binding was determined by the inclusion of 500 μM L-glutamate. KA receptors were quantitated as previously described.[17a,b] [^{3}H]-KA (10 nM, 60 Ci/mmol) binding was determined in 50 mM Tris–citrate buffer, pH 7.0, at 4 °C for 30 min. Nonspecific binding was determined by the inclusion of 100 μM unlabeled KA. QA receptors were measured with [^{3}H]-AMPA binding as described by Honore et al.[17c] Essentially, [^{3}H]-AMPA (10 nM, 27.6 Ci/mmol) is quantified in 50 mM Tris–acetate buffer, pH 7.2, containing 100 mM KSCN for 30 min at 4 °C. Nonspecific binding was determined by the inclusion of 100 μM quisqualic acid. In the inhibition studies, the conformationally defined analogues were included in the assay mixture at the appropriate concentrations (0.1–200 μM). The assays (total volume, 1.08 mL) were initiated by the addition of radiolabel and terminated by centrifugation (Beckman Microfuge, top speed, 3 min). Unbound radioligand in the supernatant was removed by suction and the radioactivity in the pellet quantitated by liquid scintillation counting. All of the binding studies were carried out as two sets of triplicates within a single experiments and each experiment was repeated at least three times.

Acknowledgment. We thank Tracy N. Blakely and Kent C. Robbins for their expert technical assistance. This work was supported by NIH Grants NS27600 and NS01227 (RCDA to A.R.C.), NIA Grant AG07918, and NICDS Grant POHD24236.

Registry No. 1, 51-35-4; *N*-Cbz-1, 13504-85-3; **2**, 130830-60-3; **2** (4-*O*-deprotected), 103667-57-8; **3**, 130830-69-2; 4, 64927-38-4; *N*-Cbz-4, 130830-73-8; 4 (dimethyl ester, Mosher amide), 130830-79-4; **5**, 130830-61-4; **5** (4-*O*-deprotected), 130930-26-6; **6**, 130830-70-5; **7**, 64769-66-0; *N*-Cbz-**7**, 130830-74-9; **7** (dimethyl ester, Mosher amide), 130830-81-8; **8**, 2584-71-6; *N*-Cbz-**8**, 130930-25-5; **9**, 130830-63-6; **9** (4-*O*-deprotected), 130930-27-7; **10**, 130830-71-6; **11**, 130830-77-2; *N*-Cbz-**11**, 130830-75-0; 11 (dimethyl ester, Mosher amide), 130830-82-9; **12**, 130830-62-5; **12** (4-*O*-deprotected), 130930-28-8; **12** (4-acetoxy analog), 130830-64-7; **13**, 130830-72-7; 14, 130830-78-3; *N*-Cbz-14, 130830-76-1; 14 (dimethyl ester, Mosher amide), 130830-80-7; *N*-(benzyloxycarbonyl)-*cis*-4-cyano-L-proline ethyl ester, 130830-65-8; *N*-(benzyloxycarbonyl)-*trans*-4-cyano-L-proline ethyl ester, 130830-66-9; *N*-(benzyloxycarbonyl)-*trans*-4-cyano-D-proline ethyl ester, 130830-67-0; *N*-(benzyloxycarbonyl)-*cis*-4-cyano-D-proline ethyl ester, 130830-68-1.

Fadrozole Hydrochloride: A Potent, Selective, Nonsteroidal Inhibitor of Aromatase for the Treatment of Estrogen-Dependent Disease

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A new class of potent, selective, nonsteroidal inhibitors of aromatase have been discovered. The most potent member of this series is fadrozole hydrochloride, CGS 16949 A, 4-(5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridin-5-yl)benzonitrile monohydrochloride, **26a**. In addition, the 6,7-dihydropyrrolo[1,2-*c*]imidazole (**21a**) and the 6,7,8,9-tetrahydroimidazo[1,5-*a*]azepine (**21b**) analogues were synthesized and evaluated. CGS 16949 A's ability to selectively inhibit aromatase (IC_{50} = 4.5 nM) over other cytochrome P-450 enzymes and suppress estrogen production when administered orally make it a suitable candidate to test the potential of an aromatase inhibitor in estrogen-dependent diseases including breast cancer.

Estrogen has been implicated in the progression of several diseases. Consequently the direct antagonism of estrogen or the lowering of its circulating level is thought to be relevant to the therapy of disorders such as estrogen-dependent breast cancer, gynecomastia, systemic lupus erythematosus, and premature labor.[1] Currently tamoxifen, an estrogen receptor antagonist, and aminoglutethimide, an aromatase inhibitor, have proven to be efficacious in the treatment of estrogen-dependent breast cancer.[2,3]

The full potential of aromatase inhibitors in the treatment of estrogen-dependent diseases is yet to be fully evaluated. This has been due to the lack of a potent, highly selective, orally active, side-effect-free inhibitor of this enzyme. From a clinical viewpoint aminoglutethimide (**6**) comes closest to satisfying these criteria. However, its relatively low potency and lack of specificity make it less than ideal. The objective of these studies has been to identify a compound which fulfills these criteria and so provide the basis for testing the hypothesis that aromatase inhibition could be beneficial in the treatment of estrogen-dependent breast cancer.

Selectivity Criteria

Aromatase occurs widely in tissues such as adipose tissue, brain, testes, and ovaries. The enzyme is a membrane-bound microsomal complex containing NADPH-cytochrome *c* reductase and cytochrome P-450 units. The mechanism by which androstenedione is converted to estrone has been studied in detail.[4-17] This conversion can

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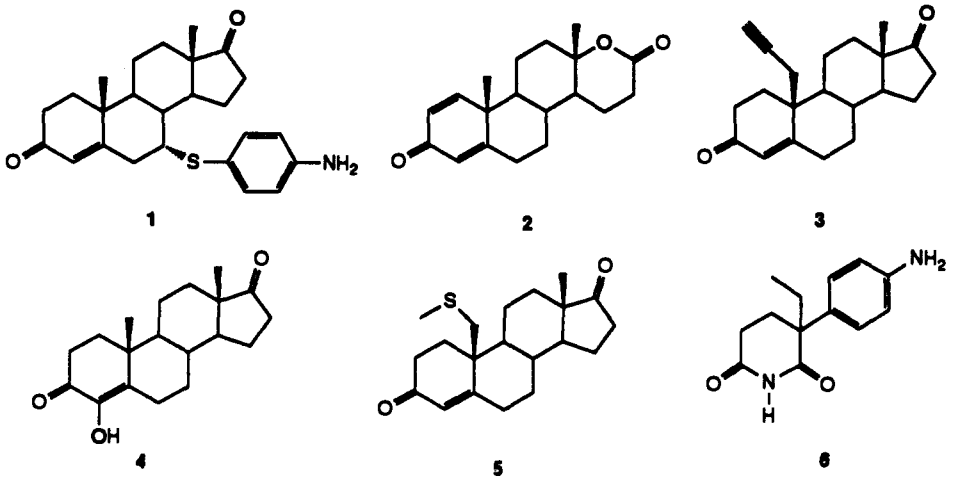
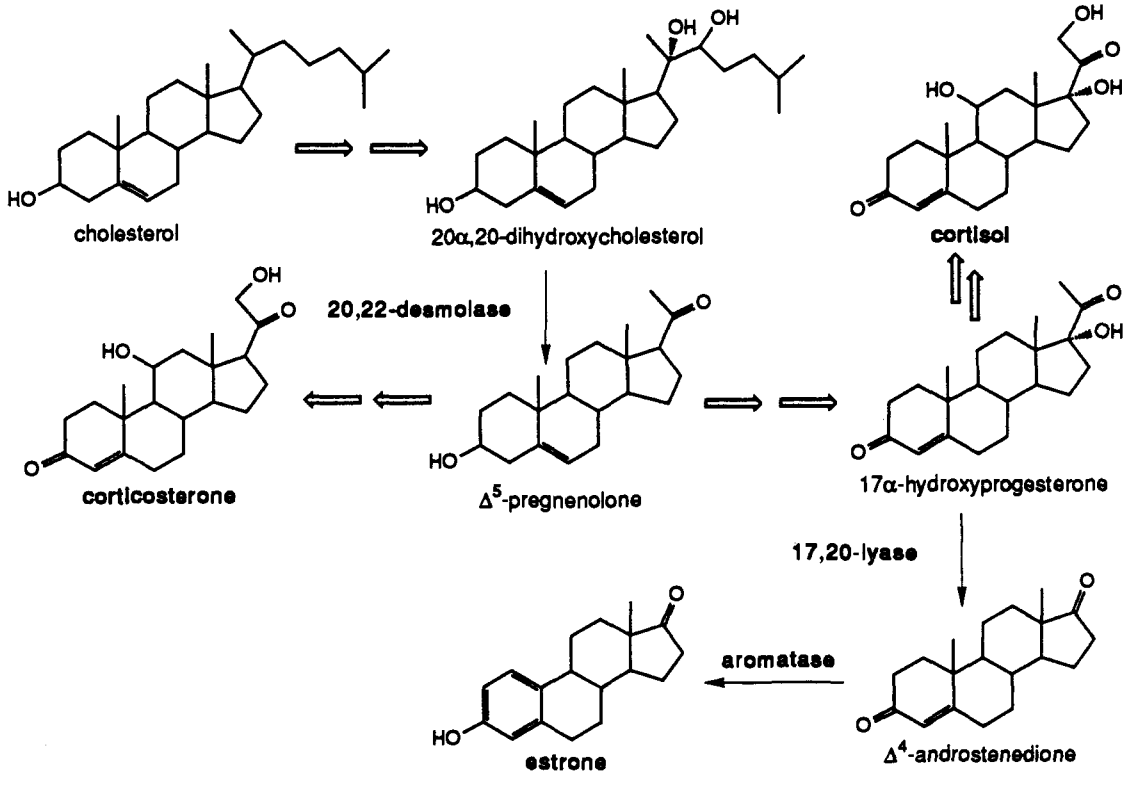


Scheme I. Steroidogenesis



be competitively inhibited by substrate mimics such as 7α-substituted 4-androstene-3,17-dione (1)[18,19] and testololactone (2)[20] and irreversibly by mechanism-based inhibitors such as 10-(2-propynyl)estr-4-ene-3,17-dione (3; MDL 18, 962)[21] and by 4-hydroxy-4-androsten-3,17-dione (4),[1] whose exact mechanism of action is still not clear. In general, these compounds produce type I high-spin optical difference spectra[20] and interfere with androgen binding to its enzyme binding site rather than by affecting the function of the heme iron atom.

