Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 536–541
Bioorganic & Medicinal Chemistry

Novel imidazo[1,2-*a*]pyrazine derivatives as potent reversible inhibitors of the gastric H^+/K^+-ATPase☆

Peter Jan Zimmermann,* Christof Brehm, Wilm Buhr, Andreas Marc Palmer, Jürgen Volz and Wolfgang-Alexander Simon

Nycomed GmbH, Byk-Gulden-Str. 2, D-78467 Konstanz, Germany

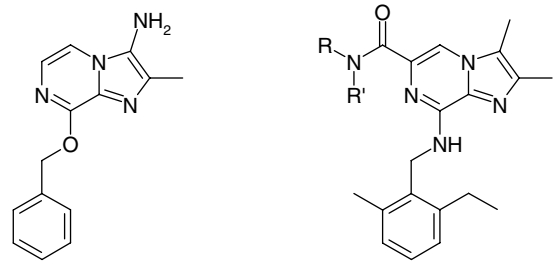
Received 3 April 2007; accepted 4 September 2007
Available online 12 September 2007

Abstract—A series of novel 6-substituted imidazo[1,2-*a*]pyrazines were synthesized via palladium catalyzed amino- or alkoxycarbonylation as key step. The anti-secretory activity of these compounds has been assessed in a binding assay against H^+/K^+-ATPase from hog gastric mucosa. Some of the compounds proved to be potent inhibitors of the gastric acid pump.

1. Introduction

The treatment of gastro-oesophageal reflux disease (GERD) and other acid related diseases relies primarily on inhibition of the gastric H^+/K^+-ATPase which effects the final step of acid secretion in the stomach. Although irreversible inhibitors of the H^+/K^+-ATPase (PPIs) are considered the treatment of choice, there is still potential for improvement in GERD and ulcer therapy.[1] The new reversible potassium competitive acid blockers (P-CABs) may offer some therapeutic advantages such as faster and better symptom control and more rapid healing.[2]

Over the past two decades, lead optimization of P-CABs has been mainly focused on the structural class of substituted imidazo[1,2-*a*]pyridines[3–6] or imidazonaphthyridines.[7] The closely related imidazo[1,2-*a*]pyrazines have been studied less extensively.[8,9] As part of our continuing efforts to identify novel P-CABs, we envisaged the known inhibitor SCH 32651 (**1**)[8] as a suitable starting point for further structural modifications. Herein, we report on the synthesis and biological evaluation of some new 6-carboxamide-substituted imidazo[1,2-*a*]pyrazines **2** and certain derivatives thereof (Fig. 1).[10]



SCH 32651 (**1**) **2**

Figure 1. Structure of SCH 32651 (**1**) and target compound **2**.

2. Chemistry

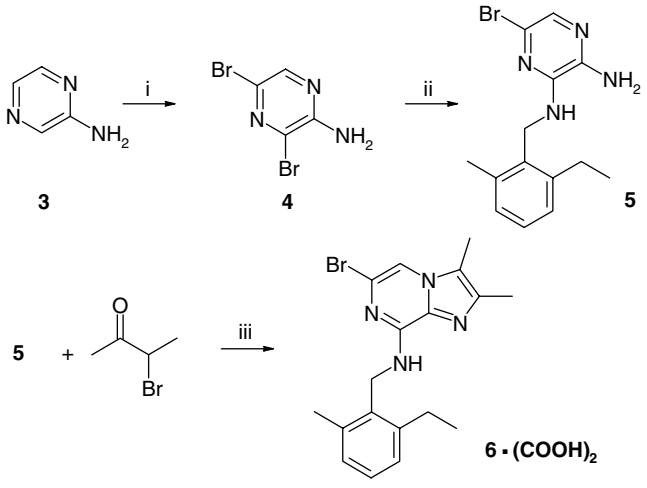
The most prevalent synthetic route toward imidazo[1,2-*a*]pyrazines comprises the condensation of aminopyrazines with α-halogenocarbonyl compounds.[11] In order to have access to the target compounds **2** and derivatives thereof, we planned to introduce the amide functionality at a late stage of the synthesis, after the remaining substituents had already been established. For this approach, we considered the palladium catalyzed carbonylation of an appropriate substituted 6-halogeno-imidazo[1,2-*a*]pyrazine to be the method of choice. Starting from commercially available aminopyrazine (**3**), bromination

Keywords: Anti-ulcer; P-CAB; H^+/K^+-ATPase; Carbonylation; Imidazo[1,2-*a*]pyrazine.
☆ Design of novel P-CABs, III. -Part II: Palmer, A. M.; Grobbel, B.; Brehm, C.; Zimmermann, P. J.; Buhr, W.; Feth, M. P.; Holst, H. C.; Simon, W. A. doi:10.1016/j.bmc.2007.08.065.
* Corresponding author. Tel.: +49 0 7531 84 4783; fax: +49 0 7531 84 94783; e-mail: pjz@lycos.de

