Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 6, pp. 543-546, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00069-0

4-(2-PYRIDYL)-5-PHENYLTHIAZOLES AS NOVEL NON-BICYCLIC REVERSIBLE INHIBITORS OF THE GASTRIC H^+/K^+-ATPase

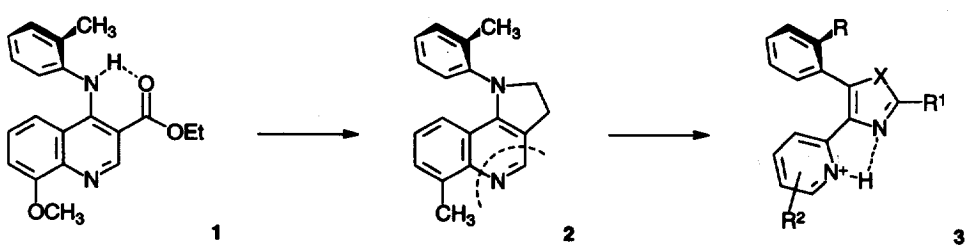
R. J. Ife*, C. A. Leach, A. J. Pope and C. J. Theobald

Chemical and Cellular Sciences, SmithKline Beecham Pharmaceuticals R & D, The Frythe, Welwyn, Herts, AL6 9AR, England

Abstract 4-(2-Pyridyl)-5-phenylthiazoles, **3** (X = S), are shown to be reversible, K^+-competitive gastric H^+/K^+-ATPase inhibitors. It is suggested that a hydrogen-bond between the protonated pyridine and the thiazole may help the molecule adopt a conformation which mimics that of the previously described quinoline based inhibitors such as **1** and **2**.

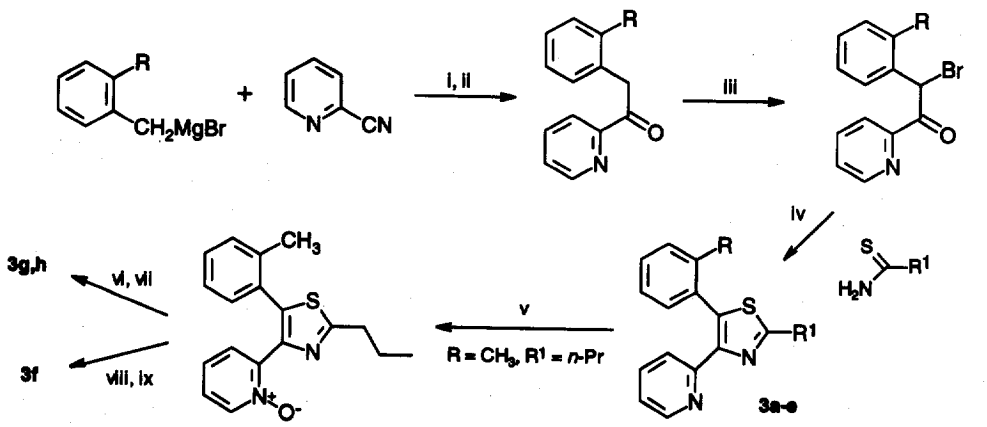
A research effort to identify reversible, short acting inhibitors of the gastric H^+/K^+-ATPase as an alternative antisecretory strategy to both histamine H_2-receptor antagonists and the long acting, irreversible inhibitors such as omeprazole has been conducted in a number of laboratories.[1] This has led to a class of compounds, the so called "K-site inhibitors", which inhibit the enzyme by binding competitively with respect to potassium to the lumenal surface of the H^+/K^+-ATPase.[2,3,4] Our efforts have centred largely on quinoline based systems and this has led to two clinical candidates, SK&F 96067[3,4] and SK&F 97574[6], both of which have proved to be effective inhibitors of acid secretion in man.

Previously we described the development of compounds based on the pyrroloquinolines such as **2** from the lead compound **1**.[4,7] In part, this was based on the proposal that hydrogen-bonding between the carbonyl oxygen and the N-H in **1** was an important factor in controlling the orientation of the arylamino moiety and ultimately activity. We reasoned that if the hydrogen-bond in **1** could be replaced by a pyrrolidine ring, then perhaps a ring already present in **1** and **2** could conversely be replaced by a hydrogen-bond. Since it has previously been shown that these compounds bind to the enzyme as protonated cations[4], compounds of general structure **3**, in which part of the quinoline nucleus has been formally replaced by a potential hydrogen-bond between a protonated pyridine and a suitable nitrogen heterocycle, were therefore considered worthy of investigation.



