# Reversible Inhibitors of the Gastric ($H^+/K^+$)-ATPase. 1. 1-Aryl-4-methylpyrrolo[3,2-*c*]quinolines as Conformationally Restrained Analogues of 4-(Arylamino)quinolines

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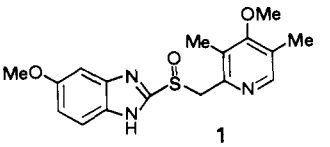
The 4-(arylamino)quinoline **4**, previously described as an antiulcer compound, is shown to be an inhibitor of the gastric ($H^+/K^+$)-ATPase. It is postulated that 1-arylpyrrolo[3,2-*c*]quinolines **6** act as conformationally restrained analogues of **4**. A series of derivatives of **6** has been prepared and shown to be potent inhibitors of the target enzyme in vitro. Substitution in the ortho position of the aryl ring is important for activity. Unsaturation in the 5-membered ring makes little difference, but introduction of heteroatoms into the same ring markedly reduces activity. In more detailed kinetic experiments, **15c** and **4** both show reversible, $K^+$-competitive binding to the enzyme, with submicromolar $K_i$ values. The compounds appear to act at the lumenal face of the enzyme and to require protonation for activity. Several compounds in the series are shown to be potent inhibitors of pentagastrin-stimulated acid secretion in the rat.

There has been considerable interest in recent years in the gastric ($H^+/K^+$)-ATPase (the "proton pump"). This is the enzyme responsible for the secretion of acid into the gastric lumen,[1] and has thus been viewed as an important target for peptic ulcer therapy. Much of the impetus for this work came from the discovery of the [(pyridylmethyl)sulfinyl]benzimidazoles (PSBs) such as omeprazole (**1**), which has been shown to be an inhibitor of the ($H^+/K^+$)-ATPase[2,3] and an effective treatment for peptic ulcer disease.[4] It has been shown by ourselves and others that their mode of action involves extensive intramolecular rearrangement,[5-7] followed by covalent modification of the enzyme.[8] However, as a consequence of their covalent interaction, the PSBs show a long duration of action in vivo, which may be responsible for the elevated levels of circulating gastrin observed after repeated administration of these compounds.[9] Since it is known that gastrin exerts a trophic effect on the gastric mucosa,[10] we felt that it would be of therapeutic interest to have ($H^+/K^+$)-ATPase inhibitors with a significantly shorter duration of action. We therefore began a search for freely reversible, noncovalent inhibitors of the target enzyme.

The first inhibitors of this type to be reported were a series of imidazo[1,2-*a*]pyridines and imidazo[1,2-*a*]-pyrazines with antiulcer and cytoprotective activity. Initial work was done mainly on the highly potent compound **2** (SCH 28080),[11] and then after this was found to be hepatotoxic attention switched to the somewhat less potent derivative **3** (SCH 32651).[12] These compounds have now been studied in some detail and have been shown to be reversible, potassium-competitive inhibitors of the ($H^+/K^+$)-ATPase.[13-15] It appeared possible that other lipophilic, basic heterocyclic molecules that had been reported to have gastric antisecretory activity might also be acting by a similar pharmacological mechanism. In practice, this view proved misleading for some classes of compounds,[16] but appears to be valid for the 4-(arylamino)quinoline-3-carboxylate esters such as **4**.[17] Biochemical studies on **4** (vide infra) suggest that this would be an appropriate lead compound for further modifications.

One approach to devising new inhibitors was based on the hypothesis that the ester group in **4** is responsible for fixing the conformation of the arylamino group, both by forming a hydrogen bond to the NH and by increasing the conjugation between the nitrogen and the quinoline ring through its effect as a $\pi$-electron-withdrawing group. We

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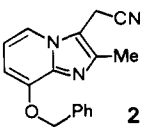


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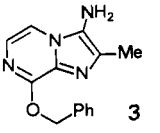


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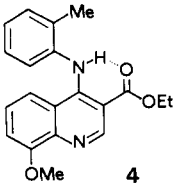


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(1) Sachs, G. *Physiology of the Gastrointestinal Tract*, 2nd ed.; Johnson, L. R., Ed.; Raven Press: New York, 1987; Vol. 1, pp 865–881.
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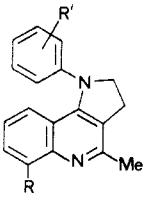
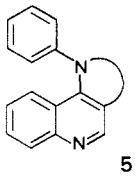
Table I. Synthesis, Structure, and Physical Properties of Some 1-Aryl-4-methyl-2,3-dihydropyrrolo[3,2-c]quinolines



| no. | R | R′ | solvent[a] | temp | reaction time | mp, °C (solvent) | yield, % | formula[b] |
|---|---|---|---|---|---|---|---|---|
| 15a | $OCH_3$ | H | 1-BuOH | reflux | 22 h | 164–5 (aq EtOH)[c] | 27 | $C_{19}H_{18}N_2O$ |
| 15b | $OCH_3$ | 2-$OCH_3$ | 1-BuOH | reflux | 6 h | 168–9 (aq MeOH) | 19 | $C_{20}H_{20}N_2O_2$ |
| 15c | $OCH_3$ | 2-$CH_3$ | 1-BuOH | reflux | 5 days | 182–3 (aq EtOH) | 22 | $C_{20}H_{20}N_2O$ |
| 15d | $CH_3$ | 2-$CH_3$ | 1-BuOH | reflux | 4 days | 126–8 (aq EtOH) | 23 | $C_{20}H_{20}N_2$ |
| 15e | H | 2-$CH_3$ | EtOH | 150 °C | 17 h | 148–50 (EtOAc) | 43 | $C_{19}H_{18}N_2$ |
| 15f | H | 2-$CH_3$-4-$OCH_3$ | EtOH | 170 °C | 18 h | 115–7 (aq MeOH) | 22 | $C_{20}H_{20}N_2O \cdot 0.4H_2O$ |
| 15g | H | 2-$CH_3$-4-OH | 1-BuOH | reflux | 20 h | 315–8 (MeOH) | 12 | $C_{19}H_{18}N_2O$ |
| 15h | H | 2,6-$(CH_3)_2$ | 2-PrOH | 140 °C | 5 days | 149–52 (aq EtOH) | 11 | $C_{20}H_{20}N_2$ |
| 15i | $OCH_3$ | 2-$CH_3$-4-$OCH_3$ | 2-PrOH | reflux | 2 days | 163–7 (aq EtOH) | 19 | $C_{21}H_{22}N_2O_2 \cdot 0.9H_2O$ |
| 15j | F | 2-$CH_3$ | 2-PrOH | 170 °C | 18 h | 209–11 (EtOAc) | 29 | $C_{19}H_{17}FN_2$ |
| 15k | OH | 2-$CH_3$ | *d* | | | 144–6 (EtOAc) | | $C_{19}H_{18}N_2O$ |