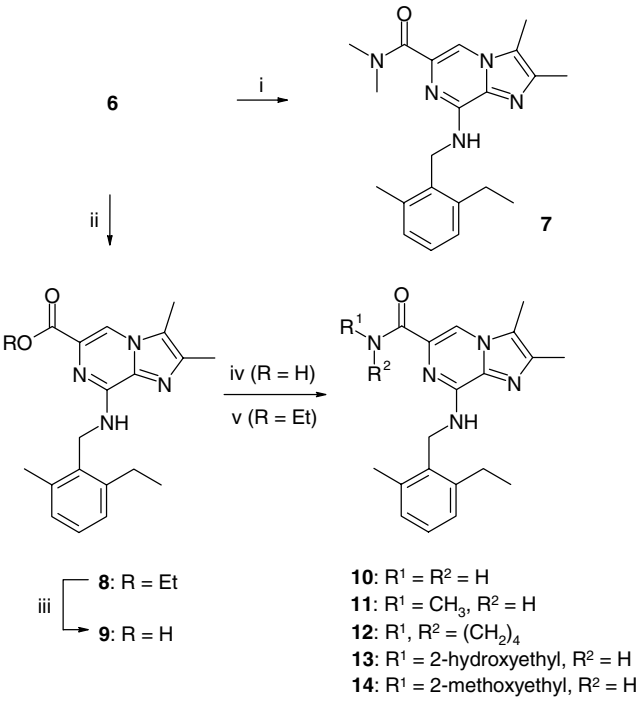
0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.09.009

with *N*-bromosuccinimide gave 2-amino-3,5-dibromopyrazine (**4**).[12] Initial attempts on the condensation of **4** with 3-bromo-2-butanone failed, in accordance with the literature reports on related reactions of 2-amino-3,5-dibromopyrazine (**4**) with α-halogenoketones.[13] Therefore, we decided to introduce the 3-aminobenzyl group first:[14] Reaction of 2-ethyl-6-methylbenzylamine with compound **4** was best accomplished under microwave-assisted conditions to yield the benzylamino derivative **5** on a multigram scale with high regioselectivity (>95%) and in 68% yield. Subsequent condensation of compound **5** with 3-bromo-2-butanone proceeded smoothly to give the 6-bromo-imidazo[1,2-*a*]pyrazine **6** which was isolated as its salt with oxalic acid in 70% yield (Scheme 1).

Having obtained the key intermediate **6**, elaboration of the carboxamide had to be accomplished. At first, as shown in Scheme 2, we examined the direct aminocarbonylation of the 6-bromo-imidazo[1,2-*a*]pyrazine **6** according to standard procedures described in the literature.[15] To our delight, heating a mixture of bromide **6**, palladium acetate (15 mol %), triphenylphosphine, and dimethylamine (2 M solution in tetrahydrofuran) under a pressure of 6 bars carbon monoxide for 16 h furnished the *N*,*N*-dimethylcarboxamide **7** in 66% yield. Although the preparation of amide **7** according to this procedure proceeded well, we considered the alkoxycarbonylation of the 6-bromo precursor **6** as more viable, since the resulting ester **8** would allow the convenient synthesis of a variety of amides. In fact, reaction of **6** under similar conditions applied for the preparation of amide **7**, but using triethylamine in ethanol rather than dimethylamine in tetrahydrofuran, delivered ethyl ester **8** in excellent yield. After saponification of ethyl ester **8** to the carboxylic acid **9**, the carboxamides **10**–**12** were obtained in good to excellent yields by reaction of **9** with the respective amine and TBTU[16] as coupling reagent. Alternatively, heating ethyl ester **8** with 2-aminoethanol or 2-methoxyethylamine provided directly the corresponding carboxamides **13** and **14**, respectively (Scheme 2).

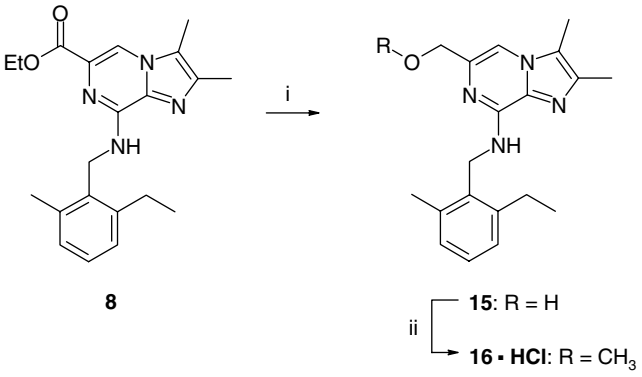


Scheme 1. Reagents and conditions: (i) *N*-bromosuccinimide, CH_2Cl_2, 63%; (ii) 2-ethyl-6-methylbenzylamine, Et_3N, CH_3CN, microwave 180 °C, 40 min, 68%; (iii) (1)dioxane, 100 °C; (2)oxalic acid, acetone, 70%.