Thiazoles (X = S) were chosen as the starting point for this work and the initial series of compounds **3a** - **3e** were prepared as outlined in Scheme 1 from the appropriately substituted α-bromo ketone and thioamide.[8] Compounds were assessed in the first instance for their ability to inhibit K^+-stimulated ATPase activity in lyophilised gastric vesicles using methods previously described.[4] Active compounds were subsequently evaluated *in vivo* for their ability to inhibit pentagastrin-stimulated gastric acid secretion in the lumen perfused rat model.[4] The results obtained are summarised in Table 1.

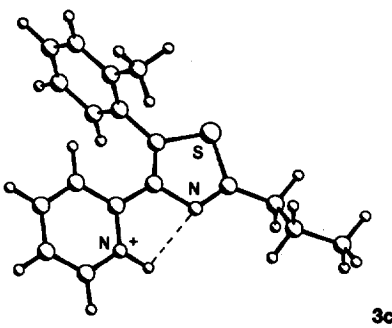
Scheme 1



Reagents: I) Et_2O, RT, overnight. II) NH_4Cl, conc. HCl, 5°C. III) 48% HBr, Br_2, 100°C, 15min. IV) EtOH, Δ, 4-5h. V) CH_2Cl_2, *m*-CPBA, RT, 16h. VI) $POCl_3$, 120°C, 1h (gave 6-Cl and 4-Cl pyridine, ~2 : 1). VII) 6-Cl cmpd., 33% CH_3NH_2/EtOH, 185°C, sealed vessel, 16h *or* 4-Cl cmpd., PhOH, NH_3 gas, 200°C, 3h. VIII) $(PhO)_2P(O)N_3$, 155-60°C, 3h. IX) EtOH, H_2, 10% Pd/C, 50psi, 2.5h.

This initial series of compounds provided active inhibitors of the gastric H^+/K^+-ATPase. The substituent R^1, which was introduced to mimic the ester side chain in compound **1**, has a marked effect on activity with potency increasing around 10 fold between R^1 = CH_3, **3a**, and R^1 = *n*-Pr, **3c**, though clearly even larger substituents can also be accommodated. The *ortho*-methyl group, R, also has a significant effect on activity, with potency increasing some 5 fold between the desmethyl compound, **3b**, and **3c**. Both of these observations are closely analogous to the SAR found in compounds related to **1** and **2**.[4,5,7] In these compounds, the *ortho*-methyl substituent could presumably be contributing to activity by encouraging the phenyl group to twist out of conjugation with the thiazole, providing less steric bulk, and thereby facilitating the pyridine and thiazole to achieve coplanarity and maximising the potential for H-bonding.

Despite hydrogen-bonded 5-membered rings being generally less favoured than 6-membered rings, because of the more acute bond angles, the presence of the postulated hydrogen-bond was supported by IR spectral studies on **3c** as both the perchlorate and hydrobromide salt.[9] In addition, MNDO calculations performed on the protonated cation of **3c** suggested a minimum energy conformation in which the o-tolyl moiety is essentially orthogonal to the thiazole (τ = 90.5°) and the pyridyl group almost planar (τ = 12.3°).[10]



3c-H⁺

It can be seen from Table 1 that replacement of the thiazole with other heterocycles, led to a loss of activity. This might be rationalised for the pyrimidine, **3j**, in terms of the smaller exocyclic bond angles in the 6-membered ring leading to increased steric crowding between the o-tolyl and the pyridyl moieties and disruption of the hydrogen-bond. In the case of **3i**, preferential protonation on the more basic imidazole leading to quite different electronic properties seems the most plausible explanation. Indeed, compound **3c** is only weakly basic (pK_a 4.2 at 25°, corresponding to protonation on pyridine, cf. pK_a **1** = 6.7[7]) and this was identified as a potential area for further improving activity.