[a] See Experimental Section for description of general methods. [b] All new compounds had C, H, N microanalyses within 0.3% of the calculated values. [c] Literature 151–151.5 °C (ref 19). [d] See Experimental Section.

therefore investigated compounds of type **5**, in which the conformational constraint is achieved covalently by formation of an additional ring. Such compounds are not particularly numerous in the literature; the largest class consists of the 1-phenyl-4-methylpyrrolo[3,2-*c*]quinolines together with their 2,3-dihydro derivatives (**6**). Such compounds have been tested for antibacterial activity,[18] but there have been no reports of their effect on gastric secretion. The results obtained with these and related compounds are the subject of the present paper.



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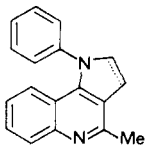

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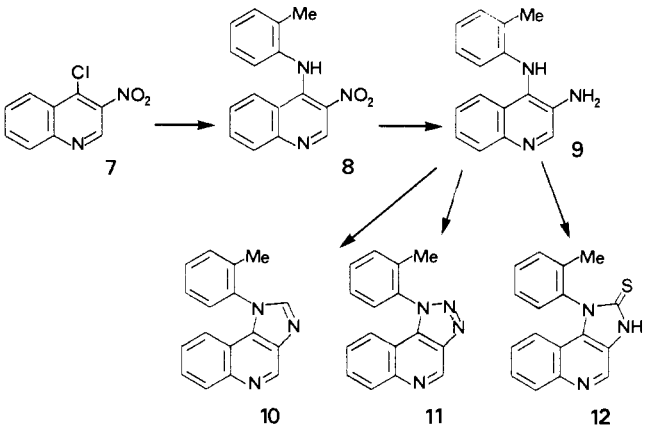
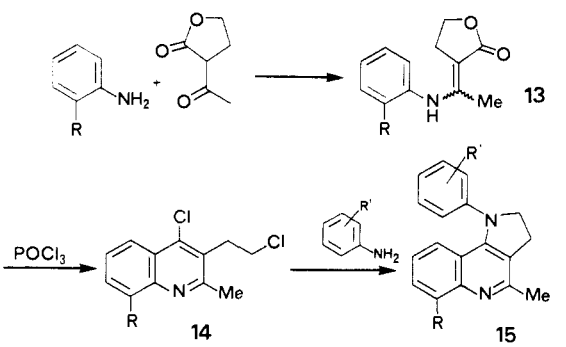
## Chemistry

The first compounds of type **5** to be prepared were a small group of imidazo- and triazoloquinolines shown in Scheme I. Compound **7** reacted smoothly with *o*-toluidine to give **8**, which could be reduced with $SnCl_2$ to the amine **9**. This served as a common intermediate to several target compounds, giving the imidazoquinoline **10** by reaction with formic acid, the triazoloquinoline **11** by diazotization, and the thione **12** by reaction with carbon disulfide.

**Scheme I**



**Scheme II**



Synthesis of the 1-aryl-4-methyl-2,3-dihydropyrrolo-[3,2-*c*]quinolines **15a–g** followed the method of Ozawa and Nagaoka[19,20] (Scheme II). Although the first two steps to give **14** could in some cases be carried out as a one-pot reaction, we generally found it preferable to isolate the intermediate **13**. In most cases a mixture of *E* and *Z* isomers of **13** was obtained, but it was found that both could be converted equally readily to **14**, so the chlorination step was most conveniently carried out on the isomer mixture. It should be noted that the conversion of **13** to

(11) Kaminski, J. J.; Bristol, J. A.; Puchalski, C; Lovey, R. G.; Elliot, A. J.; Guzik, H.; Solomon, D. M.; Conn, D. J.; Domalski, M. S.; Wong, S.-C.; Gold, E. H.; Long, J. F.; Chiu, P. J. S.; Steinberg, M.; McPhail, A. T. *J. Med. Chem.* **1985**, *28*, 876.
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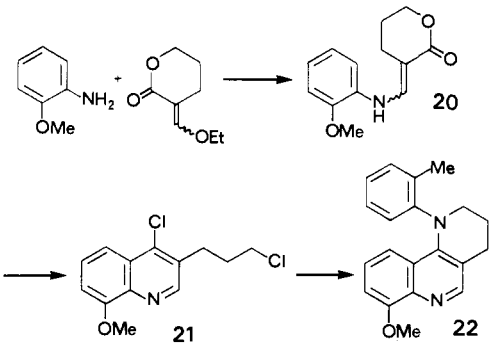
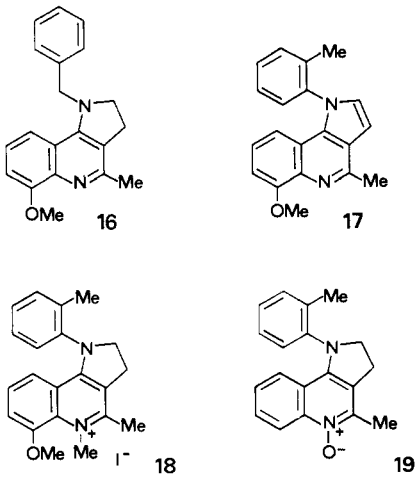
Scheme III