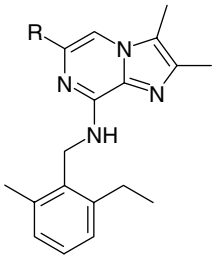
Scheme 2. Reagents and conditions: (i) $Pd(OAc)_2$, PPh_3, 2 M Me_2NH in THF, 6 bars CO, 120 °C, 16 h, 66%; (ii) $Pd(OAc)_2$, PPh_3, EtOH, Et_3N, 10 bars CO, 100 °C, 14 h, 89%; (iii) 2 N NaOH, dioxane, 80 °C, 1 h, 95%; (iv) TBTU, CH_2Cl_2; **10**: $NH_{3\ (g)}$, rt to reflux, 3 h, 66%; **11**: 8 M $MeNH_2$ in EtOH, rt, 16 h, 94%; **12**: pyrrolidine, rt, 7 h, 78%; (v) **13**: 2-aminoethanol, 80–100 °C, 1.5 h, 91%, **14**: 2-methoxyethylamine, reflux, 20 h, 49%.

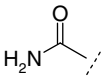
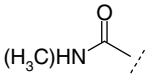
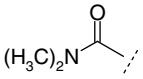
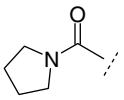
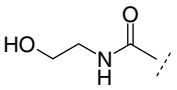
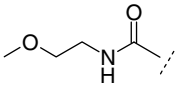
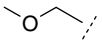
In addition to the imidazo[1,2-*a*]pyrazine-6-carboxamides **10**–**14** thus prepared, we were interested in the 6-alkoxymethyl-substituted analogues of **2** to enlarge the structure–activity relation. Consequently, ethyl ester **8** was subjected to lithium aluminium hydride reduction to give the hydroxymethyl derivative **15**, which in turn was alkylated to yield the corresponding methyl ether **16** (Scheme 3).



Scheme 3. Reagents and conditions: (i) $LiAlH_4$, THF, 0 °C, 1 h, 87%; (ii) (1)NaH, CH_3I, DMF, rt, 1 h; (2)1.5 M HCl in Et_2O, CH_2Cl_2, rt, 21%.

Table 1. Inhibition of H^+/K^+-ATPase by compounds **1**, **7**, and **10**–**16**



Compound	R	H^+/K^+-ATPase $-\log IC_{50}$
SCH 32651 (**1**)		5.9
10		n.i.[a]
11		5.5
7		6.4
12		6.9
13		5.0
14		6.0
15		5.9
16		5.5

[a] No inhibition, i.e. $-\log IC_{50} < 4$.

3. Results and discussion

All imidazo[1,2-*a*]pyrazines **7** and **10**–**16** as well as SCH 32651 (**1**)[17] were evaluated in a competitive binding assay against H^+/K^+-ATPase from hog gastric mucosa. The results are summarized in Table 1. The unsubstituted carboxamide **10** showed an approximately 100-fold reduction in activity ($-\log IC_{50} < 4$) compared to the reference compound **1**. On the other hand, the in vitro activity of compound **10** was clearly improved when one or two alkyl substituents were introduced on the amide nitrogen. A substantial increase in potency was observed when one of the amide hydrogens of **10** was replaced by a methyl group (**11**) or an extended alkyl chain (**13**, **14**). The increase in activity was even more pronounced when both of the amide hydrogens of **10** were substituted by methyl groups (**7**) or when the amide nitrogen was replaced by a pyrrolidino group (**12**). The substitution of the amide moiety by a hydroxymethyl (**15**) or methoxymethyl group (**16**) led to derivatives with an activity comparable to the monoalkyl-substituted carboxamides **14** and **11**, respectively.

4. Conclusions

In summary, we have reported a general route for the preparation of 6,8-disubstituted imidazo[1,2-*a*]pyrazines. This new approach has been exemplified by the convenient synthesis of some 6-carboxamides and related derivatives. Two compounds of this series (**7**, **12**) were shown to have a superior in vitro activity as antagonists of the gastric H^+/K^+-ATPase compared to the known P-CAB SCH 32651 (**1**).