Table 1 H^+/K^+-ATPase and Gastric Antisecretory Activity of Compounds 3

Cmpd.[a]	X	R	R^1	R^2	H^+/K^+-ATPase IC_{50} (μM) or % inhib.[d]	Rat gastric secretion % inhib. @ 10μmol/kg iv[e]
2	-	-	-	-	0.98	47 ± 3[f]
3a[b]	S	CH_3	CH_3	H	48% @ 100μM	n.t.
3b[bc]	S	H	*n*-Pr	H	42.3	21 ± 4[g]
3c[b]	S	CH_3	*n*-Pr	H	8.2	26 ± 8
3d[b]	S	CH_3	*o*-tolyl	H	4.9	insoluble
3e	S	CH_3	benzyl	H	9.6	29 ± 3
3f	S	CH_3	*n*-Pr	6-NH_2	2.9	48 ± 3
3g	S	CH_3	*n*-Pr	6-$NHCH_3$	12.5	42 ± 1[h]
3h	S	CH_3	*n*-Pr	4-NH_2	2.2	36 ± 5[f]
3i[b]	NH	CH_3	*n*-Pr	H	67% @ 100μM	n.t.
3j[b]	CH=N	CH_3	*n*-Pr	H	23% @ 30μM	n.t.

a) ^1H NMR and IR spectra where consistent with assigned structures and, unless otherwise indicated, all microanalytical values were within ±0.4% of calculated values. b) Compound prepared as the HBr salt. c) For 1HBr, C: calcd, 56.51; found, 56.05. d) Inhibition of K^+-stimulated gastric ATPase activity (ref. 4). e) Inhibition of pentagastrin-stimulated gastric acid secretion in the anaesthetised rat (ref. 4); % inhibition ± SEM, n = 4 unless otherwise indicated. f) n = 5. g) n = 3. h) n = 6.

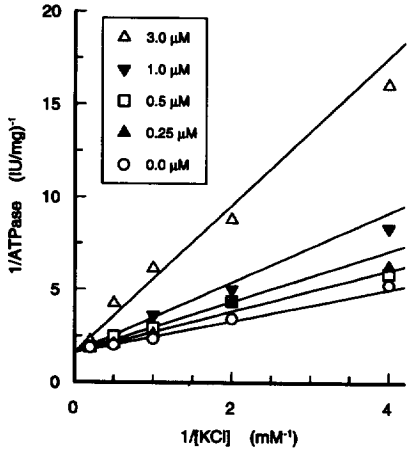
In principle, increasing the pK_a of the pyridine should affect *in vitro* activity by increasing the proportion of protonated species present at the neutral pH of the assay and improve *in vivo* activity by facilitating greater accumulation of the inhibitor into the acidic compartments of the parietal cell. This can most effectively be brought about by the introduction of 4- or 6-amino substituents. Derivatives **3f-h** were therefore prepared from **3c** via its N-oxide, Scheme 1.[8] Reaction of the N-oxide with diphenylphosphoryl azide followed by hydrogenation of the intermediate 6-azide over 10% Pd/C gave the 6-amino compound, **3f**. Treatment of the N-oxide with phosphoryl chloride gave a mixture 6- and 4-chloro pyridines (ratio ~ 2 : 1) which, after separation by chromatography, could be converted to the 4-amino compound, **3h**, and 6-methylamino compound, **3g**, by reaction with ammonia or methylamine.

The pK_a of **3f** was increased by around 1 log unit to 5.3. However, the effect of this on the *in vitro* activity of the compound was somewhat less than expected and in the case of the 6-methylamino compound, **3g**, activity fell slightly. Similarly, for the 4-amino compound, **3h** (pK_a 8.0), where the largest effect might have been anticipated, only a modest increase in activity was seen. We have previously found substitution in the equivalent ring in the quinoline based inhibitors to be detrimental in particular positions and have associated this with a deleterious steric interaction on the enzyme.[6] In these compounds, therefore, the advantage gained by increasing the pK_a of the pyridine may well have been offset by the introduction of additional steric interactions. This is certainly supported by the lower activity of the bulkier 6-methylamino analogue, **3g**, compared to the primary amino compound, **3f**.