Aromatase inhibition can also be achieved by compounds, e.g. 6, which bind directly to the cytochrome P-450 of the enzyme. Compounds acting by this mechanism exhibit type II optical difference spectra and could be viewed as potentially less selective inhibitors of cytochrome P-450 containing enzymes, since by coordinating to the iron of the porphyrin ring the enzyme complex is rendered catalytically incompetent. This is a clear disadvantage of aminoglutethimide (6), which requires hydrocortisone replacement therapy due to its potent desmolase inhibitory activity.[22] The inhibition of 20,22-desmolase, also a cy-

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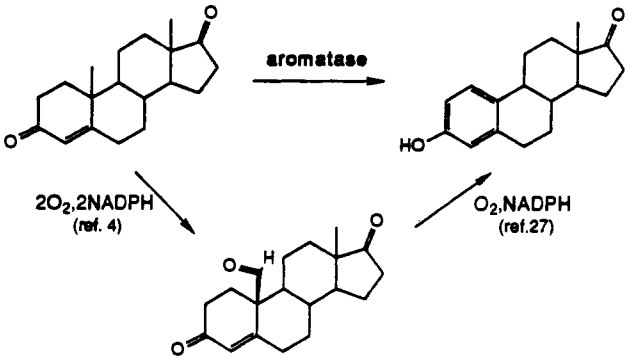
Scheme II. Androstenedione Oxidation by Aromatase



tochrome P-450 containing enzyme, prevents the oxidative side-chain cleavage of 20α,22β-dihydroxycholesterol and results in a blockade of not only estrogen biosynthesis but also the biosynthesis of other important secretory steroids (Scheme I). Since this cleavage is a step common also to cortisol biosynthesis, replacement therapy is necessary. Aromatization is the final step in the biogenesis of estrone and estradiol and is, consequently, the most effective step at which to selectively interfere without effecting the biogenesis of other steroids.

A further selectivity criterion had to be fulfilled. The toxicological potential of many heterocyclic compounds can be traced to their interaction with liver enzymes. Therefore, it was not only necessary to find selective inhibitors of steroid biosynthesis it was essential that the compounds would be much less potent as inhibitors of the ubiquitous cytochrome P-450 containing enzymes of the liver.[23]

Rationale

Since attempts to improve the potency, selectivity, and side-effects profile of aminoglutethimide (**6**)[24] and other aromatase-inhibiting steroids[21,25] had been largely unsuccessful despite an immense volume of work, it was decided that further efforts in this direction provided little chance of achieving a qualitative improvement on the already existing aromatase inhibitors.

Consequently, aminoglutethimide (**6**) and steroid analogues were intentionally avoided. Although, in the meantime, it has been possible to diminish the desmolase inhibitory activity of aminoglutethimide analogues, low potency continues to be a weakness of this series.[26] On the other hand, the abundant information concerning the mechanism of aromatization of 4-androstene-3,17-dione and its inhibition by these agents played an important part in the design of potential inhibitors.

The mechanism of aromatization has been studied in detail.[4-17] Although the exact nature of the third oxidative step of the pathway remains unclear (Scheme II),[4,27] the triple hydroxylation of the β-face of the steroid by a postulated ferroxy intermediate serves to localize the iron atom of the cytochrome P-450 complex of aromatase and suggests that a chelation to iron on the β-face of the steroid could be explored in inhibitor design. Kinetic, spectroscopic, and crystallographic studies with P-450-containing enzymes have supported the notion that the iron is proximal to the target atom for desmolase,[28] aromatase,[29,46] and P-450cam.[5] This hypothesis was further supported by the potency of 19-(methylthio)-4-androstene-3,17-dione (**5**; K_i = 1 nm) as a aromatase inhibitor and the fact that it induces a type II difference spectrum which is attributed to its binding to iron.[30] In addition, although aminoglutethimide (**6**) only inhibits aromatase at micromolar concentrations, its activity can be explained by assuming a chelation to iron through the anilino nitrogen. This is further strongly supported by aminoglutethimide's[31] type II difference spectrum, which is indicative of a low-spin iron–porphyrin complex. It seems likely that the anilino nitrogen, which possesses considerable sp^2 character, is the chelating atom. This mechanistic information stimulated a search for other structural types possessing this property.

This simple but attractive starting point did not guarantee the necessary high selectivity, since the cytochrome P-450 unit is common to many oxidative enzymes. However, simultaneous work on thromboxane A_2 synthetase,[32] also a cytochrome P-450 containing enzyme, had confirmed, at least in that case, that agents which owe their potency to direct interaction with iron can also be selective. On the basis of the selectivity of pirmazoben for TxA_2 synthetase, it was assumed that the imidazole ring was largely responsible for efficacy due to its strong interaction with the porphyrin nucleus, and the side chain provided selectivity and potency by mimicking the structure of prostaglandin H_2 in the binding site. The mimicry appears to be limited, in this case, to the carboxyl interaction with the catalytic site of TxA_2 synthetase.[32,33] It was hoped that this concept could be extended to discovering equally selective inhibitors of aromatase. Consequently, N-substituted imidazoles and 3-substituted pyridines, known inhibitors of TxA_2 synthetase, as well as other potential ligands were investigated in detail. This strategy lead to the discovery of *N*-(4-cyanobenzyl)imidazole (**9**), a potent inhibitor of human placental aromatase.[34]

Chemistry

The choice of a synthetic target was based, in part, on analogy to known TxA_2 synthetase inhibitors.[32] Further, it was shown that *N*-(4-cyanobenzyl)-5-methylimidazole (**7**), and *N*-[(4-cyanophenyl)-1-ethylmethyl]imidazole (**8**) maintain the high activity of *N*-(4-cyanobenzyl)imidazole (**9**), while *N*-(4-cyanobenzyl)-4,5,6,7-tetrahydrobenzimidazole (**10**) is 1000 times weaker.[34,35]

Consequently, rigid structures with the general structure **11** were chosen as targets which might possess improved potency and selectivity due to their conformational bias.

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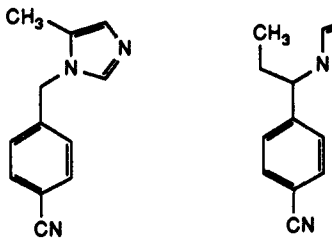

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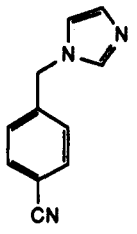

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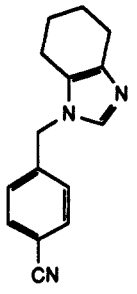

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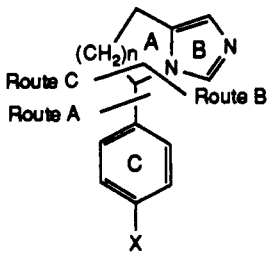

11

Three key retrosynthetic disconnections led to three synthetic pathways (Scheme III) whose complementarity provided a diverse series of compounds with which to probe the structure–activity relationship.

Route A (Scheme IV). The first approach relied on the diazotization of 4-aminobenzoic acid in the presence of pyridine[36] to yield the A and C rings of **11** (n = 2). The major product of this condensation is the desired ortho isomer. This was separated from the para- and meta-isomers, which were also present in about 10–15% yield. The imidazole ring was then constructed by a sequence beginning with activation of the pyridine ring with 40% peracetic acid and methylation of the *N*-oxide with dimethyl sulfate. The resulting crude salt was treated with potassium cyanide to yield 2,6-disubstituted pyridine **13** in 82% overall yield. The cyano group was reduced most practically at moderate pressure with Raney Ni (65%). Amine **14a** could be formylated and cyclized in one pot with formic acetic anhydride, but best yields were obtained through stepwise formylation with formic acid at 90 °C to yield **14b** in 83% yield and cyclization to **15** with phosphorus oxychloride at the same temperature, in 73% yield.

Subsequent reduction of the A ring with palladium on charcoal yielded 5,6,7,8-tetrahydroimidazo [1,5-*a*]pyridine (**16**; 85%), which was further modified by standard methods to yield the desired substituents in the 4-phenyl position. This approach is restricted to imidazopyridines by the use of pyridine in the diazotization. The synthesis of different A ring analogues demanded an alternative synthesis.

Route B (Scheme V). A more flexible, second approach was developed, which allowed the synthesis of imidazo[1,5-*a*]azepines and pyrrolo[1,2-*c*]imidazoles as well as other A ring analogues.

The protection of ethyl 4-imidazolylalkanoates **17a** with triphenylmethyl chloride and reduction with diisobutylaluminum hydride yielded the corresponding aldehydes **18** in good overall yields. The necessary aromatic ring was introduced with the lithium dianion derived from 4-bromo-*N*-*tert*-butylbenzenecarboxamide.[37] The secondary, benzylic alcohol **19** was chlorinated with 3 equiv of thionyl chloride in refluxing methylene chloride. The intramolecular cyclization proceeded in refluxing acetonitrile. The presumed quaternized intermediate was treated with refluxing methanol to remove the triphenylmethyl protecting group. Further treatment with hot thionyl chloride converted amide **20** to the important cyano derivatives **21a** and **21b**.

The starting alkanoic esters **17** are synthesized from ethyl urocanate (n = 1) by hydrogenation, from **22** (n = 1)[38] by a one-carbon homologation and from **18** by Wittig olefination[39] followed by hydrogenation (n = 3).

Route C (Scheme VI). A third, more convergent approach was developed starting with 3-(1*H*-imidazol-4-yl)-propanol (**22**).[38] This synthesis involved the regioselective, one-pot, double protection of the starting material by successive treatments with dimethylcarbamyl chloride and chlorotrimethylsilane in acetonitrile in near quantitative yield. The protected imidazole **23** was then alkylated with the appropriately para-substituted benzyl bromide, e.g. 4-(bromomethyl)benzonitrile.

The dimethylcarbamyl protecting group directs the benzylation adjacent to the propanol side chain. The resulting quaternary salt was decomposed with ammonia and the silyl group was removed hydrolytically during the workup to yield 1-[(4-cyanophenyl)methyl]-1*H*-imidazole-5-propanol (**24a**) in 55% yield.

Thionyl chloride in refluxing methylene chloride yielded the chloropropyl derivative **25** (96%), which was cyclized with 2.0 equiv of potassium *tert*-butoxide in tetrahydrofuran (86%) to yield 5-(4-cyanophenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine (**26a**).

This approach proved to be quite general except that the final cyclization conditions depended on the nature of the phenyl substituent. For example, 4-bromo derivative **25b** could be effectively cyclized only with lithium diisopropylamide in tetrahydrofuran containing 1.0 equiv of TMEDA. This base dependency is presumably a function of the electronegativity of the substituent and its resulting effect on the balance between the acidity of the benzylic protons and the nucleophilicity of the resulting anion.

Structure–Activity Relationships

1. Effect of the Aromatic Side Chain and Substituents (Table I). The original activity of *N*-(4-cyanobenzyl)imidazole (**9**) suggested that a lipophilic substituent on the aromatic ring was important for potent inhibition of placental aromatase. It was assumed that the ring mimics some part of the steroidal substrate. This was supported by the low potency of 5-(3-cyanopropyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine (**27**) compared to that of its aromatic isostere **26a**.