5. Experimental

5.1. General methods

All chemicals and solvents were commercially available and used without further purification. The reactions were monitored by thin-layer chromatography (TLC) using silica plates (HPTLC-plates Nano-SIL 20 UV_{254}, Macherey-Nagel) and spots were visualized in UV-light at $\lambda = 254$ nm. Column chromatography was performed on Merck silica gel 60. Melting points (mp) were determined on a Büchi B-540 melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a Bruker 200 MHz spectrometer with TMS as an internal standard at 300 K. HPLC–HRMS spectra were recorded in positive ion mode on an electrospray quadrupole-time of flight mass spectrometer (micrOTOF-Q, Bruker Daltonics, Bremen) that is coupled to an Agilent 1100 series HPLC. The spectra were calibrated externally with sodium formate cluster ions. Microwave syntheses were carried out with a Personal Chemistry Emrys Optimizer.

5.1.1. 2-Ethyl-6-methylbenzylamine. To a suspension of $LiAlH_4$ (10.4 g, 275 mmol) in dried Et_2O (200 mL) was slowly added a solution of 2-ethyl-6-methylbenzonitrile (20.0 g, 138 mmol) in Et_2O (60 mL) at −10 °C. After 1 h at 0 °C and 1 h at room temperature, the reaction mixture was carefully hydrolyzed with water (4 mL) and 6 N NaOH (4 mL). After 2 h at room temperature, anhydrous $MgSO_4$ was added and the reaction mixture was filtered through Celite. Evaporation of the solvent yielded 2-ethyl-6-methylbenzylamine (15.5 g, 80%) as a colorless oil which was used without further purification in the next step. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm): 1.16–1.32 (m, 5H, CH_2CH_3, NH_2), 2.40 (s, 3H, CH_3), 2.73 (q, $J = 7.5$ Hz, 2H, CH_2CH_3), 3.88 (s, 2H, CH_2NH), 6.99–7.14 (m, 3H, C_6H_3).

5.1.2. 5-Bromo-N^3-(2-ethyl-6-methylbenzyl)pyrazine-2,3-diamine (5). A mixture of 2-amino-3,5-dibromopyrazine (**4**)[12] (1.26 g, 5 mmol), 2-ethyl-6-methylbenzylamine (1.5 g, 10 mmol), Et_3N (1.5 mL), and CH_3CN (3.5 mL) in a sealed tube was irradiated in a microwave-oven for 40 min at 180 °C. The crude reaction mixtures of 10 such runs were combined, treated with saturated

aqueous $NaHCO_3$, and extracted with EtOAc. The organic phase was dried over anhydrous $MgSO_4$ and evaporated. The residue was purified by column chromatography (light petroleum ether/EtOAc, 4:1). Crystallization from dioxane yielded **5** (10.2 g, 68%) as a colorless solid. Mp 155 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 1.15 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.33 (s, 3H, CH_3), 2.67 (q, J = 7.5 Hz, 2H, CH_2CH_3), 4.40 (d, J = 4.0 Hz, 2H, CH_2NH), 6.24 (br s, 2H, NH_2), 6.44 (br t, 1H, CH_2NH), 7.06–7.22 (m, 4H, C_6H_3, 6-H); HRMS $[M+H]^+$: 321.0690, $C_{14}H_{18}BrN_4$ requires 321.0709.

5.1.3. 6-Bromo-*N*-(2-ethyl-6-methylbenzyl)-2,3-dimethylimidazo[1,2-*a*]pyrazin-8-amine oxalate (6·COOH). To a suspension of **5** (10 g, 31 mmol) in dioxane (60 mL) was added 3-bromo-2-butanone (4.90 mL, 46.7 mmol) and the resulting mixture was heated to 100 °C. After 2 h, additional 3-bromo-2-butanone (4.90 mL, 46.7 mmol) was added and heating was continued for 16 h. The mixture was cooled, diluted with CH_2Cl_2, and extracted with saturated aqueous $NaHCO_3$. The organic phase was dried over anhydrous $MgSO_4$ and evaporated. Purification of the residue by column chromatography (light petroleum ether/EtOAc, 4:1) provided a colorless oil which was dissolved in acetone and treated with a solution of oxalic acid dihydrate (3.91 g, 31 mmol) in acetone. The precipitate was collected and washed with *n*-heptane to yield **6·COOH** (10 g, 70%) as a colorless solid. Mp 163 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 1.14 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.26 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.78 (q, J = 7.5 Hz, 2H, CH_2CH_3), 4.61 (d, J = 4.8 Hz, 2H, CH_2NH), 7.00–7.17 (m, 3H, C_6H_3), 7.75 (s, 1H, 5-H); HRMS $[M+H]^+$: 373.1013, $C_{18}H_{22}BrN_4$ requires 373.1022.

