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Communications to the Editor

Exceptionally Potent Antispermatogenic Compounds from 8-Halogenation of (4a*RS*,5*SR*,9b*RS*)-Hexahydroindeno-[1,2-*c*]pyridines

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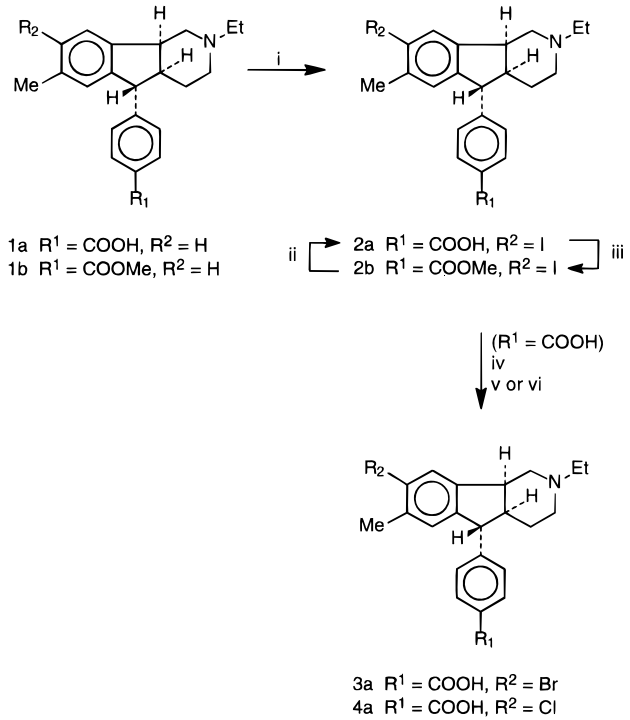
The need for new methods of male fertility control and the paucity of current chemical methods have been documented. Although a variety of nonsteroidal compounds disrupt spermatogenesis, most of them exhibit unacceptable toxic effects on other tissues.[1] Thus the report that the hexahydroindeno[1,2-*c*]pyridine Sandoz 20-438 was antispermatogenic on oral administration to mice, rats, and dogs without apparent effect on libido[2,3] and was nonmutagenic[4] led us to investigate this type of compound more thoroughly. We recently reported structure−activity relationship studies showing that the antispermatogenic activity of 5-aryl-(4a*RS*,5*SR*,9b*RS*)-hexahydroindeno[1,2-*c*]pyridines is highly stereo-, enantio-, and chemoselective. The activity was strongly sensitive to modifications of the para substituent on the 5-aryl group. When this substituent was carboxyl (**1a**, RTI-4587-054) or a group that could hypothetically be converted to carboxyl in vivo (e.g., **1b**, RTI-4587-056), compounds with excellent oral antifertility activity were obtained.[5] We have now found that the activity of these compounds is enhanced about 40-fold by halogenation at the 8-position of the hexahydroindenopyridine system.

Chemistry. Treatment of the carboxylic acid **1a** or methyl ester **1b** (either the racemate or the active enantiomer) with 1 mol of iodine/HgO in $HClO_4$/HOAc[6] led smoothly to the 8-iodinated products **2** (Scheme 1). The position of the iodine substituent was defined by the presence of two singlet peaks at 6.78 and 7.72 ppm in the aromatic region of the ^{1}H NMR for the *para*-6 and -9 protons, together with the expected AB pattern

Scheme 1[a]



[a] Reagents: (i) I_2, HgO, HOAc, $HClO_4$; (ii) HCl, H_2O; (iii) MeOH, $SOCl_2$; (iv) NaH, THF, HMPA, *t*-BuLi; (v) $BrCH_2CH_2Br$; (vi) Cl_3CCCl_3.

of the unchanged 5-aryl ring. The methyl ester **2b** (RTI-4587-073) could be hydrolyzed to the carboxylic acid **2a** (RTI-4587-074). This latter compound proved the best substrate for conversion of the 8-iodo substituent to other moieties. Formation of the sodium carboxylate with NaH followed by treatment with *tert*-butyllithium gave the 8-lithio intermediate, which reacted with 1,2-dibromoethane[7,8] or hexachloroethane[7] to yield the halogenated analogs **3a** and **4a**. All final compounds were converted to the HCl salts for analysis and biological testing.

Biology. Compounds were tested for their effects on spermatogenesis in Swiss mice. Mice were given a single oral dose as listed in Table 1, and necropsied 3 days later.[5,9] The sperm-producing ability of each testis

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Table 1. Antispermatogenic Effect of Halogenated Indenopyridine Compounds in Adult Male Swiss Mice[a]

structure	R^1	R^2	dose (μmol/kg)	SI[b] change (%)
		Racemates[c]		
1a	CO_2H	H	26	−24%*
			79	−52%*
2a	CO_2H	I	2	−67%*
			6	−66%*
			20	−76%*
2b	CO_2Me	I	2	−57%*
			6	−69%*
			20	−74%*
3a	CO_2H	Br	6	−69%*
			21	−71%*
			62	−72%*
4a	CO_2H	Cl	2	−55%*
			7	−66%*
			25	−72%*
		Chiral[d]		
(*l*)-**1b**	CO_2Me	H	3	3%
			8	−2%
			25	−33%*
			75	−64%*
(*l*)-**2b**	CO_2Me	I	0.6	−34%*
			2	−66%*
			6	−71%*
			19	−72%*

