physical Properties of Indenopyridine Analogs



compd	R^1	R^2	R^3	R^4	molecular formula[a]	4a,5/4a,9 relationship of hydrogens	mp, °C	selected ^{1}H NMR[b] resonances
8a	Et	Me	Me	H	$C_{22}H_{26}ClN$	$\Delta^{4a,5}$	257–260	4.32
8b	Me	Me	Me	H	$C_{21}H_{24}ClN$[c]	$\Delta^{4a,5}$	240–243	4.33
8c	Et	Me	H	*o*-Me	$C_{22}H_{26}ClN$[d]	$\Delta^{4a,5}$	258–260	4.33
8d	Et	Me	H	*m*-Me	$C_{22}H_{26}ClN$	$\Delta^{4a,5}$	230–232	4.34
8e	Et	Me	F	H	$C_{21}H_{23}ClFN$	$\Delta^{4a,5}$	228–230	4.38
8f	Et	Me	OMe	H	$C_{22}H_{26}ClNO$	$\Delta^{4a,5}$	244–249	4.34
8g	Et	H	H	H	$C_{20}H_{22}ClN$[d]	$\Delta^{4a,5}$	215–217	4.42
8h	Et	Me	H	H	$C_{21}H_{24}ClN$[d]	$\Delta^{4a,5}$	200–202	4.35
8i	Et	H	Me	H	$C_{21}H_{24}ClN$	$\Delta^{4a,5}$	216–219	4.37
8j	Et	Me	Me	Me	$C_{23}H_{28}ClN$[d]	$\Delta^{4a,5}$	250–253	4.38
8k	Et	Me	COOH	H	$C_{22}H_{24}ClNO_2$[e]	$\Delta^{4a,5}$	>195 (dec)	4.44
8l	Et	Et	Me	H	$C_{23}H_{28}ClN$[d]	$\Delta^{4a,5}$	208–212	4.30
8m	Et	Et	COOH	H	$C_{23}H_{26}ClNO_2$	$\Delta^{4a,5}$	>235 (dec)	4.42
3c	Et	Me	Me	H	$C_{22}H_{28}ClN$	*trans*/*trans*	220 (dec)	4.20 (J = 11.0 Hz)
9a	Et	Me	Me	H	$C_{22}H_{28}ClN$	*cis*/*cis*	237–240	4.50 (J = 8.7 Hz)
10a	Et	Me	Me	H	$C_{22}H_{28}ClN$[f]	*trans*/*cis*	>280 (dec)	4.12 (J = 10.6 Hz)
10b	Me	Me	Me	H	$C_{21}H_{26}ClN$[d]	*trans*/*cis*	277–279	4.05 (J = 11.2 Hz)
10c	Et	Me	H	*o*-Me	$C_{22}H_{28}ClN$	*trans*/*cis*	250–252	4.42 (J = 10.0 Hz)
10d	Et	Me	H	*m*-Me	$C_{22}H_{28}ClN$[d]	*trans*/*cis*	>235 (dec)	4.16 (J = 11.2 Hz)
10e	Et	Me	F	H	$C_{21}H_{25}ClFN$	*trans*/*cis*	245–248	4.13 (J = 10.3 Hz)
10f	Et	Me	OMe	H	$C_{22}H_{28}ClNO$[d]	*trans*/*cis*	272–275	4.10 (J = 11.0 Hz)
10g	Et	H	H	H	$C_{20}H_{24}ClN$	*trans*/*cis*	>284 (dec)	4.12 (J = 12.0 Hz)
10h	Et	Me	H	H	$C_{21}H_{26}ClN$	*trans*/*cis*	270	4.14 (J = 10.8 Hz)
10i	Et	H	Me	H	$C_{21}H_{26}ClN$	*trans*/*cis*	287–300	4.19 (J = 11.4 Hz)
10j	Et	Me	Me	*o*-Me	$C_{23}H_{30}ClN$[d]	*trans*/*cis*	265–270	4.40 (J = 10.7 Hz)
10k	Et	Me	COOH	H	$C_{22}H_{26}ClNO_2$[f]	*trans*/*cis*	203 (dec)	4.10 (J = 11.0 Hz)
10l	Et	Et	Me	H	$C_{23}H_{30}ClN$[d]	*trans*/*cis*	235–240	4.11 (J = 10.8 Hz)
10m	Et	Et	COOH	H	$C_{24}H_{30}ClNO_2$[d]	*trans*/*cis*	>275 (dec)	4.28 (J = 11.0 Hz)
10n	Et	Et	COOMe	H	$C_{24}H_{30}ClNO_2$[c]	*trans*/*cis*	215	4.22 (J = 11.0 Hz)
10o	Et	Me	OH	H	$C_{21}H_{26}ClNO$[c]	*trans*/*cis*	255 (dec)	4.05 (J = 10.8 Hz)
11a	H	Me	Me	H	$C_{20}H_{24}Cl$[d]	*trans*/*cis*	280–281	4.03 (J = 10.0 Hz)
11b	*n*-Pr	Me	Me	H	$C_{23}H_{30}ClN$	*trans*/*cis*	>235 (dec)	4.01 (J = 11.0 Hz)
11c	*i*-Pr	Me	Me	H	$C_{23}H_{30}ClN$	*trans*/*cis*	>250 (dec)	4.04 (J = 11.0 Hz)
12b	Et	Me	COOMe	H	$C_{23}H_{28}ClNO_2$	*trans*/*cis*	204	4.20 (J = 11.0 Hz)
12c	Et	Me	COO-*n*-Pr	H	$C_{25}H_{32}ClNO_2$[c]	*trans*/*cis*	202	4.21 (J = 11.0 Hz)
12d	Et	Me	CN	H	$C_{22}H_{25}ClN_2$[d]	*trans*/*cis*	202	4.24 (J = 10.0 Hz)
12e	Et	Me	$CONH_2$	H	$C_{22}H_{26}N_2O$	*trans*/*cis*	202–204	4.26 (J = 10.8 Hz)
12f	Et	Me	$CONMe_2$	H	$C_{24}H_{31}ClN_2O$	*trans*/*cis*	203	4.23 (J = 10.2 Hz)
12g	Et	Me	CH_2OH	H	$C_{22}H_{28}ClNO$[c]	*trans*/*cis*	218 (dec)	4.10 (J = 10.8 Hz)
12h	Et	Me	$CH_2OCONHMe$	H	$C_{24}H_{31}ClN_2O_2$[f]	*trans*/*cis*	196–197	4.19 (J = 10.8 Hz)
12i	Et	Me	CHO	H	$C_{22}H_{26}ClNO$[f]	*trans*/*cis*	190 (dec)	4.21 (J = 11.0 Hz)
13	*g*				$C_{22}H_{30}ClN$		>180 (dec)	
14	*g*				$C_{22}H_{28}ClN$[d]		>200 (dec)	
15	*g*				$C_{22}H_{29}NO$		>175 (dec)	
16a	*g*				$C_{22}H_{29}NO$		91–92	
16b	*g*				$C_{22}H_{29}NO$		95	
17	*g*				$C_{22}H_{28}ClN$[d]		220	4.49 (J = 8.6 Hz)[h]