5.1.4. 8-[(2-Ethyl-6-methylbenzyl)amino]-*N*,*N*,2,3-tetramethylimidazo[1,2-*a*]pyrazine-6-carboxamide (7). To a solution of **6** (2.3 g, 5.1 mmol, liberated from **6·COOH** as described in 5.1.5) in dimethylamine (50 mL, 2 M solution in THF) were added $Pd(OAc)_2$ (0.17 g, 0.76 mmol) and PPh_3 (0.80 g, 3.1 mmol). The mixture was transferred to an autoclave and carbonylated (6 bars CO, 120 °C) for 16 h. The reaction mixture was evaporated and the residue was dissolved in CH_2Cl_2. The organic phase was extracted with saturated aqueous NH_4Cl, dried over anhydrous $MgSO_4$, and evaporated. Purification of the residue by column chromatography (light petroleum ether/EtOAc, 1:1) yielded **7** (1.22 g, 66%) as a colorless solid. Mp 174 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 1.12 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.27 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.74 (q, J = 7.5 Hz, 2H, CH_2CH_3), 3.03 [br s, 6H, $N(CH_3)_2$], 4.65 (d, J = 5.1 Hz, 2H, CH_2NH), 6.94–7.17 (m, 4H, C_6H_3, CH_2NH), 7.75 (s, 1H, 5-H); HRMS $[M+H]^+$: 366.2271, $C_{21}H_{28}N_5O$ requires 366.2288; Anal. Calcd for $C_{21}H_{27}N_5O$: C, 69.01; H, 7.45; N, 19.16. Found: C, 68.95; H, 7.43; N, 19.11.

5.1.5. Ethyl 8-[(2-ethyl-6-methylbenzyl)amino]-2,3-dimethylimidazo[1,2-*a*]pyrazine-6-carboxylate (8). 6-Bromo-*N*-(2-ethyl-6-methylbenzyl)-2,3-dimethylimidazo[1,2-*a*]-pyrazin-8-amine oxalate (**6·COOH**, 10.0 g, 22 mmol) was treated with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic phase was separated, dried over anhydrous $MgSO_4$ and evaporated to give crude **6** as a colorless oil. After dissolving **6** in EtOH (80 mL) and Et_3N (16 mL), $Pd(OAc)_2$ (0.50 g, 2.2 mmol) and PPh_3 (1.64 g, 6.2 mmol) were added. The mixture was transferred to an autoclave and carbonylated (10 bars CO, 100 °C) for 14 h. The reaction mixture was filtered and evaporated to leave an orange oil which was dissolved in CH_2Cl_2, and extracted with water. The organic phase was dried over anhydrous $MgSO_4$ and evaporated. Crystallization of the residue from EtOAc/*n*-heptane yielded **8** (7.2 g, 89%) as a colorless solid. Mp 144 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 1.13 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.34 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 2.28 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.83 (q, J = 7.5 Hz, 2H, CH_2CH_3), 4.32 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 4.70 (d, J = 5.4 Hz, 2H, CH_2NH), 6.99–7.15 (m, 4H, C_6H_3, CH_2NH), 8.19 (s, 1H, 5-H); HRMS $[M+H]^+$: 367.2110, $C_{21}H_{27}N_4O_2$ requires 367.2129.

5.1.6. 8-[(2-Ethyl-6-methylbenzyl)amino]-2,3-dimethylimidazo[1,2-*a*]pyrazine-6-carboxylic acid (9). To a solution of **8** (4.00 g, 10.9 mmol) in dioxane (40 mL) was added 2 N NaOH (8 mL). After 1 h at 80 °C, the reaction mixture was evaporated to half of its volume and the pH was adjusted to pH 6 with 6 N HCl. The thick precipitate was collected, washed with water, and dried in vacuo over P_2O_5 to yield **9** (3.52 g, 95%) as a colorless solid. Mp 230 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 1.13 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.27 (s, 3H, CH_3), 2.40 (s, 6H, 2× CH_3), 2.78 (q, J = 7.5 Hz, 2H, CH_2CH_3), 4.73 (d, J = 5.1 Hz, 2H, CH_2NH), 6.92–7.17 (m, 4H, C_6H_3, CH_2NH), 8.20 (s, 1H, 5-H), 12.50 (br s, 1H, COOH); HRMS $[M+H]^+$: 339.1797, $C_{19}H_{23}N_4O_2$ requires 339.1816.