14 can on occasion initiate with a sudden exotherm, which can be hazardous in large-scale preparations. Reaction of 14 with anilines was often quite sluggish (Table I). Thus 14 (R = OMe) with aniline or *o*-anisidine hydrochlorides in 1-butanol gave **15a** or **15b** after 6 h at reflux, but the corresponding reaction with *o*-toluidine required 5 days to give **15c**. As expected, the reaction with the sterically hindered 2,6-dimethylaniline was particularly slow and low-yielding, though after some optimization it proved possible to isolate **15h** in acceptable amounts. Use of a secondary alcohol (2-propanol) as solvent proved essential in this case; 1-butanol reaction with the chloroquinoline more rapidly than the dimethylaniline, to give a mixture of butoxy-containing byproducts. In general, the reaction could conveniently be carried out at elevated temperature in a pressure vessel, except in cases where R = OMe, which gave rise to excessive levels of side reactions. For example, the formation of **15c** at 170 °C was accompanied by **15k** as one of several byproducts.

Synthesis of an *N*-benzyl analogue (**16**) by reaction of 14 with benzylamine was similar to the preparation of **15**.

Oxidation of the 2,3-dihydropyrrolo[3,2-*c*]quinolines to the fully aromatic system could conveniently be accomplished by dehydrogenation over palladium catalyst; thus **15c** yielded **17**. It was hoped to derivatize the 5-membered ring of **17** by electrophilic substitution. However, in contrast to indoles, Vilsmeier–Haack formylation failed, with only starting material recovered.




Derivatization of **15c** also proved difficult. Quaternization with methyl iodide gave **18** in modest yield. Attempted N-oxidation of **15c** failed totally, presumably due to steric hindrance,[21] but **15e** gave **19** in low yield. Both **18** and **19** were contaminated with a small amount of starting material which could not be removed by chromatography or recrystallization, largely due to the instability of the two compounds.

We also prepared the 6-membered ring compound **22** by a route similar to that used for the dihydropyrroloquinolines **15** (Scheme III). The dichloro compound **21** was obtained without difficulty, but as before the reaction with 2-methylaniline proved sluggish and low-yielding.

Conformational calculations on **15c** using both molecular mechanics and MNDO suggested that in its minimum-energy conformation the 1-aryl substituent should be essentially orthogonal to the plane of the pyrroloquinoline ring system, with a significant energy barrier to rotation of the aryl ring. NMR spectra of dihydropyrroloquinolines ortho substituted in the aryl ring support the idea of restricted rotation about the N–aryl bond, with chemical shifts for the prochiral hydrogen atoms on the 5-membered ring differing by about 0.4 ppm in both **15b** and **15c**. The compounds, therefore, are clearly chiral on the NMR time scale.

Isolation and characterization of the 1-arylpyrroloquinolines was made easier by the characteristic intense fluorescence of these compounds, shared by few of the byproducts. Absorption and emission spectra were recorded for several of the purified compounds, and the results are summarized in Table II. The most intense fluorescence was observed with **15c**, for which emission spectra could easily be measured at concentrations as low as 50 nM.

Table II. UV Absorption and Fluorescence Emission Spectroscopy[a]

| no. | absorption $\lambda_{max}$, nm | $\epsilon \times 10^{-4}$ | emission $\lambda_{max}$, nm | relative intensity |
|---|---|---|---|---|
| **15c** | 364 | 1.7 | 470 | 62 |
| **17** | 320 | 0.55 | 425 | 40 |
| **15f** | 360 | 1.7 | 515 | 3.2 |
| **15g** | 361 | 1.5 | 520 | 1.0 |

[a] Spectra were run in 10 mM Pipes/Tris buffer pH 7.0, containing methanol cosolvent at a concentration of 2.5% v/v for absorption spectra and <0.5% v/v for emission spectra. Each compound was excited at its absorption $\lambda_{max}$ during recording of the emission spectrum.

## Biology

The gastric ($H^+/K^+$)-ATPase can be isolated from pig gastric mucosa in the form of intact gastric vesicles.[22] The majority of these vesicles are oriented such that the ATP-hydrolyzing (cytoplasmic) face of the pump is on the outside of the vesicle and activity of the pump leads to an acidification of the vesicle interior. Thus the activity of the ($H^+/K^+$)-ATPase can be followed as a $K^+$-stimulated ATPase activity, provided that $K^+$ is allowed free access to its stimulatory site inside the vesicle. This is achieved either by the inclusion of a $K^+$ ionophore in the assay or by prior lyophilization of the vesicles which renders the membrane $K^+$-permeable. Alternatively, pump activity can be determined by following the accumulation of a weak base, such as aminopyrine, as the intravesicular space is acidified.

All compounds of interest were assessed for their ability to inhibit $K^+$-stimulated ATPase activity in lyophilized vesicles, and most were also tested against aminopyrine accumulation in intact vesicles. Compounds were dissolved in dimethyl sulfoxide, which was shown not to affect these assays at the concentrations used.

(21) Jones, G.; Baty, D. J. In *The Chemistry of Heterocyclic Compounds*; Jones G., Ed.; John Wiley: New York, 1982; Vol. 32, Part 2, pp 380–382.

(22) Saccomani, G.; Stewart, H. B.; Lewin, M.; Sachs, G. *Biochim. Biophys. Acta* **1977**, *465*, 311.