[a] All compounds were analyzed for C, H, and N with results that agreed to ±0.4% with theoretical values. [b] NMR spectra were determined at 250 MHz (on the HCl salts) in $CDCl_3$. For the $\Delta^{4,5}$ compounds, the value is the chemical shift of the 9b-H; for the others, it is the chemical shift (and coupling constant) of the 5-H (9-H in **17**). [c] This salt was hydrated with 0.75 mol of water. [d] This salt was hydrated with 0.25 mol of water. [e] This salt was hydrated with 1.0 mol of water. [f] This salt was hydrated with **0**.5 mol of water. [g] See Figure 1 for structures. [h] Although the position and coupling of this hexahydroindeno[2,1-*c*]pyridine hydrogen closely approximates those of the *cis*/*cis* hexahydroindeno[1,2-*c*]pyridine **9a**, they are consistent with *cis*/*trans* analogs of the 2,1-*c* series (ref 6).

ride (11a). To a solution of **10b** (as the free base, 12.1 g) in toluene (200 mL) was added ethyl chloroformate in 50 mL of toluene dropwise, and the resulting mixture was refluxed for 3 h. Some hydrochloride salt precipitated out. At the end of the reaction, the toluene layer was washed with water. Evaporation of toluene gave 10.7 g of an oil. This oil was dissolved in 200 mL of *n*-BuOH containing 50 g of KOH. The mixture was refluxed for 24 h. After evaporation of solvent, water and ether were added. The ether layer was separated and washed several times with water. Evaporation of dry ether (Na_2SO_4) gave 8.2 g of oil (86% yield, based on reacted starting material). A portion of this oil (2.1 g) was dissolved in 1 N methanolic HCl (200 mL). Evaporation of MeOH gave a solid. Recrystallization of this solid from EtOAc gave 2.3 g (88%, based on reacted starting material).