5.1.7. 8-[(2-Ethyl-6-methylbenzyl)amino]-2,3-dimethylimidazo[1,2-*a*]pyrazine-6-carboxamide (10). To a suspension of **9** (1.02 g, 3 mmol) in CH_2Cl_2 (20 mL) was added TBTU[16] (1.61 g, 5 mmol). After 30 min, NH_3 was passed over the mixture. After 1 h, additional TBTU (1.0 g, 3.1 mmol) was added. Stirring was continued for 1 h at room temperature and finally 1 h under reflux. The reaction mixture was extracted with 2 N NaOH and the organic phase was separated, dried over anhydrous $MgSO_4$, and evaporated. Purification of the residue by column chromatography (CH_2Cl_2/MeOH, 20:1) and crystallization from EtOAc/*n*-heptane yielded **10** (0.67 g, 66%) as a colorless solid. Mp 227 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 1.12 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.27 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.73 (q, J = 7.5 Hz, 2H, CH_2CH_3), 4.78 (d, J = 5.1 Hz, 2H, CH_2NH), 6.93 (t, J = 5.1 Hz, 1H, CH_2NH), 7.01–7.18 (m, 3H, C_6H_3), 7.55 (br s, 1H of NH_2), 7.84 (br s, 1H of NH_2), 8.06 (s, 1H, 5-H); HRMS $[M+H]^+$: 338.1960, $C_{19}H_{24}N_5O$ requires 338.1975. Anal. Calcd for $C_{19}H_{23}N_5O$: C, 67.63; H, 6.87; N, 20.76. Found: C, 67.62; H, 6.86; N, 20.83.

5.1.8. 8-[(2-Ethyl-6-methylbenzyl)amino]-*N*,2,3-trimethylimidazo[1,2-*a*]pyrazine-6-carboxamide (11). To a suspension of **9** (1.02 g, 3 mmol) in CH_2Cl_2 (20 mL) was added TBTU (1.61 g, 5 mmol). After 30 min, methylamine (1.5 mL, 12 mmol, 8 M solution in EtOH) was added and stirring was continued for 16 h. The reaction mixture was extracted with 2 N NaOH and the organic phase was separated, dried over anhydrous $MgSO_4$, and evaporated. Purification of the residue by column chromatography (light petroleum ether/EtOAc, 1:1) and crystallization from EtOAc/*n*-heptane yielded **11** (0.99 g, 94%) as a colorless solid. Mp 120 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 1.12 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.26 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.73 (q, J = 7.5 Hz, 2H, CH_2CH_3), 2.85 (d, 3H, J = 4.9 Hz, NHCH_3), 4.81 (d, J = 5.1 Hz, 2H, CH_2NH), 6.96 (t, J = 5.1 Hz, 1H, CH_2NH), 7.02–7.18 (m, 3H, C_6H_3), 8.03 (s, 1H, 5-H), 8.40 (q, J = 4.9 Hz, 1H, NHCH$_3$); HRMS $[M+H]^+$: 352.2136, $C_{20}H_{26}N_5O$ requires 352.2132. Anal. Calcd for $C_{20}H_{25}N_5O{\cdot}H_2O$: C, 65.02; H, 7.37; N, 18.96. Found: C, 64.99; H, 7.30; N, 19.00.

5.1.9. *N*-(2-Ethyl-6-methylbenzyl)-2,3-dimethyl-6-(pyrrolidin-1-ylcarbonyl)imidazo[1,2-*a*]pyrazin-8-amine (12). To a suspension of **9** (0.50 g, 1.5 mmol) in CH_2Cl_2 (10 mL) was added TBTU (0.70 g, 2.2 mmol). After 30 min, pyrrolidine (0.5 mL, 6 mmol) was added and the mixture was stirred for 7 h. The reaction mixture was extracted with 2 N NaOH and the organic phase was separated, dried over anhydrous $MgSO_4$, and evaporated. Purification of the residue by column chromatography (CH_2Cl_2/MeOH, 20:1) and crystallization from EtOAc/*n*-heptane yielded **12** (0.45 g, 78%) as a colorless solid. Mp 197 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 1.12 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.84 (m, 4H, 2× CH_2), 2.27 (s, 3H, CH_3), 2.36 (s, 6H, 2× CH_3), 2.71 (q, J = 7.5 Hz, 2H, CH_2CH_3), 3.50 (m, 2H, CH_2), 3.77 (m, 2H, CH_2), 4.67 (d, J = 5.0 Hz, 2H, CH_2NH), 6.89 (t, J = 5.0 Hz, 1H, CH_2NH), 7.01–7.18 (m, 3H, C_6H_3), 7.89 (s, 1H, 5-H); HRMS $[M+H]^+$: 392.2431, $C_{23}H_{30}N_5O$ requires 392.2445. Anal. Calcd for $C_{23}H_{29}N_5O$: C, 70.56; H, 7.47; N, 17.89. Found: C, 70.60; H, 7.47; N, 17.61.