Table III. ($H^+/K^+$)-ATPase Inhibitory and Gastric Antisecretory Activity

| no. | ATPase $IC_{50}$,[a] μM | AP accum $IC_{50}$,[a] μM | rat gastric secretion $ED_{50}$[b] | % inhibn[c] |
|---|---|---|---|---|
| 1 | | | 0.45 (0.23–0.85) | |
| 2 | 0.101 ± 0.008 | 0.013 ± 0.001 | 0.31 (0.25–0.40) | |
| 4 | 0.85 ± 0.10 | 0.037 ± 0.009 | 8.2 (4.6–11.4) | |
| 10 | 9.8 ± 0.3 | 2.23 ± 0.04 | | |
| 11 | 69 ± 21 | >30 | | |
| 12 | 22 ± 0.4 | >30 | | |
| 15a | 2.4 ± 0.1 | 0.084 ± 0.012 | toxic[d] | 17 ± 8.3 |
| 15b | 1.6 ± 0.2 | 0.23 ± 0.02 | toxic[d] | |
| 15c | 0.66 ± 0.11 | 0.036 ± 0.09 | 19.8[e] | |
| 15d | 0.42 ± 0.03 | 0.028 ± 0.000 | nd[f] | 43 ± 7.0 |
| 15e | 0.41 | 0.028 ± 0.002 | nd | 42 ± 3.7 |
| 15f | 0.53 | 0.026 ± 0.001 | nd | 51 ± 5.3 |
| 15g | 0.17 | | nd | 41 ± 3.0 |
| 15h | 1.5 | | nd | 18 ± 5.6 |
| 15i | 1.0 | | nd | 45 ± 1.2 ($n$ = 3) |
| 15j | 0.75 | | | 26 ± 5.1 |
| 15k | 1.1 | 0.061 ± 0.018 | nd | 23 ± 4.3 ($n$ = 5) |
| 16 | 11 | 1.6 ± 0.3 | toxic[d] | 0 |
| 17 | 0.64 ± 0.09 | 0.034 ± 0.005 | 7.1 (5.9–8.2) | 48 ± 0.2 ($n$ = 3) |
| 18 | 1.3 ± 0.1 | 2.4 ± 0.4[g] | | |
| 19 | 2.9 | | nd | 40 ± 2.8 |
| 22 | 3.1 ± 0.2 | 0.11 ± 0.04 | nd | 21 ± 2.7 |

[a] $IC_{50}$ ± range ($n$ = 2) or observed $IC_{50}$ ($n$ = 1). [b] $ED_{50}$ in μmol/kg iv with 95% confidence limits. [c] Observed percent inhibition of acid secretion at a dose of 10 μmol/kg iv (mean of four rats ± SEM except as noted). [d] Rats died at doses giving less than 50% inhibition. [e] Wide limits. [f] Not determined: highest dose used was 10 μmol/kg. [g] This observed activity may be due to the presence of 15c as a minor impurity.

In vivo testing for gastric antisecretory activity was carried out in the lumen-perfused rat.[23] Compounds were administered as an intravenous bolus injection to pentagastrin-stimulated rats, and acid output was measured in the perfusate of the gastric lumen.

## Results and Discussion

The compounds of the present study were tested initially for their ability to inhibit ($H^+/K^+$)-ATPase activity, and in most cases also [$^{14}$C]aminopyrine accumulation. $IC_{50}$ values in these two assays are shown in Table III.

In interpreting the results of the in vitro assays it is important to appreciate the nature of the enzyme's environment in each case. The simplest system is the ATPase assay using lyophilized vesicles; in this case, the pH of the assay medium is 7.0 both inside and outside the vesicles, and any drug substances added to the medium will have equal access to the inside and outside surfaces of the vesicle (i.e. both faces of the enzyme). The aminopyrine accumulation assay is a more complex situation, in which the outside (cytosolic) surface of the vesicle is at pH 7.0, while the inside is at an undefined acidic pH, typically around 2 when the enzyme is maximally stimulated.[24] This is clearly closer to the physiological situation than in the ATPase assay, but as with in vivo data, interpretation of the results of the assay is complicated by the need to consider how the drug is distributed.

Two types of compound in particular would be expected to show substantially different apparent potencies in the two in vitro screens. Firstly, compounds that acted on the lumenal face of the enzyme, but could not penetrate membranes for some reason, would show very low potency

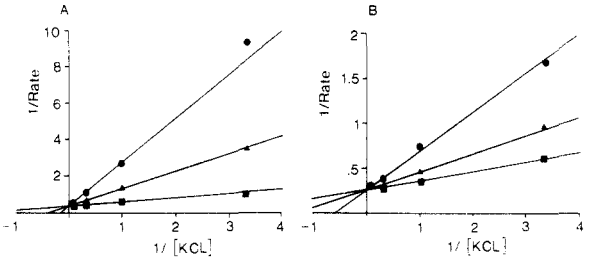



Figure 1. $K^+$-stimulated ATPase activity was determined at pH 7.0 in the presence of 2 mM MgATP and 0.03 to 10 mM KCl. Data were plotted as the reciprocal of the enzyme rate (μmol $min^{-1}$ (mg of protein)$^{-1}$) vs the reciprocal of the KCl concentration (mM). (A) Compound 4 was present at 0 (■), 1 μM (▲), and 3 μM (●). (B) Compound 15c was present at 0 (■), 0.3 μM (▲), and 1 μM (●). Lines show the best fit to a competitive pattern of inhibition for a representative experiment in each case.

in the AP accumulation assay. Secondly, in the AP assay, basic compounds would be expected to accumulate in the acidic compartment of the vesicle interior, in their protonated form. How this affects activity would depend to an important degree on whether the protonated or neutral form of the compound was responsible for enzyme binding. In practice, although the AP assay is in principle much more relevant to the in vivo situation in the pumping parietal cell, the number of variables was such that a detailed analysis of the results proved difficult.

Initial results on conformationally restrained analogues of 4, with the triazolo- and imidazoquinolines **10–12**, were moderately encouraging; the compounds clearly had measurable activity in the ATPase assay, although $IC_{50}$ values were significantly greater than for the lead compound 4. In contrast to 4, which showed a much lower apparent $IC_{50}$ value in the AP accumulation assay than against ATPase activity, **10** showed little difference in the two screens, and **11** and **12** were almost inactive against AP accumulation. Assuming that the compounds are acting in the same way, this could suggest possible membrane penetration problems for **11** and **12**; the lower $pK_a$ of these compounds may also be a factor.

It appeared likely that **10** would be the most lipophilic and most basic compound of the three. The finding that it was also the most potent encouraged us to go on to investigate the dihydropyrroloquinolines **15**. As is clear from Table III, compounds of this class proved to be highly effective inhibitors of the target enzyme, with potency similar to that of 4. In contrast to the situation with **10**, **11**, and **12**, nearly all the dihydropyrroloquinolines were also found to show an enhanced potency in the AP accumulation assay, to an extent very similar to that of the lead compound 4. This suggested that it was the accumulated (protonated) form of these basic compounds that acted upon the lumenal face of the enzyme to cause inhibition.