Para substitution of the aromatic ring was clearly preferred.[33] Furthermore the choice of the aromatic substituent influenced both the in vitro enzyme inhibition and

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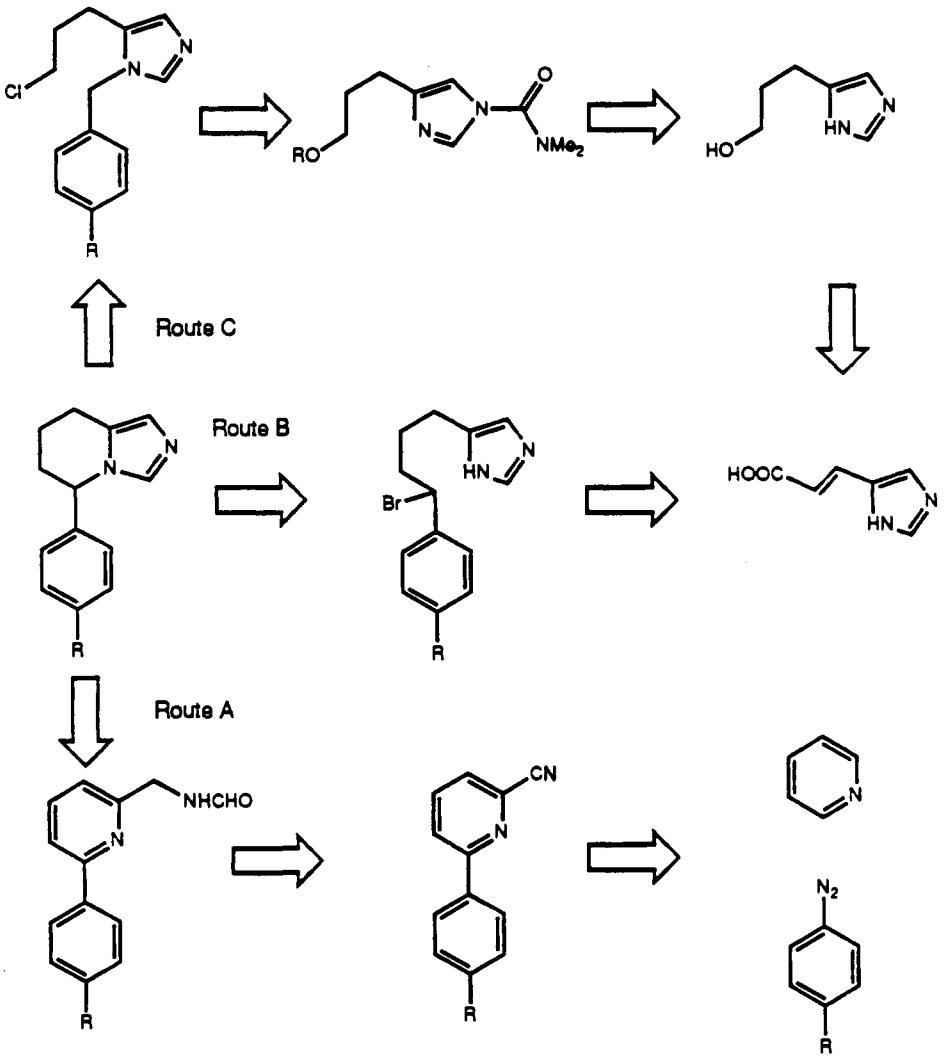
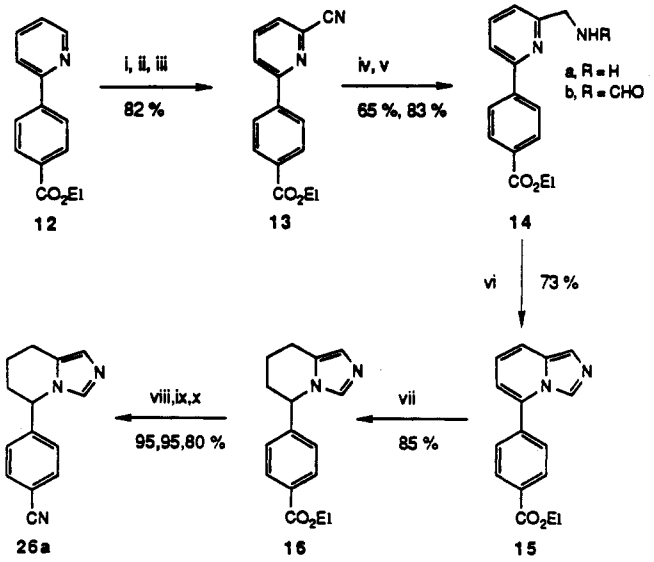
Scheme III. Retrosynthetic Pathways.



Scheme IV.[a] Synthetic Route A to Tetrahydroimidazo[1,5-*a*]pyridines



[a] (i) 40% CH_3CO_3H; (ii) Me_2SO_4; (iii) KCN; (iv) H_2, Pd/C; (v) HCO_2H, $PhCH_3$, 90 °C; (vi) $POCl_3$, toluene, 90 °C; (vii) H_2, Pd/C; (viii) NaOH, EtOH; (ix) $SOCl_2$, reflux; NH_3; (x) $POCl_3$, $CHCl_3$, reflux.

the minimum effective oral dose to approximately the same extent.

The 4-cyano and 4-bromo derivatives **26a** and **26b** proved to be the most potent inhibitors of the enzyme and more importantly their in vitro enzyme inhibitory activity was a reliable predictor of their in vivo potency. Other electron-withdrawing groups (**16** and **26c**) in the aromatic ring led to compounds with inhibitory activity in the 10 nM range in vitro. However, their in vivo potency tended to be significantly weaker. In general, it appears that hydrophilic electron-withdrawing para substituents are less effective in vivo. The more hydrophilic acid derivative **26e** was effective neither in vitro nor in vivo. This is in contrast to TxA_2 synthetase inhibitors which require an acid functional group in a similarly positioned side chain[32] for optimal activity.

The most active member of this series, fadrozole hydrochloride (CGS 16949 A, **26a**), was approximately 1000 times more potent than aminoglutethimide both in vitro and in vivo (Figure 1).[40]

2. Effect of Ring A Size and Flexibility (Table II). It was hypothesized that the potency of *N*-(4-cyanobenzyl)imidazole (**9**; IC_{50} = 15 nM) might be improved by increasing its conformational rigidity. This assumption was tested by the three conformationally restrained derivatives **21a**, **21b**, and **26a**. Both tetrahydroimidazo[1,5-*a*]pyridine (**26a**) and 6,7-dihydropyrrolo[1,2-*c*]imidazole (**21a**) proved to be approximately 1 order of magnitude

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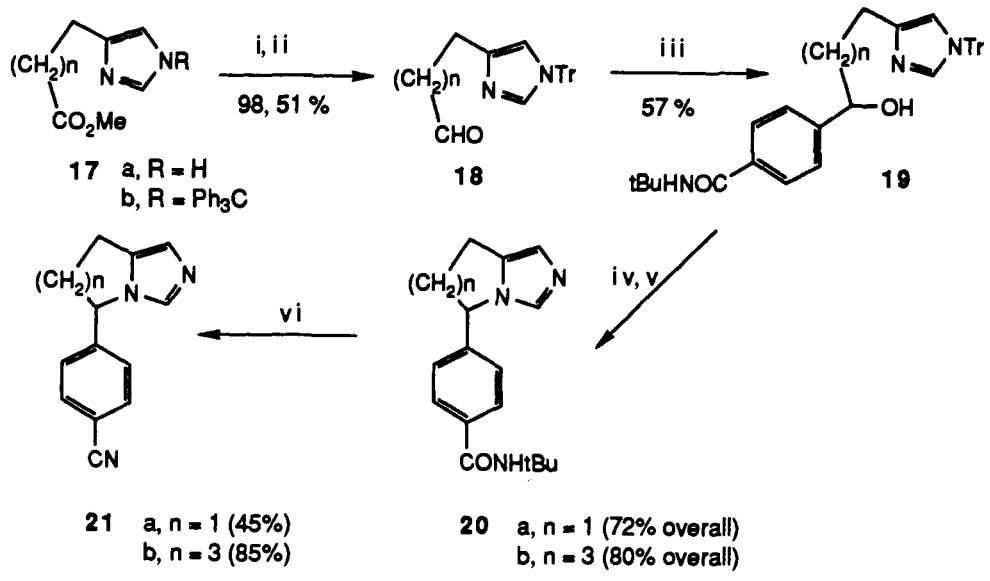
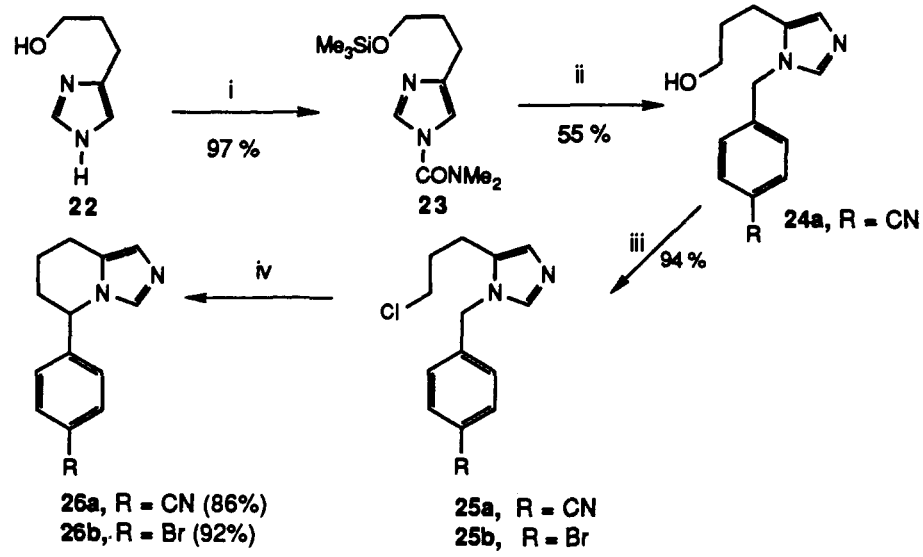
Scheme V.[a] Synthetic Route B to Ring Fused Imidazoles



[a] (i) Ph_3CCl, Et_3N, CH_2CN, reflux; (ii) 2.0 equiv of DIBAH, CH_2Cl_2, −78 °C; (iii) 4-LiPhCONLi-Bu-*t*, THF, −78 °C; (iv) 3.0 equiv of $SOCl_2$, CH_2Cl_2, reflux; (v) CH_3CN, reflux; (vi) $SOCl_2$, reflux.

Scheme VI.[a] Synthetic Route C to Tetrahydroimidazo[1,5-*a*]pyridines



[a] (i) Me_2NCOCl, Et_3N, CH_3CN; (ii) Me_3SiCl, Et_3N, CH_3CN; (iii0 4-bromomethylbenzonitrile, CH_3CN, reflux; NH_3, 0 °C; (iv) $SOCl_2$, CH_2Cl_2, reflux; (v) (A) 2.0 equiv of KOtBu, THF, (b) 2.0 equiv of LDA, TMEDA, THF, −78 °C.

more potent than their uncyclized progenitor **9**. This in vitro superiority correlated to an improved in vivo potency.

The corresponding seven-membered A ring analogue, 6,7,8,9-tetrahydroimidazo[1,5-*a*]azepine (**21b**), showed no significant superiority to *N*-(4-cyanobenzyl)imidazole. The additional flexibility of the seven-membered ring, and the resulting detrimental effect on the entropy term in the free energy equation, is a plausible explanation for its reduced potency in comparison to **21a** and **26a**.