[a] A single dose of indenopyridine (as the HCl salt) or vehicle was given to mice by gavage at 10 mL/kg. Vehicle was 90% water, 7% Tween-20, and 3% ethanol. Necropsy was conducted on day 3, beginning about 72 h postdosing. [b] Spermatogenic index (percent change from vehicle control). Values were calculated from the means ($n = 5$) as [100(test − control)/control]. [c] All racemic compounds were tested at the same time. SI for vehicle control was 5.8 ± 0.2 (SE). [d] Chiral compounds were compared in the same assay. The vehicle was 1% Tween 20 in water. SI for vehicle control was 5.7 ± 0.2 (SE). *Significantly different from vehicle control; Dunnett's one-tailed *T*-test, $p < 0.05$. Statistical analysis was performed on the raw data before conversion to percent change.

was rated using the spermatogenic index of Fail,[10] which is based upon the histologic integrity of the seminiferous tubules. With an 8-iodo-7-methyl-4′-carboxy or -4′-carbomethoxy substituent pattern (**2a** and **2b**, Scheme 1), an oral dose of 2 μmol/kg (1 mg/kg) of the racemate resulted in a 57−67% decrease in the spermatogenic index and was at least as effective as a 79 μmol/kg (30 mg/kg) dose of the analog without the 8-iodo substituent. In the case of the 8-bromo or 8-chloro analogs (**3a**, **4a**), the lowest dose tested (6 or 2 μmol/kg) was also at least as effective as the 79 μmol/kg dose of the nonhalogenated analog (see Table 1). Comparison of the active (levo) enantiomer of the 8-iodo-7-methyl-4′-carbomethoxy analog with the active enantiomer of the 8-H-7-methyl-4′-carbomethoxy analog showed the former to have the same or greater effect at 0.6 and 2 μmol/kg (0.3 and 1 mg/kg) as the latter at 25 and 75 μmol/kg (10 and 30 mg/kg). Thus a 40-fold increase in molar potency was achieved by halogenation of the 8-position. The new analogs, like those previously reported,[5] also significantly reduced testis weight (data not shown).

The very specific structural requirements for antispermatogenic activity in the hexahydroindenopyridines and the lack of marked central effects have led us to postulate that these compounds exert their effects by interaction with a specific site in a specific macromolecule within the testis.[5] The enhanced activity of the iodo compound suggests that in the form of its radioactive analogs it will be useful for identifying the binding site and molecule. In addition the potent oral activity of these halogenated derivatives (significant effect of (*l*)-**2b** at a single dose of 10 μg per mouse) should enhance possibilities for control of male fertility with this series of compounds.

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Supporting Information Available: Experimental details (3 pages). Ordering information is given on any current masthead page.

References

(1) Christin-Maitre, S.; Guérin, J.-F.; Bouchard, Philippe. La Contraception Masculine. *Médecine/Sciences* **1995**, *11*, 579−90.
(2) Suter, K. E.; Hodel, C.; Gradient, F.; Fluckiger, E. Antispermatogenic Activity of an Indenopyridine Derivative. *Experientia* **1977**, *33*, 810.
(3) Hodel, C.; Suter, K. Reversible Inhibition of Spermatogenesis with an Indenopyridine (20−438). *Arch. Toxicol., Suppl. 1*, **1978**, 323−326.
(4) Matter, B. E.; Jaeger, I.; Suter, W.; Tsuchimoto, T.; Deyssenroth, H. Actions of an Antispermatogenic, but Non-mutagenic, Indenopyridine Derivative in Mice and *Salmonella typhimurium*. *Mutat. Res.* **1979**, *66*, 113−127.
(5) Cook, C. E.; Wani, M. C.; Jump, J. M.; Lee, Y.-W.; Fail, P. A.; Anderson, S. A.; Gu, Y.-Q.; Petrow, V. Structure-Activity Studies of 2,3,4,4a,5,9b-Hexahydroindeno[1,2-c]pyridines as Antispermatogenic Agents for Male Contraception. *J. Med. Chem.* **1995**, *38*, 753−763.
(6) Boja, J. W.; Kuhar, M. J.; Kopajtic, T.; Yang, E.; Abraham, P.; Lewin, A. H.; Carroll, F. I. Secondary Amine Analogues of 3β-(4'-Substituted phenyl)tropano-2β-carboxylic Acid Esters and N-Norcocaine Exhibit Enhanced Affinity for Serotonin and Norepinephrine Transporters. *J. Med. Chem.* **1994**, *37*, 1220−1223.
(7) Snieckus, V. Directed Ortho Metalation. Tertiary Amide and O-Carbamate Directors in Synthetic Strategies for Polysubstituted Aromatics. *Chem. Rev.* **1990**, *90*, 879−933.
(8) Miah, M. A. J.; Snieckus, V. Directed Ortho Metalation of O-Pyridyl Carbamates. Regiospecific Entries into Polysubstituted Pyridines. *J. Org. Chem.* **1985**, *50*, 5436−5438.
(9) Fail, P. A.; Anderson, S. A.; Wani, M. C.; Lee, D.; Cook, C. E. (1991). Response of Mouse Testis to Sandoz 20 438 (S20438). *Biol. Reprod. (Suppl. 1)* **1991**, *44* (1), 175 (Abstract 490).
(10) Whitsett, J. M.; Noden, P. F.; Cherry, J.; Lawton, A. D. Effect of Transitional Photoperiods on Testicular Development and Puberty in Male Deer Mice (*Peromyscus maniculatus*). *J. Reprod. Fertil.* **1984**, *72*, 277−286.

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