It was therefore of interest to compare one of this class of compounds, for example **15c**, with compound 4 in a study of the kinetics of inhibition of ATPase activity. Assay time courses in the presence of 4 or **15c** were linear over the 30-min incubation period. This lack of time dependence suggested reversible inhibition of the enzyme. This was confirmed directly in the case of 4 where dialysis of inhibited (33% of control) enzyme resulted in the recovery of activity to 92 ± 6% of control (mean and range, $n$ = 2). Steady-state enzyme kinetics showed both compounds to be competitive with respect to the activating cation, $K^+$ (Figure 1). The $K_i$ values for these interactions were the same, being 0.29 ± 0.03 μM (SE, $n$ = 3) for compound 4 and 0.29 ± 0.02 μM (range, $n$ = 2) for compound **15c**. Since the activation of ATPase activity by $K^+$ occurs

(23) Parsons, M. E.; Owen, D. A. A.; Ganellin, C. R.; Durant, G. J. *Agents Actions* **1977**, *7*, 31.
(24) Keeling, D. J. Unpublished results.

from the lumenal (extracellular) side of the membrane containing the enzyme, this competition suggests that these compounds interact with the enzyme at the lumenal face. The fluorescent properties of compounds such as **15c** may make it possible to define the binding site more closely in future experiments.

As can be seen from Table III, differences in in vitro potency between the various pyrroloquinolines tested were relatively small. The biggest differences were seen when varying the substitution on the phenyl ring. Thus, the *o*-methyl derivative **15c** is somewhat more active than the parent compound **15a**; the *o*-methoxy derivative **15b** appears to be intermediate in activity, though the anomalously low potency in the AP accumulation assay suggests that other, as yet unexplained, factors are involved here. A second *o*-methyl group appears to be unfavorable (**15h** vs **15e**).

The decrease in activity on going to the *N*-benzyl derivative **16** suggests that the positioning of the phenyl ring is also important. The increased ring size in the tetrahydropyridino[3,2-*c*]quinoline **22** would also be expected to significantly affect the conformation; this compound is slightly less active than any of the compounds **15**, and although the significance of omitting the methyl substituent adjacent to the basic nitrogen has not been firmly established, it appears unlikely that the larger ring confers any real advantage.

Introduction of additional unsaturation into **15c** to give **17** had no effect on in vitro activity. Although **17** ($pK_a$ 7.4) is less basic than **15c** ($pK_a$ 9.5), both compounds would clearly be extensively protonated under the low-pH conditions present in the parietal cell. At the pH of the ATPase assay (7.0), the degree of protonation would be around 70% for **17** and 99.7% for **15c**.

Support for the view that the cation is the active form of the molecule came from the quaternized derivative **18**, which had similar activity to the parent compound in the ATPase assay. It was expected that, as a potentially impermeable derivative, **18** would also give information about the site of action of the molecule when tested in the AP accumulation assay, where it is necessary for the compound to cross the membrane in order to reach the lumenal face of the enzyme. In fact, **18** showed almost no activity in this assay, suggesting that it acts exclusively from the lumenal side; as noted earlier, this is also the side from which $K^+$ activation occurs. This finding is consistent with results previously obtained for **2**.[15] The precise $IC_{50}$ of **18** is difficult to determine, as it proved impossible to isolate the quaternary salt free of traces of **15c**; the sample used appeared by NMR and HPLC to contain around 2% of unquaternized material, and this would be sufficient to account for all the inhibition of AP accumulation seen. The *N*-oxide **19** is also an ($H^+/K^+$)-ATPase inhibitor, though of somewhat lower potency than the parent compound.[25]

Selected compounds were examined for their effect on gastric acid secretion in vivo (Table III). On testing the early compounds in the dihydropyrroloquinoline series, it was noted that some were acutely toxic; doses below the $ED_{50}$ caused a rapid fall in blood pressure, leading in some cases to death within minutes.[26] However, this toxicity appears not to correlate with the antisecretory activity, and varying the substitution pattern gave compounds for which no toxic effects were observed. Full dose–response curves could thus be established for **15c** and **17**. Although in vivo activity clearly falls some way short of the most potent of the Schering compounds, **2**, this confirmed **17** as being at least as potent as the lead compound **4**. As expected, the compounds displayed a relatively short duration of action, contrasting sharply with the results observed with omeprazole. For example, a 30 μmol/kg dose of **17** administered as an iv bolus gave a peak inhibition of 76%, but with 78% recovery at 2 h postdose (mean of four rats); a 1 μmol/kg dose of omeprazole gave a similar degree of inhibition, but with no recovery at 2 h.

In general, in vivo activity showed a reasonable correlation with that seen in vitro. The clearest outlier is the *N*-oxide **19**, which is possibly metabolized to **15c** before achieving its in vivo effect. Compound **15g** may be less potent in vivo than expected, as a result of its metabolically labile phenolic OH group, but the effect is slight.

## Conclusions

The present study has established the 1-phenylpyrrolo[3,2-*c*]quinolines as a novel class of inhibitors of the gastric ($H^+/K^+$)-ATPase. Both **4** and **15c** have been shown to be competitive with respect to $K^+$, with submicromolar $K_i$ values.

This activity is reflected in vivo in their ability to inhibit pentagastrin-stimulated gastric acid secretion in the rat. As expected from their mechanism of action, this inhibition is relatively short-lived after bolus dosing.

## Experimental Section

**Chemistry.** NMR spectra were obtained as $CDCl_3$ or $d_6$-DMSO solutions on a Bruker AM250 spectrometer. NMR spectra were obtained for all compounds made. UV spectra were recorded on a Beckmann DU-50 spectrophotometer. Fluorescence spectra were recorded on a Perkin-Elmer LS5 spectrophotometer. Solutions were dried over $Na_2SO_4$. Chromatography (analytical and preparative) was carried out on silica gel, eluted with varying concentrations of methanolic ammonia in dichloromethane; all the pyrroloquinolines had extraordinarily low mobility in the absence of ammonia. All compounds were checked for purity by HPLC (μBondapack-$C_{18}$ column, acetonitrile gradients in pH 6.0 ammonium acetate buffer, UV detection).