(4a*RS*,5*SR*,9b*RS*)-2-*n*-Propyl-7-methyl-2,3,4,4a,5,9b-hexahydro-5-(4-methylphenyl)-1*H*-indeno[1,2-*c*]pyridine Hydrochloride (11b). **11a** (300 mg) was mixed with 1-bromopropane (200 mg) and 4 g of K_2CO_3 in 50 mL of acetone. The mixture was refluxed for 12 h. After the filtration of solid, evaporation of solvent gave an oil which was

subjected to column chromatography. The free base obtained from the column was converted to the HCl salt (315 mg, 89%).

(4a*RS*,5*SR*,9b*RS*)-2-Ethyl-7-methyl-2,3,4,4a,5,9b-hexahydro-5-(4-carbomethoxyphenyl)-1*H*-indeno[1,2-*c*]pyridine Hydrochloride (12b). To a solution of **10k** (3.6 g, 9.69 mmol) in methanol (50 mL) at −10 °C was added thionyl chloride (1.1 mL, 14.5 mmol) over a 10 min period. The resulting solution was allowed to stand in a refrigerator at 5 °C for 68 h during which time the product had begun to crystallize out as fine white needles. Three crops were obtained and combined to yield 2.65 g of **12b**.

(4a*RS*,5*SR*,9b*RS*)-2-Ethyl-7-methyl-2,3,4,4a,5,9b-hexahydro-5-[4-(*n*-propyloxy)carbonyl]phenyl]-1*H*-indeno[1,2-*c*]pyridine Hydrochloride (12c). The carboxylic acid **10k** (83.1 mg, 0.22 mmol) was converted into the corresponding acid chloride by reaction with thionyl chloride (5 mL) at room temperature for 2 h. Upon removal of excess $SOCl_2$ *in vacuo*, *n*-propanol (3 mL) was added and the solution was stirred for 4 days at room temperature. The solvent was removed *in vacuo*, and the crude material was passed through a column of silica gel with chloroform−methanol (20:1) as eluent. Exposure to ethereal hydrogen chloride yielded 90.5 mg (99%) of **12c** as a white solid.

(4a*RS*,5*SR*,9b*RS*)-2-Ethyl-7-methyl-2,3,4,4a,5,9b-hexahydro-5-(*p*-cyanophenyl)-1*H*-indeno[1,2-*c*]pyridine Hydrochloride (12d). **10k** (504 mg, 1.36 mmol) was converted to the corresponding acid chloride in the same manner as above. To this material was added 3-sulfolane (3 mL) and sulfamide (376 mg, 3.91 mmol), and the mixture was heated to 120 °C. During the course of the reaction, a finely divided precipitate formed (presumably sulfamic acid). After 3 h, the mixture was cooled to room temperature, diluted with 5% NaOH, and extracted with ether (3 × 30 mL). The combined ether extracts were washed with water (3 × 20 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude material was purified via column chromatography using chloroform−methanol as eluent and afforded **12d** as the free base (378 mg, 88%). A portion of this material was converted to the hydrochloride salt by use of 5% HCl in methanol.

(4a*RS*,5*SR*,9b*RS*)-2-Ethyl-7-methyl-2,3,4,4a,5,9b-hexahydro-5-*p*-benzamido-1*H*-indeno[1,2-*c*]pyridine Hydrochloride (12e). A mixture of carboxylic acid **10k** (310 mg, 0.83 mmol) and thionyl chloride (5 mL) was stirred at room temperature for 3 h at which time excess thionyl chloride was removed *in vacuo* to leave acid chloride **12a** as an off-white amorphous solid. To this material was added concentrated ammonium hydroxide (5 mL), and the resulting heterogeneous mixture was stirred overnight. The mixture was diluted with water (10 mL) and extracted with $CHCl_3$. The organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give the free base **12e** (190 mg). This material was recrystallized from ether−EtOAc as needles (145 mg; mp 223 °C dec). Anal. ($C_{22}H_{26}N_2O$) C, H, N. Exposure of a solution of this material to anhydrous hydrogen chloride in ether and removal of the solvent gave **12e** as its hydrochloride salt.