3. Effect of the Enantiomers of CGS 16949 A. The enantiomers of CGS 16949 A could be separated by HPLC on β-cyclodextrin-bonded silica gel to yield the *d* and *l* enantiomers with $[\alpha]^{25}_D = +85.02°$ and $[\alpha]^{25}_D = -89.2°$, respectively. The IC_{50}s of the *d* and *l* compounds were 39 and 4.6 nM, respectively. While it was apparent that contamination of the *d* enantiomer with its antipode could explain its significant activity, the potency of the *l* isomer which is equivalent to the racemate clearly indicated that the aromatase is sensitive to the absolute stereochemistry of the inhibitor.[47]

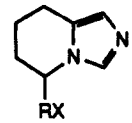
Pharmacology

The potency and selectivity with which fadrozole hydrochloride (**26a**) inhibits the aromatase enzyme in vitro has been described in detail. It was shown that fadrozole hydrochloride is a very potent inhibitor of both human placental and rat ovarian aromatase[40] and that in hamster ovarian slices fadrozole hydrochloride inhibited the production of estrogen with an IC_{50} of 0.03 μM.[41] The production of progesterone (an index of side chain cleaving enzyme activity) was only inhibited with a much higher IC_{50} of 120 μM. On the other hand, aminoglutethimide inhibited the production of estrogen and progesterone with the same IC_{50} of 30 μM. Thus, fadrozole hydrochloride was approximately 1000 times more selective than aminoglutethimide in inhibiting aromatase.

In vivo, fadrozole hydrochloride was able to inhibit the aromatase-mediated androstenedione-induced uterine

(41) Haeusler, A.; Schenkel, L.; Kraehenbuehl, C.; Monnet, G.; Bhatnagar, A. S. *J. Steroid Biochem.* **1989**, *33*, 125.

Table I. The Effect of the Aromatic Side Chain



no.	R	X	IC_{50} (conf limits),[a] nM	ED_{min},[b] μmol/kg	mp, °C	formula[c]
26a	C_6H_4	4-CN	6.4 (5.5, 7.4)	0.01	231–233	$C_{14}H_{13}N_3$·HCl
26b	C_6H_4	4-Br	7.6 (5.3, 10.9)	0.50	216–217	$C_{13}H_{13}BrN_2$·HCl
26c	C_6H_4	4-CH_2OH	14.3 (10, 20)	0.50	150–153	$C_{14}H_{16}N_2O$·$C_4H_4O_4$
26d	C_6H_4	4-CH_3	17.6 (12, 25)	ND	173–175	$C_{14}H_{16}N_2$·HCl
26e	C_6H_4	4-COOH	>1000	ND	309–310[d]	$C_{14}H_{14}N_2O_2$
27	nPr	3-CN	92.9 (76, 114)	ND	oil	$C_{11}H_{15}N_3$
16	C_6H_4	4-COOEt	21.0 (17, 25)	>10.0	164–166	$C_{16}H_{18}N_2O_2$·HCl

[a] IC_{50} (method 1) and asymptotic 95% confidence limits determined by logistic regression of percent inhibition vs log dose. [b] Minimal dose of compound which significantly ($p < 0.05$) inhibits ovarian estrogen synthesis. ND = not determined. [c] All C, H, N within ±0.4% of the theoretical values. [d] Decomposition.

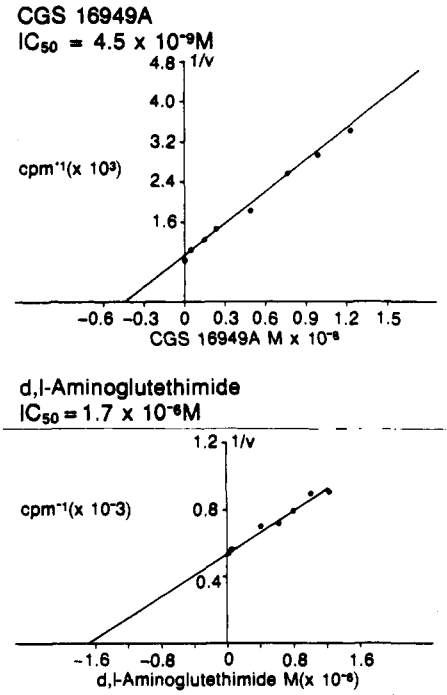
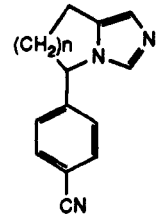


Figure 1. Determination of half-maximal inhibition of human placental microsomal aromatase by CGS 16949 A and aminoglutethimide phosphate in product isolation assay (method 2).

Table II. The Effect of the Ring Size



no.	*n*	IC_{50} (conf limits),[a] nM	% inhibition[b] 1.0	0.5	0.1	0.05	mp, °C	formula[c]
21a	1	2.0 (1.8, 2.3)		77	66	19[d]	227–228	$C_{13}H_{11}N_3$·HCl
26a	2	6.4 (5.5, 7.4)	94	87	65	33	231–233	$C_{14}H_{13}N_3$·HCl
21b	3	14.1 (13, 15)	52				153–155	$C_{15}H_{15}N_3$·$C_6H_6O_6$

[a] IC_{50} (method 1) and asymptotic 95% confidence limits determined by logistic regression of percent inhibition vs. log dose. [b] Inhibition at the listed doses in μmol/kg. [c] All C, H, N within ±0.4% of the theoretical valves. [d] Did not significantly differ from vehicle control ($p > 0.05$).

hypertrophy in immature female rats with an ED_{50} of 0.03 mg/kg when given orally.[42] In the same model, aminoglutethimide elicited the same effect with an ED_{50} of 30 mg/kg when given orally. Thus, fadrozole hydrochloride is 1000 times as potent as aminoglutethimide in this in vivo assay for aromatase. In intact, cyclic, female rats, fadrozole hydrochloride effectively reduced circulating levels of estradiol and suppressed uterine weight.

While fadrozole hydrochloride is highly specific for suppressing aromatase activity at low doses, in vitro and in vivo studies in animals and man have indicated that synthesis of other cytochrome P-450 dependent steroids can be suppressed to various degrees with higher doses of fadrozole hydrochloride. Uterine suppression was observed in vivo with doses of fadrozole hydrochloride which, unlike aminoglutethimide, did not induce adrenal hypertrophy[40] and thus indicate the lack of an inhibitory effect of fadrozole hydrochloride on corticosterone synthesis at doses which maximally suppressed estrogen synthesis. At a dose greater than 10 times the dose required to maximally suppress uterine weights in rats, a blunting of the corticosterone response to exogenous ACTH was observed in vivo. Using rat adrenal slices, the IC_{50}'s for inhibiting ACTH-stimulated corticosterone and aldosterone production were 100 and 1 μM, respectively.[49] In vivo, fadrozole hydrochloride suppressed plasma levels of aldosterone in ACTH-stimulated rats in a dose-dependent fashion, while necessitating 100-fold greater doses to suppress corticosterone.[49] At higher doses than that required for aromatase inhibition, fadrozole hydrochloride inhibited adrenal but not testicular mitochrondrial cholesterol side-chain-cleavage activity.[50] In postmenopausal women, fadrozole hydrochloride has been found to be a potent inhibitor of aromatase activity, a weaker inhibitor of aldosterone production, and an even weaker inhibitor of cortisol production.[51] The antitumor efficacy of fa-

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(44) Van Wauwe, J. P.; Janssen, P. A. J. *J. Med. Chem.* **1989**, *32*, 2231.
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(46) Osawa, Y.; Nobutake, Y.; Fronckowjak, M.; Kitawaki, J. *Steroids* **1987**, *50*, 11.
(47) Subsequent detailed studies on absolute stereochemistry will be published in the near future.
(48) Private communication to be published later by Professor Hartmann, University of Saarland.
(49) Haeusler, A.; Monnet, G.; Borer, C.; Bhatnagar, A. S. *J. Steroid Biochem.* **1989**, *34*, 567.
(50) Santen, R. J.; Langecker, P.; Santner, S. J.; Sikka, S.; Rajfer, J.; Swerdloff, R. *Endocr. Res.* **1990**, *16*, 77.
(51) Santen, R. J.; Demers, L. M.; Adlercreutz, H.; Harvey, H.; Santner, S.; Sanders, S.; Lipton, A. *J. Clin. Endocrinol. Metab.* **1989**, *68*, 99.

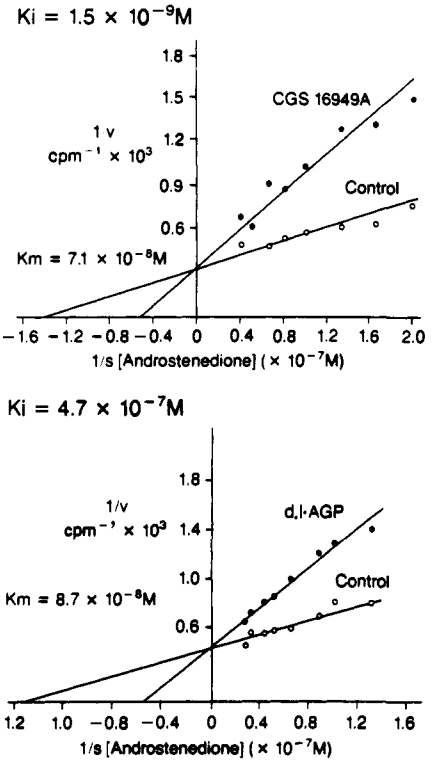

Figure 2. Double-reciprocal plots showing competitive nature of the inhibition by CGS 16949 A and aminoglutethimide phosphate of human placental aromatase activity in the product isolation assay (method 2).

drozole hydrochloride has been demonstrated in rats bearing DMBA-induced mammary tumors. It was shown that in intact female rats bearing DMBA-induced mammary tumors, oral administration of fadrozole hydrochloride caused almost complete regression of palpable tumors and almost totally suppressed the appearance of new tumors.[43] The ED_{50} for these effects was 0.1 mg/kg per day. A dose of 2 mg/kg per day resulted in maximal suppression of the growth of the DMBA-induced mammary tumors.

Mechanism of Action of Fadrozole Hydrochloride

Fadrozole hydrochloride is a competitive, reversible inhibitor of aromatase. Its Lineweaver–Burk plot is shown in Figure 2. It is a type II inhibitor of aromatase inducing a type II difference spectrum upon interaction with a cytochrome P-450 enzyme.[48] This indicates that fadrozole hydrochloride coordinates with the iron of the porphyrin nucleus, a feature it has in common with aminoglutethimide and other imidazoles, triazoles, and pyridines. By this mechanism, it competes with the binding of molecular oxygen to iron and reversibly inactivates the enzyme (Figure 2).

While this interaction of fadrozole hydrochloride with the porphyrin nucleus explains its mechanism of action, the high potency and selectivity of the compound must depend also on its complementarity with the enzyme active site. Since fadrozole hydrochloride does not obviously resemble the endogenous substrate androstenedione, it is merely speculation to propose how these chemical entities might overlap with one another in the active site (Figure 3). The low K_i = 1.5 nM (Figure 2) indicates a high affinity for aromatase which cannot be explained solely by the iron–imidazole interaction and must represent strong interactions between the aromatic ring and the porphyrin nucleus or the peptide tertiary structure of the enzyme. The former seems less likely to be of major importance since it would be likely to contribute little selectivity to

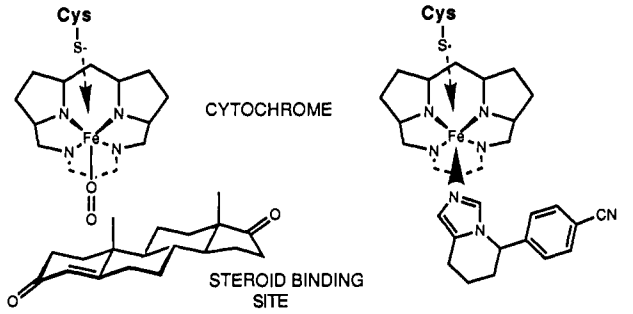

Figure 3. Proposed binding model of CGS 16949 A to the cytochrome P-450–aromatase complex.

the inhibitor-enzyme interaction. It seems more plausible that the 4-cyanophenyl group binds strongly to a part of the amino acid sequence of aromatase which is not shared with other cytochrome P-450 containing enzymes, such as desmolase. This could endow fadrozole hydrochloride with selectivity for aromatase. The saturated piperidine ring may also contribute to this interaction and hence selectivity, or it may simply serve to anchor the imidazole and phenyl rings in a near optimum conformation for binding in the active site. Although a full understanding of this interaction will depend on further investigation, it seems, for this class of inhibitors, the heterocyclic ring provides efficacy as an inhibitor and the additional functionality, a cyanophenyl ring in this case, imparts potency and selectivity for aromatase.