**4-[(2-Methylphenyl)amino]-3-nitroquinoline (8).** 4-Chloro-3-nitroquinoline[27] (2.08 g, 0.01 mol) and 2-methylaniline (2.14 g, 0.02 mol) were dissolved in THF (50 mL), and the mixture was stirred at reflux for 16 h, during which time a pale yellow solid was deposited. After cooling, the solvent was evaporated and the residue dissolved in 2 M HCl (80 mL). The acidic solution was extracted with $CHCl_3$ (×3) and the chloroform solution washed with aqueous $NaHCO_3$ and water. Drying and evaporation of the chloroform gave an orange-yellow solid which was crystallized from absolute ethanol to give **8** (1.2 g, 43%) as orange needles, mp 137–138 °C. Anal. ($C_{16}H_{13}N_3O_2$) C, H, N.

**3-Amino-4-[(2-methylphenyl)amino]quinoline (9).** Compound **8** (5.2 g, 18.6 mmol) was dissolved in EtOAc (250 mL) and stannous chloride (20.9 g, 93 mmol) added. The mixture was stirred at room temperature for 2 h and filtered through Celite and the solvent evaporated to give a solid residue. This was triturated with $CHCl_3$, filtered, and dissolved in water. The aqueous solution was basified with NaOH (to pH 12) and extracted with $CHCl_3$. The organic extracts were combined, washed with water, dried, and evaporated to a yellow solid, which was recrystallized several times from ethanol/petroleum ether to give

(25) The *N*-oxide of **4** was also prepared and shown to be active in the enzyme assay. As this compound was significantly more stable than **19**, we were able to isolate pure material and measure a $pK_a$ of 3.81. The compound is thus weakly basic and could act in the protonated form under physiological conditions; however, at the pH of the ATPase assay (7.0) the proportion of protonated form would be very small.

(26) The toxicological properties of **15a** and **15c** have subsequently been investigated further (Gabel, R. A.; Forman, D. L.; Macia, R. A.; Matthews, W. D. Unpublished results); details will be published separately.

(27) Wright, G. C.; Gray, J. E.; Yu, C.-N. *J. Med. Chem.* **1974**, *17*, 245.

9 as the base, mp 156–158 °C. A portion of this base (0.47 g) was treated with ethanolic HCl (30 mL) and filtered and the solution evaporated. Recrystallization from ethanol/ether gave a pale yellow solid (0.43 g), mp 303–305 °C. Anal. ($C_{16}H_{15}N_3$·1.75HCl·0.02EtOH) C, H, N.

**1-(2-Methylphenyl)imidazo[4,5-*c*]quinoline Hydrochloride (10).** Compound 9 (0.9 g, 3.6 mmol) and formic acid (10 mL) were mixed at room temperature and then stirred at reflux for 3 h, cooled, and evaporated to dryness. The residual oil was partitioned between water and $CHCl_3$, and the chloroform solution was washed with aqueous $NaHCO_3$ and water and then dried and evaporated to give a dark viscous oil (0.85 g). This was dissolved in ethanolic HCl (50 mL) and the solution evaporated to dryness. Recrystallization from ethanol/ether gave 10 as a white solid (0.55 g, 59%), mp 266–270 °C. Anal. ($C_{17}H_{13}N_3$·HCl·0.2$H_2O$) C, H, N.

**1-(2-Methylphenyl)triazolo[4,5-*c*]quinoline (11).** A solution of sodium nitrite (0.82 g, 12 mmol) in water (10 mL) was added dropwise to a stirred suspension of 9 (2.0 g, 8 mmol) in 2 M HCl (80 mL) at 0–5 °C. The solid gradually dissolved to give a pale orange solution, which deposited a solid after about 1 h. After warming to room temperature the solid was collected, washed with water, and recrystallized from aqueous ethanol with charcoaling, to give 11 as a white solid (0.55 g, 26%), mp 140–142 °C. Anal. ($C_{16}H_{12}N_4$) C, H, N.

**1-(2-Methylphenyl)-2(3*H*)-thioimidazo[4,5-*c*]quinoline (12).** Compound 9 (2.3 g, 9.23 mmol) was mixed with $CS_2$ (10 mL), triethylamine (2 mL), and EtOH (60 mL). The solution was warmed at 42 °C (oil bath temperature) for 16 h, during which time a solid was deposited. The solid was collected (1.4 g) and recrystallized from aqueous ethanol to give 12 as a white solid, mp 288–290 °C. Anal. ($C_{17}H_{13}N_3S$) C, H, N, S.

**4-Chloro-3-(2-chloroethyl)-8-fluoro-2-methylquinoline (14, R = F).** A mixture of 2-fluoroaniline (9.65 mL, 0.1 mol) and 2-acetyl-γ-butyrolactone (10.76 mL, 0.1 mol) was heated to 120 °C for 1 h and then at 160 °C for 2 h. The mixture was cooled, phosphoryl chloride (100 mL) added,[28] and the resulting solution heated at reflux for 1.5 h. After cooling, the mixture was poured onto ice and extracted into $CH_2Cl_2$, which was washed with aqueous $NaHCO_3$, water, and brine, dried, and evaporated. Trituration of the residue with $Et_2O$, followed by recrystallization from EtOH, gave 4-chloro-3-(2-chloroethyl)-8-fluoro-2-methylquinoline (5.48 g, 22%), mp 101–103 °C. Anal. ($C_{12}H_{10}Cl_2FN$) C, H, N, Cl.