(4a*RS*,5*SR*,9b*RS*)-2-Ethyl-7-methyl-2,3,4,4a,5,9b-hexahydro-5-(*p*-*N*,*N*-dimethylbenzamido)-1*H*-indeno[1,2-*c*]pyridine Hydrochloride (12f). **10k** (106 mg, 0.28 mmol) was converted into the corresponding acid chloride in the same manner as above. To this material was added dimethylamine (2.0 mL of a 40% solution in water), and the resulting mixture was stirred for 85 min at room temperature. The crude material was concentrated *in vacuo*, taken up in chloroform, filtered over Celite, and purified by column chromatography using chloroform−methanol (20:1) as eluent. The product was converted to the hydrochloride salt with ethereal hydrogen chloride and yielded **12f** (72 mg, 64%) as a white solid.

(4a*RS*,5*SR*,9b*RS*)-2-Ethyl-7-methyl-2,3,4,4a,5,9b-hexahydro-5-[4-(hydroxymethyl)phenyl]-1*H*-indeno[1,2-*c*]pyridine Hydrochloride (12g). To a slurry of **10k** (175 mg, 0.47 mmol) in THF (1 mL) at 0 °C was added BH_3−THF (1 mmol, 1 mL of a 1.0 M solution in THF) dropwise over a 10 min period. The resulting mixture was warmed to room temperature and stirred for 5 h, at which time the reaction was quenched by the slow addition of 3 M NaOH (15 mL) and the mixture allowed to stir for 16 h. The solution was saturated with potassium carbonate, extracted with ether (3 × 20 mL), dried (Na_2SO_4), and concentrated *in vacuo* to afford a white solid which was purified by flash column chromatography using 10% MeOH−$CHCl_3$. The yield of **12g** was 117.2 mg (70%).

(4a*RS*,5*SR*,9b*RS*)-2-Ethyl-7-methyl-2,3,4,4a,5,9b-hexahydro-5-[4-[[(*N*-methylcarbamoyl)oxy]methyl]phenyl]-1*H*-indeno[1,2-*c*]pyridine Hydrochloride (12h). To a solution of **12g** (78.3 mg, 0.22 mmol) in chloroform (1 mL) was added phosgene (0.3 mL of a 1.93 M solution in toluene, 0.57 mmol) at room temperature, and the resulting mixture was stirred for 3 h. The crude intermediate was concentrated to dryness *in vacuo*, and methylamine (2 mL of a 40% solution in water) was added. The mixture was stirred overnight and concentrated *in vacuo*. The crude carbamate was purified by column chromatography (SiO_2) with chloroform−methanol (20:1) as eluent. The material obtained was treated with ethereal hydrogen chloride to yield 63 mg (69%) of **12h** as a white solid.

(4a*RS*,5*SR*,9b*RS*)-2-Ethyl-7-methyl-2,3,4,4a,5,9b-hexahydro-5-(4-formylphenyl)-1*H*-indeno[1,2-*c*]pyridine Hydrochloride (12i). To a solution of **12g** (62.6 mg, 0.18 mmol) in CH_2Cl_2 (4 mL) was added Dess−Martin[12] periodinane (300 mg) portionwise until thin layer chromatography (10:1 $CHCl_3$:MeOH on silica gel) indicated that the starting material had been completely consumed (20 min). The reaction mixture was passed through a short column of silica gel with CH_2Cl_2 as eluent, and the solvent was removed *in vacuo* to yield 31.6 mg (51%) of **12i** (Scheme 3) as a white solid.

***N*-Ethyl-4-(*m*-tolyl-*p*-tolylmethylidene)piperidine (14).** To a solution of *p*-tolylmagnesium bromide (45 mL, 1.0 M solution in ether) at 0 °C was added a solution of *N*-ethyl-4-(3-methylbenzoyl)piperidine (170 mg, 0.74 mmol) in ether (50 mL). The reaction mixture was stirred at 0 °C for 3 h, poured in a 5% solution of NH_4Cl (40 mL), and extracted with ether (3 x 100 mL). The ether extract was dried (Na_2SO_4) and evaporated to give a white solid which without purification was dehydrated by use of 5% HCl in MeOH (15 mL) at 80 °C for 2 h. The olefin **14** was obtained after column chromatography (SiO_2) with $CHCl_3$ as eluent (0.185 g, 82%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.19 (3H, t, J = 7.2 Hz), 2.29 (6H, s), 2.44−2.60 (10H, m), 6.91−7.25 (8H, m, aromatic). Anal. ($C_{22}H_{28}ClN{\cdot}0.25H_2O$) C, H, N.