Conclusion

The pharmacological evaluation of fadrozole hydrochloride proves it to be a very potent inhibitor which effectively inhibits aromatase in vivo with an ED_{50} = 0.03 mg/kg po,[42] leading to significant reductions in estrogen levels. The excellent selectivity for aromatase over desmolase supports the working hypothesis that the strong binding to iron is responsible for the higher potency of the imidazoles and that the complementarity of the inhibitor with the steroid binding site, while enhancing this potency, more importantly provides selectivity for aromatase over desmolase and other cytochrome P-450 enzymes.

It is reasonable to assume that selective inhibitors of cytochrome P-450 containing enzymes of any type can be discovered provided that differences in the tertiary structure of the binding site can be exploited by incorporating sufficiently strong, but specific, additional ligand–protein interactions. By this approach, the nonselective activity of imidazole could be fine-tuned to any enzyme's structural requirements and yield a family of cytochrome P-450 inhibitors.[44]

The potency, selectivity, and oral activity of fadrozole hydrochloride make it a suitable candidate to test the potential of an aromatase inhibitor in estrogen-dependent diseases, especially breast cancer.

Experimental Section

Biology. In Vitro: Aromatase Tritiated Water Assay (Method 1). Human placental microsomes were prepared as described by Thompson and Siiteri[5] and the aromatase assay was conducted as described by these same investigators. Briefly, the assay measures the loss of tritium to the aqueous phase of the incubation medium during the aromatization of androstenedione labeled in positions 1 and 2. The microsomes are added to [1,2-^{3}H]androstenedione in buffer containing NADPH and incubated at 37 °C for 20 min. The reaction is terminated by the addition of 10 vol of chloroform. Following centrifugation, the aqueous phase is isolated and treated with twice the volume of a 5% aqueous suspension of charcoal to remove any remaining substrate not extracted by the chloroform. An aliquot of the remaining aqueous phase is assayed for radioactivity. Compounds

are tested for aromatase inhibitory activity by adding them to the incubation medium prior to the addition of the microsomes.

In Vitro: Product Isolation Assay For Aromatase Activity (Method 2). The assay was performed in a total volume of 1 mL at 37 °C as previously described.[40] Briefly, 120 μg of human placental microsomal protein was incubated with 11 μg of [4-^{14}C]androstene-3,17-dione ([4-^{14}C]A), 2.4×10^{-4} M NADPH (tetrasodium salt), and the appropriate concentration of inhibitor. The [4-^{14}C]A was added as a solution in 1.7% ethanol in 0.05 M potassium phosphate buffer (pH 7.4) so that the final concentration of ethanol did not exceed 0.02% (v/v). The reaction was started by the addition of enzyme and stopped after 20 min by the addition of 7 vol of ethyl acetate. Following extraction and centrifugation, the aqueous phase was reextracted with 7 vol of ethyl acetate. The extract was evaporated to dryness and dissolved in acetone prior to being chromatographed for 65 min on silica gel 60 thin-layer plates using ethyl acetate/isooctane (140:60, v/v) or toluene/chloroform/methanol (70:140:20). The radioactive estradiol and estrone peaks were identified by comparison with authentic standards and quantified with a liquid scintillation counter. The mean K_m for [4-^{14}C]A was 0.11 ± 0.06 μM (SD).[40]

In Vivo: Ovarian Estrogen Assay. Twenty-one-day-old female rats were injected (sc) with 10 IU of pregnant mare serum gonadotropin (PMSG). Two days later, the same rats were injected (sc) with 30 IU of hCG and, 24 h later, treated with either propylene glycol (0.2 mL/po) or the aromatase inhibitor in propylene glycol. One hour later, all of the rats were treated subcutaneously with 2.25 mg of androstenedione (A) in 0.1 mL of sesame oil. Four hours after the injection of A, the rats were killed. Their ovaries were freed of adhering tissue and stored at −40 °C prior to measuring their estrogen content.

To determine the total estrogen content of the ovaries, 1.5 mL of 0.05 M phosphate-buffered saline (pH 7.4) and 0.2 mL of 0.1 N NaOH were added to each pair of ovaries. The ovaries were homogenized with a Polytron, and the homogenate was extracted with 15 mL of ether. Aliquots (5 mL) were dried under N_2 and assayed in duplicate with the antiserum (Cat. No. 1630) purchased from Radioassay Systems Laboratories (Carson, CA). This antiserum had 100% cross-reactivity with E_1 and E_2. The results were expressed as total estrogen content (ng) per pair of ovaries. The sensitivity of the assay, defined as the smallest amount of E_2 causing a significant change in the percent bound, was consistently less than 10 pg. The total estrogen content of the samples was interpolated from the standard curve between the 10 and 500 pg standards. The mean intraassay coefficients of variation (CV) for samples read at these points were 8.1 and 9.2%, respectively. The mean interassay CV at these same points were 9.9 and 10.6%, respectively.

Chemistry. The melting points were measured with a Thomas melting point apparatus and are uncorrected. All temperatures are expressed in degrees centigrade. The NMR spectra were recorded on a Hitachi Perkin-Elmer R-600 or a Perkin-Elmer R-12 instrument unless stated otherwise. The IR spectra were recorded on a Perkin-Elmer 281B infrared spectrophotometer. Mass spectra were recorded on a Hewlett-Packard 5985B in the CI or EI mode. Chromatographies were carried out with 70–230 mesh silica gel from E. Merck, Darmstadt, as stationary phase, or with 2000 μm, 20 × 20 cm silica chromatography plates from Analtech. Metalations were carried out in tetrahydrofuran distilled from lithium aluminum hydride, with *n*-butyllithium in hexane from Alfa. Alkylating agents were freshly distilled prior to use. All compounds had elemental analyses for C, H, and N within ±0.4% of the theoretical value unless otherwise indicated.

6-Cyano-2-[4-(ethoxycarbonyl)phenyl]pyridine (13). Peracetic acid (40%, 8.9 mL, 64 mmol) was added dropwise to 14.08 g (65 mmol) of neat 2-[4-(ethoxycarbonyl)phenyl]pyridine[36] so as to maintain the reaction temperature between 80 and 85 °C. After the addition was complete, the reaction mixture was heated at 90 °C for 3 h and allowed to cool to room temperature. The excess peracetic acid was destroyed with aqueous sodium sulfite solution. The solvent was evaporated and the residue was taken up in methylene chloride and refiltered through Celite. Evaporation yielded 2-[4-(ethoxycarbonyl)phenyl]pyridine *N*-oxide, which was treated with 8.66 g (69 mmol) of dimethyl sulfate in 62 mL of toluene at 90 °C for 3 h. The volatile organics were evaporated, and the residue was redissolved in an ice-cold mixture of 8 mL of water and 9.3 mL (9.3 mmol) of 1 N sodium hydroxide. A solution of 13.65 g (0.21 mmol) of potassium cyanide in 10 mL of water was added slowly and the reaction mixture was maintained at 0 °C for 24 h. Extraction with methylene chloride, drying over sodium sulfate, and evaporation of solvent yielded 12.9 g (82%) of 6-cyano-2-[4-(ethoxycarbonyl)phenyl]pyridine as an amorphous powder: mp 85–87 °C; IR (CH_2Cl_2) ν 2260, 1715, 1588, 1450, 1270, 1110 cm^{-1}; NMR ($CDCl_3$) δ 1.40 (t, J = 7.0 Hz, 3 H), 4.42 (q, J = 7.0 Hz, 2 H), 7.5–8.3 (m, 7 H).

6-(Aminomethyl)-2-[4-(ethoxycarbonyl)phenyl]pyridine (14a). 6-Cyano-2-[4-(ethoxycarbonyl)phenyl]pyridine (12.0 g, 49 mmol) was hydrogenated at atmospheric pressure in 254 mL of methanol with 12.9 mL of concentrated hydrochloric acid and 2.63 g of 10% palladium on charcoal until 2 molar equiv of hydrogen had been consumed. Sodium methoxide (6.9 g, 128 mmol) was added, the catalyst was filtered off, and the solvent was evaporated. The residue was redissolved in 20 mL of methylene chloride, and the salts were removed by filtration. Evaporation of the solvent yielded a solid which was recrystallized from chloroform to yield 4.5 g (65%) of 6-(aminomethyl)-2-[4-(ethoxycarbonyl)phenyl]pyridine: mp 141–143 °C; IR (Nujol) ν 1710, 1685 cm^{-1}; NMR ($CDCl_3$) δ 1.4 (t, J = 7.0 Hz, 3 H), 4.14 (s, 2 H), 4.35 (q, J = 7.0 Hz, 2 H), 6.05 (s, 2 H), 7.3–8.3 (m, 7 H).

2-[4-(Ethoxycarbonyl)phenyl]-6-[(formylamino)methyl]pyridine (14b). A solution of 0.76 g (3.0 mmol) of 6-(aminomethyl)-2-[4-(ethoxycarbonyl)phenyl]pyridine in 10 mL of formic acid was heated at 90 °C for 15 h. The reaction mixture was cooled to 0 °C, made basic with excess saturated ammonium hydroxide solution, and extracted with chloroform. The organic extracts were dried and evaporated to yield 0.7 g (83%) of 2-[4-(ethoxycarbonyl)phenyl]-6-[(formylamino)methyl]pyridine which was recrystallized from toluene: mp 119.5–120.5 °C; IR (Nujol) ν 1710, 1685 cm^{-1}; NMR ($CDCl_3$) δ 1.35 (t, J = 7.0 Hz, 3 H), 4.28 (q, J = 7.0 Hz, 2 H), 5.25 (s, 2 H), 6.9 (br s, 1 H), 7.3–8.3 (m, 7 H).

5-[4-(Ethoxycarbonyl)phenyl]imidazo[1,5-*a*]pyridine (15). A solution of 9.8 g (35 mmol) of 2-[4-(ethoxycarbonyl)phenyl]-6-[(formylamino)methyl]pyridine and 11.15 g (72 mmol) of phosphorus oxychloride in 26 mL of toluene was heated at 90 °C for 15 h. The solvent was evaporated and the residue taken up in 50 mL of methylene chloride, cooled to 0 °C, and made basic with excess ice-cold, saturated ammonium hydroxide solution. The organic phase was separated, dried, and evaporated. The residual solid was passed through 100 g of silica gel with ethyl acetate as eluant (R_f = 0.48) to yield 6.8 g (73%) of 5-[4-(ethoxycarbonyl)phenyl]imidazo[1,5-*a*]pyridine: mp 118–119 °C; IR (CH_2Cl_2) ν 1714, 1610 cm^{-1}; NMR ($CDCl_3$) δ 1.42 (t, J = 8.0 Hz, 3 H), 4.40 (q, J = 8.0 Hz, 2 H), 6.4–7.0 (m, 2 H), 7.35–7.80 (m, 4 H), 8.10–8.35 (m, 3 H).