**1-Aryl-4-methyl-2,3-dihydropyrrolo[3,2-*c*]quinolines (15). General Method.** A solution of the 2-methyl-3-(2-chloroethyl)-4-chloroquinoline (1 equiv) and the appropriate aniline hydrochloride (1 equiv) in an alcohol solvent was heated for the appropriate time, either at reflux or in a pressure vessel (for details see Table I). The solvent was then evaporated, aqueous $NaHCO_3$ added, and the crude product extracted into $CH_2Cl_2$. The organic solution was dried and evaporated and then the product isolated by chromatography. Recrystallization from a suitable solvent (see Table I) gave material of analytical purity, in the yields shown.

**6-Hydroxy-4-methyl-1-(2-methylphenyl)-2,3-dihydropyrrolo[3,2-*c*]quinoline (15k).** 4-Chloro-3-(2-chloroethyl)-8-methoxy-2-methylquinoline (54 g, 0.2 mol), 2-methylaniline hydrochloride (28.7 g, 0.2 mol), and 1-butanol (500 mL) were heated to 170 °C in a pressure vessel for 10 h. The solvent was evaporated, the residue taken up in $CH_2Cl_2$, and the organic solution washed with aqueous $NaHCO_3$, dried, and evaporated. Chromatography gave 15c in the early fractions. Later fractions contained mainly 15k, though this proved difficult to purify. Repeated recrystallization from ethyl acetate eventually yielded an analytically pure sample of 15k (1.4 g, 2.4%). Anal. ($C_{19}H_{18}N_2O$) C, H, N.

**1-Benzyl-6-methoxy-4-methyl-2,3-dihydropyrrolo[3,2-*c*]quinoline (16).** A mixture of 4-chloro-3-(2-chloroethyl)-8-methoxy-2-methylquinoline (1.40 g, 5.2 mmol), benzylamine hydrochloride (1.5 g, 10.4 mmol) and ethanol (50 mL) was heated to 160 °C for 16 h. The solvent was evaporated, the residue taken up in $CH_2Cl_2$, washed with aqueous $NaHCO_3$, and dried, and the solvent evaporated. Chromatography and recrystallization from EtOAc gave 16 (0.61 g, 61%), mp 150–152 °C. Anal. ($C_{20}H_{20}N_2O$) C, H, N.

**8-Methoxy-4-methyl-1-(2-methylphenyl)pyrrolo[3,2-*c*]quinoline (17).** A solution of 15c (4.57 g, 15 mmol) in diphenyl ether (50 mL) was heated at reflux with 10% palladium on charcoal (1.0 g) for 1 h. After removal of the catalyst on Celite, chromatography and recrystallization from EtOAc/petroleum ether gave 17 (2.2 g, 48%), mp 143–145 °C. Anal. ($C_{20}H_{18}N_2O$) C, H, N.

**4,5-Dimethyl-6-methoxy-1-(2-methylphenyl)-2,3-dihydroquinolinium Iodide (18).** Compound 15c (0.5 g, 1.6 mmol) was dissolved in a mixture of ethanol (5 mL) and acetone (1 mL), iodomethane (0.5 mL, 8.0 mmol) added, and the solution heated at reflux for 20 h. The solvent was evaporated and the crude product recrystallized successively from acetone/ether, acetone, and acetone/methanol. HPLC showed 2.5% starting material by peak area at 260 nm, and this could not be reduced further. 18: yield 0.11 g; mp 186–188 °C. Anal. ($C_{21}H_{23}IN_2O$) C, H, N; I: calcd, 28.43; found, 27.60.

**4-Methyl-1-(2-methylphenyl)-5-oxo-2,3-dihydropyrrolo[3,2-*c*]quinoline (19).** Compound 15e (15.0 g, 55 mmol) was stirred in chloroform (200 mL) with ice cooling and a solution of *m*-chloroperoxybenzoic acid (9.5 g, 55 mmol) in chloroform (100 mL) added dropwise, while the temperature was kept below 5 °C. After stirring of the mixture for a further 30 min, the solvent was evaporated. The residue was chromatographed and crystallized from ether to obtain 19 (1.8 g, 11%), mp 212–214 °C. Anal. ($C_{19}H_{18}N_2O$) H, N; C: calcd, 78.59; found, 77.14. HPLC indicated 1.1% of starting material by peak area at 260 nm, which could not be reduced further.

**4-Chloro-3-(3-chloropropyl)-8-methoxyquinoline (21).** The sodium salt of 3-(hydroxymethylene)tetrahydropyran-2-one[29] (25 g, 0.17 mol) and 2-methoxyaniline hydrochloride (32 g, 0.2 mol) in ethanol (750 mL) were warmed to 70 °C with stirring for 2 h. The mixture was filtered hot, the filtrate evaporated, and the crude product taken up in dichloromethane, washed with dilute hydrochloric acid, dried, and evaporated. Trituration with ether gave 20 (21.5 g, 55%) as a mixture of *E* and *Z* isomers. This mixture was suitable for use in the next step without separation.

A solution of 20 (11.67 g, 50 mmol) in phosphoryl chloride (50 mL) was heated at reflux for 1 h. After being cooled and poured onto ice, the mixture was heated to boiling, charcoaled, and filtered through Celite. Subsequent cooling and addition of sodium hydroxide solution gave a solid, which was filtered off and recrystallized from aqueous ethanol to yield 21 (5.6 g, 41%), mp 86–89 °C.

**7-Methoxy-1-(2-methylphenyl)-1,2,3,4-tetrahydropyridino[3,2-*c*]quinoline (22).** A solution of 21 (6.4 g, 23.6 mmol) and 2-methylaniline (5.0 mL, 47 mmol) in ethanol (250 mL) was heated at reflux for 4 days. After removal of the solvent, $CH_2Cl_2$ and aqueous $NaHCO_3$ were added to the residue, and the organic layer was washed with water, dried, and evaporated. Chromatography followed by recrystallization from EtOAc gave 22 (1.92 g, 27%), mp 160–161 °C. Anal. ($C_{20}H_{20}N_2O$) C, H, N.