***m*-Tolyl-*p*-tolyl[4-(1-ethyl)piperidinyl]methane (13).** The olefin **14** (170 mg, 5 mmol) in absolute ethyl alcohol (25 mL) containing 10% Pd on carbon and 5 drops of concentrated HCl was hydrogenated at 50 °C for 18 h. The reaction mixture was filtered through a Celite pad and washed with absolute ethyl alcohol. The solvent was removed, and the pale yellow solid was chromatographed (SiO_2) with 5% MeOH in $CHCl_3$ as eluent. The final product (**13**) was obtained as a pale golden compound (195 mg, 95%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.39 (3H, t, J = 7.2 Hz), 1.68−1.93 (4H, m), 2.18−2.29 (7H, bs), 2.47−2.56 (2H, bt), 2.93 (2H, 9), 3.39 (2H, bd), 3.53 (1H, d, J = 11.0 Hz). Anal. ($C_{22}H_{30}ClN$) C, H, N.

Resolution of Sandoz 20-438. Racemic compound (5.1 g, 15 mmol) was partitioned between ethyl acetate and aqueous ammonia and the organic phase dried over sodium sulfate and evaporated to give 4.5 g of free base. This material was dissolved in methanol (150 mL) and treated with an equivalent of (*S*)-(+)-BNPPA (5.1 g, 14.7 mmol). The mixture was warmed in a water bath to a clear solution. After standing at 4 °C for 2 days, the white crystals were filtered and washed with cold methanol. The collected crystals were recrystallized from hot methanol to give 3.55 g of a diastereomeric salt. From this salt, the free base of (−)-Sandoz 20-438 [(*l*)-**10a**] was extracted into the organic phase on partitioning between ethyl acetate and aqueous ammonia. The residue from evaporation of the ethyl acetate extract was acidified with methanolic HCl. The hydrochloride thus obtained (1.75 g) was recrystallized from methanol/ethyl acetate to give 630 mg of colorless crystals. The mother liquor was diluted with ethyl acetate and concentrated to give 1.1 g of a white solid [(*l*)-**10a**]. The optical purity was established by HPLC analysis on a Chiralcel-OD column (J. T. Baker) with 2-propanol as the eluent at a flow rate of 0.1 mL/min and with a UV detector at 254 nm.

A single peak was obtained at a retention time of 31.67 min. Addition of 3% of the (+)-isomer gave a second peak. Optical rotation: $[\alpha]_{589} = -1.6°$ (c = 0.5, $CHCl_3$).

The original mother liquor from the above described separation of the (−)-enantiomer was concentrated and partitioned between ethyl acetate and aqueous ammonia. The residue from the dry organic phase (2.4 g) was dissolved in methanol and treated with an equivalent of (*R*)-(−)-BNPPA (2.75 g, 7.9 mmol). After 2 days at 4 °C, the crystals were collected and washed with cold methanol. The collected crystals were recrystallized from hot methanol to give the diastereomeric salt (3.85 g). The salt was partitioned between ethyl acetate and aqueous ammonia, and the ethyl acetate extract was dried and concentrated to give the free base (1.8 g), which was acidified and recrystallized from methanol/ethyl acetate to give 650 mg of (+)-Sandoz 20-438 [(*d*)-**10a**] as colorless crystals. The mother liquor was diluted with ethyl acetate and concentrated to provide 1.1 g of white solid. The optical purity was confirmed by chiral HPLC analysis as above which gave a single peak with a retention time of 29.95 min. Optical rotation: $[\alpha]_{589} = +1.6°$ (c = 0.5, $CHCl_3$).