5-[4-(Ethoxycarbonyl)phenyl]-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine Hydrochloride (16). A solution of 2.0 g (7.5 mmol) of 5-[4-(ethoxycarbonyl)phenyl]imidazo[1,5-*a*]pyridine in 120 mL of anhydrous ethanol, containing 30 mL of concentrated hydrochloric acid, was hydrogenated with 1.0 g of 10% palladium on charcoal at 40 psi of hydrogen and 60 °C for 4 h. The catalyst was filtered and the solvent was evaporated to yield a solid which was recrystallized from 2-propanol and ether to provide 1.7 g (85%) of 5-[4-(ethoxycarbonyl)phenyl]tetrahydroimidazo[1,5-*a*]pyridine hydrochloride: mp 164–166 °C; IR (Nujol) ν 1725, 1595 cm^{-1}; NMR ($CDCl_3$) δ 1.4 (t, J = 6.5 Hz, 3 H), 1.6–3.2 (m, 6 H), 4.32 (q, J = 6.5 Hz, 2 H), 5.6–6.0 (m, 1 H), 7.1–7.4 (m, 3 H), 7.95 (d, J = 7.5 Hz, 2 H), 8.4 (s, 1 H). Anal. ($C_{16}H_{18}N_2O_2$·HCl) C, H, N.

5-[4-(Hydroxymethyl)phenyl]-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine (26c). A solution of 0.40 g (1.48 mmol) of 5-[4-(ethoxycarbonyl)phenyl]-5,6,7,8-tetrahydroimidazo[1,5-*a*]-pyridine in 20 mL of methylene chloride was cooled to −70 °C under nitrogen and 4.0 mL of a 1.53 M diisobutylaluminum hydride solution in toluene was added dropwise. The reaction mixture was allowed to warm to room temperature, quenched with 3.2 mL of methanol and 15 mL of water, and filtered through Celite. The layers were separated, dried over sodium sulfate, and evaporated to yield 0.32 g (95%) of 5-[4-(hydroxymethyl)-phenyl]tetrahydroimidazo[1,5-*a*]pyridine: mp 142–145 °C. Its fumarate was prepared in ethanol and recrystallized from acetone: mp 150–153 °C; IR (CH_2CH_2) ν 3595, 3160, 2950, 1487, 1100, 810 cm^{-1}; NMR (DMSO) δ 1.5–2.5 (m, 4 H), 2.82 (t, J = 7.0 Hz, 2 H),

4.50 (s, 2 H), 5.2–5.6 (m, 1 H), 6.13 (s, 2 H), 7.3 (d, J = 7.5 Hz, 2 H), 7.34 (d, J = 7.5 Hz, 2 H), 8.1 (s, 1 H). Anal. ($C_{14}H_{16}N_2O \cdot C_4H_4O_4$) C, H, N.

5-(4-Methylphenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine Hydrochloride (26d). A solution of 0.36 g (1.6 mmol) of 5-[*p*-(hydroxymethyl)phenyl]imidazo[1,5-*a*]pyridine in 25 mL of ethanol and 6.4 mL of concentrated hydrochloric acid was hydrogenated with 0.15 g of 10% palladium on charcoal at 40 psi of hydrogen and 60 °C for 4 h. The reaction mixture was filtered and evaporated and the residue was partitioned between methylene chloride and sodium bicarbonate solution. The organic phase was dried over sodium sulfate and evaporated to an oil which was purified by preparative-layer chromatography on silica with ethyl acetate. The hydrochloride salt was prepared with 1.1 molar equiv of ethereal hydrogen chloride to yield 0.24 g (63%) of 5-(4-methylphenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine hydrochloride: mp 173–175 °C; IR (CH_2CH_2) ν 2970, 1595, 1530, 1515, 1300 cm^{-1}; NMR ($CDCl_3$) δ 1.75–2.50 (m, 4 H), 2.37 (s, 3 H), 2.7–3.2 (m, 2 H), 5.3–5.7 (m, 1 H), 6.9–7.4 (m, 5 H), 7.94 (s, 1 H). Anal. ($C_{14}H_{16}N_2 \cdot HCl$) C, H, N.

5-(4-Carboxyphenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine (26e). A solution of 0.66 g (2.4 mmol) of 5-[4-(ethoxycarbonyl)phenyl]-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine in 8.0 mL of ethanol and 8.0 mL of 1 N sodium hydroxide was refluxed for 3 h, cooled, and evaporated. The residue was partitioned between water and ethyl acetate. The aqueous phase was adjusted to pH 5 with concentrated sulfuric acid and the solid was filtered and air-dried to yield 0.55 g (95%) of 5-(4-carboxyphenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine: mp 315–316 °C dec; IR (Nujol) ν 3400, 3200, 1710, 1470 cm^{-1}; NMR (DMSO) δ 1.6–2.4 (m, 4 H), 2.8–3.2 (m, 2 H), 5.2–5.8 (m, 1 H), 7.14–7.45 (m, 3 H), 7.9–8.3 (m, 3 H). Anal. ($C_{14}H_{14}N_2O_2$) C, H, N.

5-(4-Carbamylphenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine. A solution of 5.42 g (22.4 mmol) of 5-(4-carboxyphenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine in 75 mL of $SOCl_2$ was refluxed for 30 min and the excess $SOCl_2$ was removed by distillation. The residue was placed under N_2 and dissolved in 125 mL of methylene chloride at 0 °C. The solution was treated with gaseous ammonia for 2 h and kept under an ammonia atmosphere for 15 h. The resulting solid was filtered, washed with water, and dried to yield 5.15 g (95%) of 5-(4-carbamylphenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine: mp 181–183 °C; IR (Nujol) ν 1670, 1615, 1563, 1460, 1378 cm^{-1}; NMR (DMSO) δ 1.5–2.4 (m, 4 H), 2.6–3.0 (m, 2 H), 5.6–5.9 (m, 1 H), 6.95–7.4 (m, 3 H), 7.7–8.1 (m, 3 H). Anal. ($C_{14}H_{15}N_3O$) C, H, N.

5-(4-Cyanophenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine Hydrochloride (26a). A solution of 1.13 g (4.7 mmol) of 5-(4-carbamylphenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine and 1.0 mL (1.65 g, 10.7 mmol) of phosphorus oxychloride in 30 mL of chloroform is refluxed for 15 h, cooled, and evaporated with toluene. The resulting oil is redissolved in 30 mL of methylene chloride and cooled to 0 °C and 30 mL of an ice-cold solution of 50% ammonium hydroxide solution is added. The organic phase is separated, dried, and evaporated to an oil. Filtration through 20 g of silica with ethyl acetate (R_f = 0.17) yields 0.9 g (85%) of 5-(4-cyanophenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine which is dissolved in 20 mL of acetone and treated with 1.2 mL of 3 N ethereal hydrogen chloride to yield 0.98 g (80%) of its hydrochloride: mp 209–210 °C; IR (Nujol) ν 2235, 1608, 1529, 1461, 1469, 1380, 1300 cm^{-1}; NMR (DMSO) δ 1.7–2.5 (m, 4 H), 2.8–3.2 (m, 2 H), 5.87 (m, 1 H), 7.42 (d, J = 7.5 Hz, 2 H), 7.56 (d, J = 1.3 Hz, 1 H), 7.92 (d, J = 7.5 Hz, 2 H), 8.85 (d, J = 1.3 Hz, 1 H). Anal. ($C_{14}H_{13}N_3 \cdot HCl$) C, H, N.

***N,N*-Dimethyl-4-[3-[(trimethylsilyl)oxy]propyl]-1*H*-imidazole-1-carboxamide (23).** To a suspension of 51.8 g (0.41 mmol) of 1*H*-imidazole-4-propanol[45] in 500 mL of acetonitrile was added 50.0 g (0.49 mmol) of triethylamine followed dropwise by 48.6 g (0.45 mmol) of dimethylcarbamyl chloride. When the addition was complete, the mixture was refluxed for 21 h and then cooled to 0 °C, whereupon triethylamine hydrochloride precipitated. An additional 50 g (0.49 mmol) of triethylamine followed by 54.0 g (0.50 mmol) of chlorotrimethylsilane was added and stirring was resumed for 1 h. The mixture was diluted with an equal volume of ether and filtered. The filtrate was evaporated to an oil which was triturated with ether and filtered to remove additional triethylamine hydrochloride. This filtrate was evaporated to yield 106.0 g (97%) of *N,N*-dimethyl-4-[3-[(trimethylsilyl)oxy]propyl]-1*H*-imidazole-1-carboxamide as an oil. The product was used without further purification: NMR ($CDCl_3$) δ 0.12 (s, 9 H), 1.7–2.3 (m, 2 H), 2.7–3.2 (m, 2 H), 3.12 (s, 6 H), 3.5–3.9 (m, 2 H), 7.01 (s, 1 H), 7.85 (s, 1 H).

1-[(4-Cyanophenyl)methyl]-1*H*-imidazole-5-propanol (24a). A solution of 97.0 g (0.42 mmol) of *N,N*-dimethyl-4-[3-[(trimethylsilyl)oxy]propyl]-1*H*-imidazole-1-carboxamide and 72.0 g (0.37 mmol) of α-bromo-*p*-tolunitrile in 500 mL of acetonitrile was refluxed for 10 h. The solution was cooled to 0 °C in an ice bath and ammonia gas was bubbled in for 15 min. The mixture was evaporated in vacuo to give a semifold, which was dissolved in 500 mL of 1 N hydrochloric acid. The solution was allowed to stand at room temperature for 15 min and then was extracted with ether. The aqueous phase was adjusted to pH 9 with 50% sodium hydroxide and the mixture was then extracted with methylene chloride. The methylene chloride extracts were washed with water, dried over sodium sulfate, and evaporated to give a semisolid which was triturated with cold acetone to yield 49.1 g (55%) of 1-[(4-cyanophenyl)methyl]-1*H*-imidazole-5-propanol as a white solid: mp 128–136 °C; IR (Nujol) 3200–3110, 2230, 1498, 1378, 1106, 815 cm^{-1}; NMR (DMSO) δ 1.56–1.65 (2 H, m), 2.39 (2 H, t, J = 7.6 Hz), 3.38 (2 H, t, J = 6.2 Hz), 7.69 (1 H, s), 7.80–7.8 (2 H, m); MS m/e 242 (M + 1). Anal. ($C_{14}H_{15}N_3O$) C, H, N.