5.1.10. 8-[(2-Ethyl-6-methylbenzyl)amino]-*N*-(2-hydroxyethyl)-2,3-dimethylimidazo[1,2-*a*]pyrazine-6-carboxamide (13). A suspension of **8** (1.1 g, 3 mmol) in 2-aminoethanol (10 mL) was heated at 80 °C for 30 min. Then the reaction mixture was diluted with 2-aminoethanol (10 mL) and the temperature was raised to 100 °C. After 1 h, the reaction mixture was cooled and the precipitate was collected and washed with water. The colorless solid was dried in vacuo over P_2O_5 to yield **13** (1.04 g, 91%). Mp 229 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 1.13 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.27 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.74 (q, J = 7.5 Hz, 2H, CH_2CH_3), 3.36–3.59 (m, 4H, 2× CH_2), 4.80 (m, 3H, CH_2NH, OH), 7.02–7.18 (m, 4H, C_6H_3, CH_2NH), 8.05 (s, 1H, 5-H), 8.39 (t, J = 5.7 Hz, 1H, CONHCH$_2$); HRMS $[M+H]^+$: 382.2220, $C_{21}H_{28}N_5O_2$ requires 382.2238. Anal. Calcd for $C_{21}H_{27}N_5O_2$: C, 66.12; H, 7.13; N, 18.36. Found: C, 66.13; H, 7.16; N, 18.29.

5.1.11. 8-[(2-Ethyl-6-methylbenzyl)amino]-*N*-(2-methoxyethyl)-2,3-dimethylimidazo[1,2-*a*]pyrazine-6-carboxamide (14). A solution of **8** (1.00 g, 2.73 mmol) in 2-methoxyethylamine (10 mL) was refluxed for 20 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The organic phase was dried over anhydrous $MgSO_4$ and evaporated. Purification of the residue by column chromatography (light petroleum ether/EtOAc, 1:1) and crystallization from diisopropyl ether yielded **14** (0.53 g, 49%) as a colorless solid. Mp 111 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 1.12 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.27 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.74 (q, J = 7.5 Hz, 2H, CH_2CH_3), 3.29 (s, 3H, OCH_3), 3.48 (m, 4H, 2× CH_2), 4.80 (d, J = 5.1 Hz, 2H, CH_2NH), 7.02–7.18 (m, 4H, C_6H_3, CH_2NH), 8.05 (s, 1H, 5-H), 8.37 (br t, 1H, CONHCH$_2$); HRMS $[M+H]^+$: 396.2383, $C_{22}H_{30}N_5O_2$ requires 396.2394. Anal. Calcd for $C_{22}H_{29}N_5O_2$: C, 66.81; H, 7.39; N, 17.71. Found: C, 66.89; H, 7.39; N, 17.86.

5.1.12. {8-[(2-Ethyl-6-methylbenzyl)amino]-2,3-dimethylimidazo[1,2-*a*]pyrazin-6-yl}methanol (15). To a suspension of $LiAlH_4$ (0.31 g, 8.2 mmol) in dried THF (10 mL) was slowly added a solution of **8** (1.0 g, 2.7 mmol) in THF (20 mL) at 0 °C. After 1 h at 0 °C, the reaction mixture was successively hydrolyzed with water (0.2 mL), 6 N NaOH (0.4 mL), and water (1 mL). After 1 h at room temperature, anhydrous $MgSO_4$ was added and the reaction mixture was filtered through Celite. On evaporation of the filtrate, a precipitate was obtained which was collected, washed with Et_2O, and dried in vacuo to yield **15** (0.77 g, 87%) as a colorless solid. Mp 166 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 1.13 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.24 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.76 (q, J = 7.5 Hz, 2H, CH_2CH_3), 4.43 (d, J = 5.6 Hz, 2H, CH_2OH), 4.63 (d, J = 5.1 Hz, 2H, CH_2NH), 5.20 (t, J = 5.6 Hz, 1H, OH), 6.55 (t, J = 5.1 Hz, 1H, CH_2NH), 7.00–7.17 (m, 3H, C_6H_3), 7.39 (s, 1H, 5-H); HRMS $[M+H]^+$: 325.2014, $C_{19}H_{25}N_4O$ requires 325.2023. Anal. Calcd for $C_{19}H_{24}N_4O$: C, 70.34; H, 7.46; N, 17.27. Found: C, 70.22; H, 7.45; N, 17.29.

5.1.13. *N*-(2-Ethyl-6-methylbenzyl)-6-(methoxymethyl)-2,3-dimethylimidazo[1,2-*a*]pyrazin-8-amine (16·HCl). To a suspension of **15** (0.50 g, 1.5 mmol) in dried DMF (5 mL) was added NaH (0.18 g, 4.5 mmol, 60% dispersion in mineral oil) in portions at room temperature. After 30 min, methyl iodide (0.12 mL, 2 mmol) was slowly added. After 30 min, the reaction mixture was carefully hydrolyzed with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic phase was dried over anhydrous $MgSO_4$ and evaporated. Purification of the residue by column chromatography (light petroleum ether/EtOAc, 4:1) yielded 0.18 g of a colorless oil which was dissolved in CH_2Cl_2 and treated with HCl (1.5 M solution in Et_2O). Evaporation of all volatiles yielded **16·HCl** (0.12 g, 21%) as a colorless solid. Mp 177 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 1.13 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.24 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.77 (q, J = 7.5 Hz, 2H, CH_2CH_3), 3.37 (s, 3H, OCH_3), 4.33 (s, 2H, CH_2OCH$_3$),