Nevertheless, the amino compounds, **3f-g**, were more potent than the parent compound **3c**, *in vivo*. Indeed, the overall profile of **3f** (ATPase IC_{50} 2.9μM, 48% inhibition of acid secretion @ 10μmol/kg iv) is not dissimilar to that of the pyrroloquinoline, **2**, used as the starting point for this work and supports the validity of this approach.

The 4-amino compound, **3h**, was further characterised and shown to be selective against the hog kidney Na^+/K^+-ATPase (IC_{50} 44μM). Steady-state enzyme kinetics showed compound **3h**, like compounds **1** and **2**, inhibited the gastric H^+/K^+-ATPase competitively with respect to the activating cation K^+, Figure 1. A K_i of 0.84μM was obtained for this compound.

Figure 1 Inhibition of K^+-stimulated ATPase Activity by Compound **3h**.



K^+-Stimulated ATPase activity[4] was determined at the concentrations of **3h** shown and plotted as a double reciprocal plot where the ordinate is the reciprocal of enzyme rate and the abscissa is the reciprocal of the K^+ concentration. The graph shows a single representative experiment and depicts the result of least squares fitting of the data to a competitive pattern of inhibition. From two independent experiments the following parameters were calculated (± = range): V_{max} = 0.636 ± 0.013 IU/mg, K_m = 0.560 ± 0.029 mM and K_i = 0.838 ± 0.017 μM.

The compounds described in this paper represent a new class of reversible gastric H^+/K^+-ATPase inhibitors. The replacement of a postulated hydrogen-bond by a cyclic structure is a strategy often used in medicinal chemistry. Here we have shown that the concept can be applied in reverse to give access to new types of structures and may represent a generally applicable approach.

Acknowledgement

The authors would like to acknowledge contributions made by members of SmithKline Beecham Analytical Sciences for pK_a and IR studies and GI Pharmacology for the provision of *in vivo* screening data.

References and Notes

1. For a more detailed rationale of this approach see; Parsons, M. E.; Pope, A. J. *Trends in Pharmacol. Sci.* **1993**, *14*, 323.
2. Keeling, D. J.; Laing, S. M.; Senn-Bilfinger, J. *Biochem. Pharmacol.* **1988**, *37*, 2231.
3 Keeling, D. J.; Malcolm, R. C.; Laing, S. M.; Ife, R. J.; Leach. C. A. *Biochem. Pharmacol.* **1991**, *42*, 123.
4. Brown, T. H.; Ife, R. J.; Keeling, D. J.; Laing, S. M.; Leach, C. A.; Parsons, M. E.; Price, C. A.; Reavill, D. R.; Wiggall, K. J. *J. Med. Chem.* **1990**, *33*, 527.
5. Ife, R. J.; Brown, T. H.; Keeling, D. J.; Leach, C. A.; Meeson, M. L.; Parsons, M. E.; Reavill, D. R.; Theobald, C. J.; Wiggall, K. J. *J. Med. Chem.* **1992**, *35*, 3413.
6. Ife, R. J.; Leach C. A.; Parsons M. E. *7th RSC/SCI Medicinal Chemistry Symposium*, **1993**, Abstr. no. P 37. Full paper to be submitted to *J. Med. Chem.*
7. Leach, C. A.; Brown. T. H.; Ife, R. J.; Keeling, D. J.; Laing, S. M.; Parsons, M. E.; Price, C. A.; Wiggall, K. J. *J. Med. Chem.* **1992**, *35*, 1845.
8. For further experimental details see Ife, R. J.; Leach, C. A. PCT Publ. no. WO 93/15071, **1993**.
9. In contrast to pyridinium perchlorate, where the free υ_{N^+-H} occurs as a strong sharp band at 3268 cm^{-1}, the IR spectrum of **3c**-$H^+ClO_4^-$ in $CHCl_3$ contains a broad band at ~3000cm^{-1} which did not change on dilution, supportive of an intramolecular hydrogen-bond. Similar results were obtained for the HBr salt though in this case the situation is complicated by the potential for intermolecular hydrogen-bonding to Br^-.
10. This compares with earlier calculations performed on pyrroloquinolines related to compound **2** which also placed the o-tolyl group orthogonal to the plane of the quinoline ring; see reference 4.

(*Received in Belgium* 7 *October* 1994)