5-(3-Chloropropyl)-1-[(4-cyanophenyl)methyl]-1*H*-imidazole (25a). To a solution of 5.2 g (43.7 mmol) of thionyl chloride in 80 mL of methylene chloride was added in portions 8.4 g (35 mmol) of solid 1-[(4-cyanophenyl)methyl]-1*H*-imidazole-5-propanol. The rate of addition was regulated to control the foaming that occurred. When addition was complete, the solution was refluxed for 1.5 h, cooled in ice, and filtered to obtain 9.7 g (94%) of 5-(3-chloropropyl)-1-[(4-cyanophenyl)-methyl]-1*H*-imidazole hydrochloride as a buff-colored solid, mp 190–191 °C. The salt was partitioned between methylene chloride and saturated sodium bicarbonate. The organic extracts were washed, dried over sodium sulfate, and evaporated to yield 8.2 g (96%) of the free base as an oil which was characterized as the hydrochloride salt: mp 191–192 °C; IR (Nujol) ν 3085, 2740–2640, 2580–2560, 2218, 1611, 1601, 1380, 822, 820, 630 cm^{-1}; NMR (DMSO) δ 1.88–1.97 (2 H, m), 2.62 (2 H, t, J = 7.4 Hz), 3.62 (2 H, t, J = 6.4 Hz), 5.63 (2 H, s), 7.46–7.49 (2 H, m), 7.60 (1 H, d, J = 1.5 Hz), 7.87–7.89 (1 H, m), 9.40 (1 H, d, J = 1.5 Hz); MS m/e 260 (M + 1). Anal. ($C_{14}H_{14}ClN_3 \cdot HCl$) C, H, N.

5-(4-Cyanophenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine (26a). A solution of 8.1 g (31.2 mmol) of 5-(3-chloropropyl)-1-[(4-cyanophenyl)methyl]-1*H*-imidazole in 50 mL of tetrahydrofuran was cooled to 0 °C in an ice bath. To this was added 7.0 g (62.4 mmol) of potassium *tert*-butoxide as a solid in portions. The mixture was stirred at room temperature for 2 h, neutralized with 10% acetic acid, and partitioned between methylene chloride and water. The organic layer was washed with water, dried over magnesium sulfate, and evaporated to yield 6.8 g of an oil which was dissolved in a small volume of acetone and neutralized with ethereal hydrogen chloride. On cooling, 4.9 g of the hydrochloride as a white solid was obtained. Concentration of the mother liquors gave an additional 1.1 g of white solid. The combined yield of 5-(4-cyanophenyl)-5,6,7,8-tetrahydroimidazo-[1,5-*a*]pyridine hydrochloride was 6.0 g (86%), mp 201–203 °C. The hydrochloride can be recrystallized from 2-propanol and dried at 100 °C under reduced pressure (0.3 mmHg) to yield analytically pure material: mp 231–233 °C; IR (Nujol) ν 3124, 3070, 3000–2780, 2227, 1605, 1527, 1462, 1377, 1297; ^{1}H NMR (Bruker AM-300, DMSO) δ 1.75 (p, J = 5.9 Hz, 2 H), 1.95–2.10 (m, 1 H), 2.22–2.40 (m, 1 H), 2.90 (t, J = 6.2 Hz, 2 H), 5.79 (dd, J = 7.1, 5.4 Hz, 1 H), 7.40 (d, J = 8.3 Hz, 2 H), 7.52 (d, J = 1.3 Hz, 1 H), 7.89 (d, J = 8.3 Hz, 2 H), 8.81 (d, J = 1.3 Hz, 1 H); ^{13}C NMR (Bruker AM-300, DMSO) δ 16.64, 19.61, 30.47, 58.42, 111.08, 115.11, 118.43, 129.81, 131.01, 132.84, 134.00, 145.56; MS m/e 223 (M^+), 194, 140, 129, 115, 81. Anal. ($C_{14}H_{13}N_3 \cdot HCl$) C, H, N. The free base is regenerated from the hydrochloride by neutralizing with 1.1 molar equiv of sodium hydroxide in water, extracting with methylene chloride, and drying over sodium sulfate: mp 117–118 °C; IR (Nujol) ν 2228, 1604, 1481, 1237, 830, 800 cm^{-1}; NMR (DMSO) δ 1.65–1.77 (2 H, m), 1.82–1.93 (1 H, m), 2.19–2.29 (1 H, m), 2.78 (2 H, t, J = 6.4 Hz), 5.25 (1 H, t, J = 5.8), 6.76 (1 H, d, J = 0.98 Hz), 7.06 (1 H, d, J = 0.98 Hz), 7.06–7.08 (2 H, m), 7.53–7.56 (2

H, m); MS *m/e* 224 (M + 1). Anal. ($C_{14}H_{13}N_3$) C, H, N.

5-(4-Bromophenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine (26b). A solution (0.85 mmol) of lithium diisopropylamide, prepared at 0 °C from 0.12 mL of diisopropylamine and 0.33 mL of *n*-butyllithium (2.5 M) in 2 mL of tetrahydrofuran under nitrogen, was added to a solution of 0.13 mL of *N,N,N',N'*-tetramethylethylenediamine and 0.124 g (0.4 mmol) of 5-(3-chloropropyl)-1-[(4-bromophenyl)methyl]-1*H*-imidazole in 2 mL of tetrahydrofuran at −78 °C. The reaction mixture was stirred for 3.5 h, quenched at −78 °C with saturated ammonium chloride solution, and extracted with methylene chloride (3 × 10 mL). The organic extracts were dried over sodium sulfate and evaporated to yield 1.06 g (92%) of 5-(4-bromophenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine: mp 103–105 °C (acetone). The hydrochloride salt was prepared in acetone with 1.1 molar equiv of ethereal hydrogen chloride: mp 216–217 °C; IR (CH_2Cl_2) ν 3035, 2960, 2930, 1588, 1105, 1068, 1015, 815 cm^{-1}; NMR ($CDCl_3$) δ 1.6–2.5 (m, 4 H), 2.88 (t, J = 7.0 Hz, 2 H), 5.05–5.35 (m, 1 H), 6.85 (s, 1 H), 6.95 (d, J = 9.0 Hz, 2 H), 7.13 (s, 1 H), 7.48 (d, J = 9.0 Hz, 2 H). Anal. ($C_{13}H_{13}N_2Br$·HCl) C, H, N.

Methyl 3-[1-(Triphenylmethyl)-1*H*-imidazol-4-yl]-propionate (17b). A solution of 6.0 g (31.5 mmol) of methyl 3-(1*H*-4-imidazolyl)propionate and 11 mL (8.0 g, 79.0 mmol) of dry triethylamine in 31 mL of dimethylformamide was treated with a solution of 9.65 g (34.6 mmol) of triphenylmethyl chloride in 10 mL of dimethylformamide for 2 h at room temperature under nitrogen. The reaction mixture was poured onto 700 g of ice, and the resulting solid was collected by filtration and recrystallized from ether to yield 12.2 g (98%) of methyl 3-[1-(triphenylmethyl)-1*H*-imidazol-4-yl]propionate: IR (CH_2Cl_2) ν 2920, 1710, 1585 cm^{-1}; NMR ($CDCl_3$) δ 2.75 (m, 4 H), 3.05 (s, 3 H), 6.5–7.5 (m, 17 H).

3-[1-(Triphenylmethyl)-1*H*-imidazol-4-yl]propionaldehyde (18a). A solution of 44.4 mmol of diisobutylaluminum hydride in 29 mL of toluene was added to a solution of 8.79 g (22.2 mmol) of methyl 3-[1-(triphenylmethyl)-1*H*-imidazol-4-yl]propionate in 175 mL of methylene chloride at −72 °C under nitrogen. After 5 min the reaction was quenched by adding 14 mL of methanol followed by 90 mL of water. The reaction was allowed to warm to room temperature and was filtered through Celite. The organic phase was separated, dried over sodium sulfate, and evaporated to a yellow oil, which was chromatographed on 280 g of silica with ether (R_f = 0.26) to yield 4.13 g (51%) of 3-[1-(triphenylmethyl)-1*H*-imidazol-4-yl]propionaldehyde as an oil: IR ($CDCl_3$) ν 2830, 2740, 1730 cm^{-1}; NMR ($CDCl_3$) δ 2.7–3.0 (m, 4 H), 6.52 (s, 1 H), 7.0–7.6 (m, 16 H), 9.7 (s, 1 H).

4-[3-[4-[(*tert*-Butylamino)carbonyl]phenyl]-3-hydroxyprop-1-yl]-1-(triphenylmethyl)-1*H*-imidazole (19). A solution of 25 mmol of *n*-butyllithium in 10 mL of hexane was added dropwise to a solution of 3.19 g (13.0 mmol) of *N*-*tert*-butyl-4-bromobenzamide in 250 mL of tetrahydrofuran at −70 °C under argon. After 30 min, a solution of 3.74 g (10.2 mmol) of 3-[1-(triphenylmethyl)-1*H*-imidazol-4-yl]propionaldehyde in 100 mL of tetrahydrofuran was added slowly. The reaction was stirred at −70 °C for 30 min, allowed to warm to 25 °C, stirred at 25 °C for 2.5 h, and quenched with excess saturated ammonium chloride solution. The aqueous layer was separated and extracted with methylene chloride (2 × 100 mL). The combined organic extracts were dried over sodium sulfate and evaporated. The residue was chromatographed on 220 g of silica with 5:1 ether/ethyl acetate (R_f = 0.14) to yield 3.17 g (57%) of 4-[3-[4-[(*tert*-butylamino)carbonyl]phenyl]-3-hydroxyprop-1-yl]-1-(triphenylmethyl)-1*H*-imidazole as an oil: IR (CH_2Cl_2) ν 1660 cm^{-1}; NMR ($CDCl_3$) δ 1.40 (s, 9 H), 1.80–2.30 (m, 2 H), 2.4–2.7 (m, 2 H), 4.5–4.8 (m, 1 H), 5.95–6.10 (m, 1 H), 6.50 (s, 1 H), 7.0–7.7 (m, 20 H).

4-[3-[4-[(*tert*-Butylamino)carbonyl]phenyl]-3-chloroprop-1-yl]-1-(triphenylmethyl)-1*H*-imidazole. A solution of 3.21 g (6.0 mmol) of 4-[3-[4-[(*tert*-butylamino)carbonyl]-phenyl]-3-hydroxyprop-1-yl]-1-(triphenylmethyl)-1*H*-imidazole and 1.5 mL (2.5 g, 21.0 mmol) of thionyl chloride in 50 mL of methylene chloride was refluxed for 1 h, cooled, and poured into 5 mL of ice-cold sodium bicarbonate solution. The organic phase was separated, dried over sodium sulfate, and evaporated to yield 3.14 g of crude 4-[3-[4-[(*tert*-butylamino)carbonyl]phenyl]-3-chloroprop-1-yl]-1-(triphenylmethyl)-1*H*-imidazole as a white foam, which was used without purification: NMR ($CDCl_3$) δ 1.45 (s, 9 H), 4.30 (t, J = 6.0 Hz, 2 H).