**Biology. Preparation of Gastric Vesicles.** Gastric vesicles were prepared from pig fundic mucosa as previously described.[3,30] Briefly, tissue was homogenized in isotonic medium and a microsomal fraction obtained by differential centrifugation. This material was separated on a discontinuous density gradient and that fraction at the interface between the 0.25 M sucrose and 0.25 M sucrose plus 9% Ficoll layers was taken. The interface fraction, comprising intact gastric vesicles, was diluted in an equal volume of 60% (w/v) sucrose and was stored at −70 °C. Where free access to the lumenal face of the enzyme was required, the interface fraction was spun down in hypotonic medium and was freeze-dried overnight. These lyophilized gastric vesicles were resuspended in 10 mM Tris/Cl buffer, pH 7.0, and were stored at −70 °C.

(28) Care should be taken in the chlorination step, particularly when working on a larger scale, as the reaction can sometimes initiate with a sudden exotherm. This was noted particularly with anilines more electron-rich than the *o*-fluoro derivative.

(29) Leonard, N. J.; Cundall, R. L. *J. Am. Chem. Soc.* **1974**, *96*, 5909.

(30) Keeling, D. J.; Fallowfield, C.; Underwood, A. H. *Biochem. Pharmacol.* **1987**, *36*, 339.

Following lyophilization, $K^+$ was able to stimulate ATPase activity independently of the presence of the $K^+/H^+$-ionophore nigericin, thus demonstrating the increased permeability in the vesicle membrane.

**$K^+$-stimulated ATPase activity** was determined at 37 °C in the presence of 10 mM Pipes/Tris buffer, pH 7.0, lyophilized gastric vesicles (3–6 μg of protein/mL), 2 mM $MgSO_4$, 2 mM ATP, with and without 1 mM KCl. At the end of the incubation (30 min) the inorganic phosphate hydrolyzed from ATP was determined spectrophotometrically at 310 nm following molybdate-complex formation and extraction into *n*-butyl acetate.[31] Basal ATPase activity, in the absence of KCl, was usually approximately 5% of the total activity.

In experiments to assess the degree of $K^+$ competition of selected inhibitors, the KCl concentration was varied between 0 and 10 mM at different concentrations of inhibitor and the $K^+$-stimulated activities so obtained analyzed by standard kinetic methods.[32] Control experiments were performed to ensure that the enzyme rates measured were true initial rates.

**Aminopyrine accumulation ratio** was determined in the presence of 10 mM Pipes/Tris buffer, pH 7.0, intact gastric vesicles (25 μg of protein/mL), 2 mM $MgSO_4$, 2 mM ATP, 150 mM KCl, 9 μM valinomycin, 3 μM [*dimethylamine*-$^{14}$C]aminopyrine (100–120 mCi/mmol, Amersham), and 0.1 mg/mL bovine serum albumin. Total incubation volume was 200 μL. After incubation at room temperature for 30 min, 3 mL of ice-cold washing solution (150 mM KCl containing 10 mM Pipes/Tris buffer, pH 7.0) was added, and the vesicles were harvested by rapid filtration on Whatman GF/B filters. Each filter was washed with two further 3-mL aliquots of washing solution, after which the radioactivity that had accumulated into the vesicle was counted with use of 10 mL Picofluor-15 scintillant. In order to calculate an aminopyrine accumulation ratio (the ratio of the aminopyrine concentration inside the vesicle to that outside), it was assumed that the intravesicular volume was 2 μL/mg of protein.[22] Control aminopyrine accumulation ratios were routinely in the range 800–1200.

**Rat Gastric Secretion.** Studies were carried out by using the stomach lumen perfusion technique in the anesthetized rat, with continuous monitoring of the effluent perfusate using a microflow glass electrode. Acid secretion was stimulated by an intravenous infusion of pentagastrin, and the inhibitors were given intravenously when a submaximal plateau of secretion had been achieved. Percentage inhibition was calculated from a comparison of the acid secretion at peak inhibition compared to the secretory plateau immediately prior to administration.

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**Registry No.** 4, 77156-88-8; 7, 39061-97-7; 8, 123438-64-2; 9, 123438-65-3; 9·2HCl, 123438-82-4; 10, 123438-66-4; 11, 123438-67-5; 12, 123438-68-6; (*E*)-13 (R = OMe), 123438-75-5; (*Z*)-13 (R = OMe), 64620-33-3; (*E*)-13 (R = Me), 123438-76-6; (*Z*)-13 (R = Me), 64620-31-1; (*E*)-13 (R = H), 123438-77-7; (*Z*)-13 (R = H), 64620-59-3; (*E*)-13 (R = F), 123438-78-8; (*Z*)-13 (R = F), 123438-79-9; (*E*)-13 (R = OH), 123438-80-2; (*Z*)-13 (R = OH), 123438-81-3; 14 (R = OMe), 57521-19-4; 14 (R = Me), 107622-79-7; 14 (R = H), 57521-15-0; 14 (R = F), 122456-61-5; 14 (R = OH), 91398-78-6; 15a, 113569-38-3; 15b, 122456-44-4; 15c, 122456-25-1; 15d, 122456-28-4; 15e, 122456-29-5; 15f, 122456-30-8; 15g, 123438-69-7; 15h, 122456-50-2; 15i, 122456-49-9; 15j, 122456-65-9; 15k, 122456-26-2; 16, 123438-70-0; 17, 122456-27-3; 18, 123438-71-1; 19, 122456-62-6; (*E*)-20, 123438-73-3; (*Z*)-20, 123438-74-4; 21, 122456-53-5; 22, 122456-31-9; *o*-$MeC_6H_4NH_2$, 95-53-4; *o*-$MeOC_6H_4NH_2$, 90-04-0; $PhNH_2$, 62-53-3; *o*-$FC_6H_4NH_2$, 348-54-9; *o*-$OHC_6H_4NH_2$, 95-55-6; ATPase, 9000-83-3; (*E*)-3-(ethoxymethylene)tetrahydro-2(6*H*)-pyranone, 123438-72-2; (*Z*)-3-(ethoxymethylene)tetrahydro-2(6*H*)-pyranone, 123438-83-5; 3-acetyldihydro-2(3*H*)-furanone, 517-23-7; 4-methoxy-2-methylaniline, 102-50-1; 4-hydroxy-2-methylaniline, 2835-99-6; 2,6-dimethylaniline, 87-62-7.

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