Resolution of 12b. To a solution of free base **12b** (2.11 g, 6.04 mmol) in 5% methanol in $CHCl_3$ (ca. 150 mL) was added (*R*)-(−)-mandelic acid (0.924 g, 6.04 mmol). The resulting mixture was warmed on a steam bath to effect solution, concentrated under reduced pressure to dryness, and taken up in acetone (ca. 75 mL). This mixture was heated to boiling to effect solution, rapidly filtered, and concentrated on a steam bath to ca. 60 mL. After about 1 h, crystals began to form. After standing at −10 °C for 2 days, the crystals were collected with suction (966 mg): $[\alpha]^{19}_D = -53.9°$ (c = 1.24, $CHCl_3$); mp 171.0−172.0 °C. The HCl salt was made by partitioning a sample of the above crystals between ether and water, basifying with 30% NaOH to pH 12, and acidifying the ether extract with 3% HCl in MeOH. The optical rotation of the HCl salt (*l*)-**12b** from the (*R*)-(−)-mandelic acid was $[\alpha]^{19}_D = -5.0°$ (c = 0.96, $CHCl_3$). Chiral HPLC analysis indicated this material to be optically pure. The antipode was obtained by liberating the free base from the mother liquor from above, treating with an equivalent of (*S*)-(+)-mandelic acid, and recrystallizing from acetone (20 mL) in a similar fashion (1.02 g): $[\alpha]^{19}_D = +52.9°$ (c = 1.26, $CHCl_3$); mp 170.5−171.5 °C. For the HCl salt (*d*)-**12b** obtained from the (*S*)-(+)-mandelic acid, $[\alpha]^{19}_D = +5.6°$ (c = 1.07, $CHCl_3$). Chiral HPLC analysis indicated this material to be optically pure.

Synthesis of (*d*)-10k. A sample of the resolved **12b** (*R*)-(−)-mandelate salt (960 mg, 1.9 mmol) was liberated as the free base and treated with a mixture of THF (6 mL), water (5 mL), 10 N NaOH (950 L), and enough MeOH to effect solution (3.5 mL). After 4 h at room temperature, the mixture was acidified to pH 0 with concentrated HCl and concentrated to dryness under reduced pressure. The residue was taken up in absolute EtOH, and the NaCl was filtered away. The filtrate was concentrated to dryness under reduced pressure and recrystallized from EtOAc−MeOH (ca. 8 mL). This gave 525 mg (74%) (*d*)-**10k**. $[\alpha]^{22}_D = +17.0°$ (c = 1.0, MeOH).

Synthesis of (*l*)-10k. The procedure used was essentially the same as that used for (*d*)-**10k**, beginning with the resolved **12b** (*S*)-(+)-mandelate salt. (*l*)-**10k** exhibited $[\alpha]^{22}_D = -16.5°$ (c = 1.27, MeOH).

Screen for Antispermatogenic Activity. The experimental animals were CD-1® (ICR)BR (cesarian derived, Institute of Cancer Research, barrier reared), VAF (viral antibody free) outbred Swiss albino mice (Charles River Laboratories, Inc., Raleigh, NC). The animals were 7 weeks old upon arrival. During quarantine (2−5 weeks), animals were housed singly in solid-bottom poly(propylene) or poly(carbonate) cages (11.5 in. × 7 in. × 5 in.) with stainless steel wire lids (Laboratory Products, Rochelle Park, NJ). Ab-Sorb-Dri bedding (Laboratory Products, Garfield, NJ) was used. Cages were changed at least once weekly. Deionized/filtered water and ground feed (NIH-07 rodent chow, Zeigler Brothers, Gardner, PA) in glass feeders (Wahmann Manufacturing, Timonium, MD) were made available *ad libitum*. The animal rooms were maintained at mean values of 22.2 °C and 58% relative humidity. Light/dark cycles were automatically controlled at 14 h light/10 h dark. All animals were uniquely identified by means of a stainless steel ear tag (AIMS, Inc., Piscataway, NY) and a cage card. Animals (5/group) were assigned randomly to dose level and compound group. No more that 75 mice were used for any experiment. For each screen, a positive control group (30 mg/kg **10a**) and a vehicle (Table 4, footnote a, and Table 5, footnote a) control group were included. Animals were observed daily for clinical signs. Animals were gavaged or injected sc with a dosing volume of 10 mL/kg on day 0. On day 3 (72 h later) they were euthanized (CO_2). Testes were removed, weighed, and sectioned for histology.[10]

Antifertility Experiments. Animals, care, and treatment were as described above. Male mice were given the test compounds or vehicle by gavage for 35 days. On the next day, each male was cohabited with two females for up to 5 days and checked each morning for the presence of a copulatory plug in the vagina to verify that mating had occurred. Mating was considered gestational day 0, and the male was removed from the cage.

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