5-[4-[(*tert*-Butylamino)carbonyl]phenyl]-6,7-dihydro-5*H*-pyrrolo[1,2-*c*]imidazole (20a). A solution of 3.13 g (5.6 mmol) of 4-[3-[4-[(*tert*-butylamino)carbonyl]phenyl]-3-chloroprop-1-yl]-1-(triphenylmethyl)-1*H*-imidazole in 150 mL of acetonitrile was refluxed for 15 h and cooled to room temperature and 150 mL of methanol was added. The reaction mixture was refluxed an additional 15 h and evaporated to dryness. The residue was partitioned between ether and water. The ether layer was separated and washed with 1 N HCl (2 × 15 mL). The combined aqueous extracts were adjusted to pH = 8 and extracted with methylene chloride which was dried over sodium sulfate, filtered, and evaporated to a white foam. The product was crystallized from ether to yield 1.30 g (72%) of 5-[4-[(*tert*-butylamino)-carbonyl]phenyl]-6,7-dihydro-5*H*-pyrrolo[1,2-*c*]imidazole: mp 136–139 °C; IR (CH_2Cl_2) ν 3440, 2980, 1670, 1615, 1525, 1501, 1220 cm^{-1}; NMR ($CDCl_3$) δ 1.46 (s, 9 H), 2.2–3.6 (m, 4 H), 5.10–5.55 (m, 1 H), 6.0–8.8 (m, 6 H). Anal. ($C_{17}H_{21}N_3O$) C, H, N.

5-(4-Cyanophenyl)-6,7-dihydro-5*H*-pyrrolo[1,2-*c*]imidazole Hydrochloride (21a). A solution of 1.25 g (4.4 mmol) of 5-[4-[(*tert*-butylamino)carbonyl]phenyl]-6,7-dihydropyrrolo[1,2-*c*]imidazole in 10 mL (16.3 g, 137 mmol) of thionyl chloride was refluxed for 1 h, cooled, and evaporated. The residue was redissolved in 10 mL of chloroform at 0 °C and 10 mL of ice-cold, concentrated ammonium hydroxide was slowly added. The aqueous layer was separated and extracted with chloroform (3 × 20 mL), and the combined organic extracts were dried over sodium sulfate. Filtration, evaporation, and chromatography on 45 g of silica with 5% ammonium hydroxide in ethyl acetate provided an oil which was treated with 1 molar equiv of ethereal hydrogen chloride to yield 0.5 g (45%) of 5-(4-cyanophenyl)-6,7-dihydro-5*H*-pyrrolo[1,2-*c*]imidazole hydrochloride: mp 227–228 °C; IR (Nujol) ν 2218, 1609, 1520, 1460, 1378, 860 cm^{-1}; NMR (DMSO) δ 2.5–3.4 (m, 4 H), 5.8–6.2 (m, 1 H), 7.52 (d, J = 9.0 Hz, 2 H), 7.98 (s, 1 H), 7.90 (d, J = 9.0 Hz, 2 H), 9.04 (s, 1 H). Anal. ($C_{13}H_{11}N_3$·HCl) C, H, N.

5-(4-Cyanophenyl)-6,7,8,9-tetrahydro-5*H*-imidazo[1,5-*a*]azepine Fumarate (21b). A solution of 1.29 g (4.1 mmol) of 5-[4-[(*tert*-butylamino)carbonyl]phenyl]-6,7,8,9-tetrahydro-5*H*-imidazo[1,5-*a*]azepine in 10 mL (16.3 g, 137 mmol) of thionyl chloride was refluxed for 1 h, cooled, and evaporated. The residue was partitioned between methylene chloride and ice-cold sodium bicarbonate solution. The aqueous layer was separated and extracted with methylene chloride (3 × 15 mL). The combined organic layers were dried over sodium sulfate and evaporated. The resulting oil was chromatographed on 26 g of silica with 5% methanol in methylene chloride. The product was treated with 1 molar equiv of fumaric acid in ethanol to yield 1.2 g (83%) 5-(4-cyanophenyl)-6,7,8,9-tetrahydro-5*H*-imidazo[1,5-*a*]azepine fumarate salt: mp 153–155 °C; IR (CH_2Cl_2) ν 2950, 2230, 1610, 1485 cm^{-1}; NMR (DMSO) δ 1.0–3.3 (m, 8 H), 6.0 (br s, 1 H), 6.67 (s, 3 H), 7.17 (d, J = 8.0 Hz, 2 H), 7.90 (d, J = 8.0 Hz, 2 H), 9.8 (s, 1 H). Anal. ($C_{15}H_{15}N_3$·$C_6H_6O_6$) C, H, N.

Ethyl 5-[1-(Triphenylmethyl)-1*H*-imidazol-4-yl]-1-pent-2-enoate. A solution of 5.6 mL (4.0 g, 40 mmol) of diisopropylamine in 150 mL of tetrahydrofuran at −70 °C under nitrogen was treated with 14.5 mL (36.2 mmol) of 2.5 M *n*-butyllithium for 30 min and 7.2 mL (8.1 g, 36.2 mmol) of triethyl phosphonoacetate was added dropwise. After 30 min, a solution of 10.09 g (27.6 mmol) of 3-[1-(triphenylmethyl)-1*H*-imidazol-4-yl]propionaldehyde in 50 mL of tetrahydrofuran was added slowly. The reaction mixture was allowed to warm slowly to room temperature, stirred for 15 h, and quenched with excess saturated ammonium chloride solution. The aqueous layer was separated and extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were dried over sodium sulfate and evaporated to an oil (15.35 g), which was chromatographed on 450 g of silica with ether (R_f = 0.71) to yield 9.61 g (80%) of ethyl 5-[1-(triphenylmethyl)-1*H*-imidazol-4-yl]-1-pent-2-enoate: mp 86–88 °C; IR (CH_2Cl_2) ν 3045, 2990, 1716, 1659, 1601, 1563, 1291, 1040 cm^{-1}; NMR ($CDCl_3$) δ 1.27 (t, J = 7.0 Hz, 3 H), 2.4–3.9 (m, 4 H), 4.20 (q, J = 7.0 Hz, 2 H), 5.80 (d, J = 14.0 Hz, 1 H), 6.5–7.6 (m, 18 H). Anal. ($C_{29}H_{28}N_2O_2$) C, H, N.

Ethyl 5-[1-(Triphenylmethyl)-1*H*-imidazol-4-yl]-1-pentanoate. A solution of 9.20 g (21.1 mmol) of ethyl 5-[1-(triphenylmethyl)-1*H*-imidazol-4-yl]-1-pent-2-enoate in 460 mL of

anhydrous ethanol was hydrogenated with 1.88 g of 10% palladium on charcoal at atmospheric pressure for 20 min. The catalyst was removed by filtration through Celite. Evaporation provided a solid which was recrystallized from hexane to yield 8.64 g (94%) of ethyl-5-[1-(triphenylmethyl)-1*H*-imidazol-4-yl]-1-pentanoate: mp 84–86 °C; IR (CH_2Cl_2) ν 2940, 1728, 1490, 1445 cm^{-1}; NMR ($CDCl_3$) δ 1.2 (t, J = 7.0 Hz, 3 H), 1.45–1.95 (m, 4 H), 2.1–2.8 (m, 4 H), 4.1 (q, J = 7.0 Hz, 2 H), 6.50 (s, 1 H), 7.0–7.5 (m, 16 H). Anal. ($C_{29}H_{30}N_2O_2$) C, H, N.

5-[4-[(*tert*-Butylamino)carbonyl]phenyl]-6,7,8,9-tetrahydroimidazo[1,5-*a*]azepine (20b). A solution of 3.93 g (6.6 mmol) of 4-[5-[4-[(*tert*-butylamino)carbonyl]phenyl]-5-chloropent-1-yl]-1-(triphenylmethyl)-1*H*-imidazole in 180 mL of acetonitrile was refluxed for 15 h and cooled to room temperature, and 90 mL of methanol was added. The reaction mixture was refluxed an additional 15 h and evaporated to dryness. The residue was partitioned between ether and water. The ether layer was separated and washed with 1 N HCl (3 × 20 mL). The combined aqueous extracts were adjusted to pH 8 and extracted with methylene chloride which was dried over sodium sulfate, filtered, and evaporated to a white foam. The product was chromatographed on 90 g of silica gel (ethyl acetate/NH_4OH 98:2, R_f = 0.4) to yield 1.65 g (80%) of 5-[4-[(*tert*-butylamino)-carbonyl]phenyl]-6,7,8,9-tetrahydro-5*H*-imidazo[1,5-*a*]azepine, which crystallized from ether: mp 176–178 °C; IR (CH_2Cl_2) ν 3435, 2940, 1663, 1530, 1497 cm^{-1}; NMR ($CDCl_3$) δ 1.46 (s, 9 H), 1.5–3.2 (m, 8 H), 5.4–5.7 (m, 1 H), 6.83 (s, 1 H), 6.98 (d, J = 8.0 Hz, 2 H), 7.20 (s, 1 H), 7.72 (d, J = 8.0 Hz, 2 H). Anal. ($C_{19}H_{25}N_{30}$) C, H, N.

(+)- and (−)-5-(4-Cyanophenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine Hydrochloride. Racemic 5-(4-cyanophenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine hydrochloride was applied, in 20-mg aliquots, to a 4.6 × 250 mm β-cyclodextrin-bonded silica gel column using 7:3 water/methanol as the eluant at a flow rate of 0.8 mL/min. The separate fractions were evaporated under vaccum to yield (−)-5-(4-cyanophenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine hydrochloride (mp 82–83 °C (amorphous); $[\alpha]^{25}_D$ = −89.2°) and (+)-5-(4-cyanophenyl)-5,6,7,8-tetrahydroimidazol[1,5-*a*]pyridine hydrochloride (mp 218–220 °C; $[\alpha]^{25}_D$ = +85.02°).

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4′-Hydroxy-3-methoxyflavones with Potent Antipicornavirus Activity

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4′-Hydroxy-3-methoxyflavones are natural compounds with known antiviral activities against picornaviruses such as poliomyelitis and rhinoviruses. In order to establish a structure–activity relationship a series of analogues were synthesized, and their antiviral activities and cytotoxicities were compared with those of flavones from natural origin. The 4′-hydroxyl and 3-methoxyl groups, a substitution in the 5 position and a polysubstituted A ring appeared to be essential requirements for a high activity. The most interesting compound was 4′,7-dihydroxy-3-methoxy-5,6-dimethylflavone possessing in vitro TI_{99} values of >1000 and >200 against poliovirus type 1 and rhinovirus type 15, respectively. This compound was also active against other rhinovirus serotypes (2, 9, 14, 29, 39, 41, 59, 63, 70, 85, and 89) tested, having MIC_{50} values ranging from 0.016 to 0.5 μg/mL. Finally in contrast to quercetin it showed to be not mutagenic in concentrations up to 2.5 mg in the Ames test.

Several flavonoids have shown to inhibit the replication of picornaviruses. Two classes of compounds can be distinguished according to their antiviral spectrum and mechanism of action. Some chalcones and flavans inhibit selectively different serotypes of rhinoviruses.[1-3] Compounds such as 4′-ethoxy-2′-hydroxy-4,6′-dimethoxychalcone and 4′,6-dichloroflavan interact directly with specific sites on the viral capsid proteins, thereby uncoating and consequently liberation of viral RNA.[4-6] The sensitivity of the virus depends on the serotype and the compounds. Aza analogues such as 2*H*-pyrano[2,3-*b*]pyridines and *N*-benzylbenzamides show the same activity.[7]

A second class of compounds consists of flavones. Various substituted derivatives, which are active against a wide range of picornaviruses, except Mengo virus, and vesicular stomatitis virus was isolated from several plants.[8-11] They interfere with an early step in the viral RNA synthesis. Although the molecular mechanism is not completely understood yet, they probably inhibit the formation of minus-strand RNA of poliovirus by interacting with one of the proteins involved in the binding of the virus replication complex to vesicular membranes at which poliovirus replication takes place.[12-15]

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