4.64 (d, J = 5.2 Hz, 2H, CH_2NH), 6.65 (t, J = 5.2 Hz, 1H, CH$_2$NH), 7.00–7.16 (m, 3H, C_6H_3), 7.45 (s, 1H, 5-H); HRMS $[M+H]^+$: 339.2158, $C_{20}H_{27}N_4O$ requires 339.2179. Anal. Calcd for $C_{20}H_{26}N_4O$·HCl: C, 64.07; H, 7.26; N, 14.94; Cl, 9.46. Found: C, 63.85; H, 7.34; N, 14.67; Cl, 9.40.

5.2. H^+/K^+-ATPase inhibition assay

The malachite green assay modified from Yoda and Hokin[18] was used for the determination of H^+/K^+-ATPase IC_{50}.[19] Given data are the mean IC_{50} values from two to three independent determinations. Pipes [piperazine-1,4-bis(2-ethanesulfonic acid], sucrose, nigericin, Na-ATP, and malachite green were purchased from Sigma–Aldrich, Tris [tris(hydroxymethyl)aminomethane], KCl, and ammoniumheptamolybdate tetrahydrate from Merck, and $MgCl_2$ from Fluka. Final assay concentrations: 4 mM Pipes/8 mM Tris buffer, pH 7.4, 0.25 M sucrose, 1 mM KCl, 1 mM $MgCl_2$, 0.5–1 μg/100 μL nigericin (1:1 ratio with enzyme), 0.5–1 μg/100 μL enzyme (dependent on K^+-stimulated, specific activity), and 1 mM Na-ATP (high grade), reaction volume: 101 μL. Preparation of malachite green reagent: two parts malachite green stock solution (1.2 M in H_2O, protected from light and used within 12 weeks) were mixed with one part ammoniumheptamolybdate tetrahydrate stock solution (42 g/L in 4 N HCl) and kept for 30 min at room temperature prior to use. *Procedure:* A Pipes/Tris buffer-based solution with sucrose and $MgCl_2$ was prepared. Nigericin and enzyme were added to reach abovementioned final concentrations. Eighty microliters per well of this mixture was placed into 96-well flat-bottommed plates (clear, polystyrol, Greiner bio-one). Ten microliters per well KCl (1 mM final) was used for stimulation of H^+/K^+-ATPase activity. Test substances were dissolved as 10 mM solutions in 100% DMSO. One microliter of substance solution was added in dilutions ranging from 10^{-4}–10^{-9} M (final). The enzymatic reaction was started by addition of 10 μL ATP (1 mM final). The assay was incubated for 30 min at room temperature. The reaction was stopped by addition of 150 μL malachite green reagent and incubated for another 15 min prior to photometric reading of the plate at 680 nm in a PowerWave HT Microplate spectral photometer (BioTek). The results were analyzed with GraphPad Prism software (Version 4.02) to calculate IC_{50} values by sigmoidal curve fitting. 'Enzyme' refers to H^+/K^+-ATPase-containing vesicles prepared from hog gastric mucosa.[20]

Acknowledgments

We are grateful to Ms. S. Chrismann, Ms. I. Hartig-Beecken and Mr. K. Hägele for technical assistance.

References and notes

1. (a) Parsons, M. E.; Keeling, D. J. *Expert Opin. Invest. Drugs* **2005**, *14*, 411–421; (b) Fass, R.; Shapiro, M.; Dekel, R.; Sewell, J. *Aliment. Pharmacol. Ther.* **2005**, *22*, 79–94; (c) Vakil, N. *Aliment. Pharmacol. Ther.* **2004**, *19*, 1041–1049.
2. Andersson, K.; Carlsson, E. *Pharmacol. Ther.* **2005**, *108*, 294–307.
3. Kaminski, J. J.; Bristol, J. A.; Puchalski, C.; Lovey, R. G.; Elliott, 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*, 876–892.
4. Wurst, W.; Hartmann, M. *Yale J. Biol. Med.* **1996**, *69*, 233–243.
5. (a) Äbelö, A.; Andersson, M.; Holmberg, A. A.; Karlsson, M. *Eur. J. Pharm. Sci.* **2006**, *29*, 91–101; (b) Gedda, K.; Briving, C.; Svensson, K.; Maxvall, I.; Andersson, K. *Biochem. Pharmacol.* **2007**, *73*, 198–205.
6. Zimmermann, P. J.; Buhr, W.; Brehm, C.; Palmer, A. M.; Feth, M. P.; Senn-Bilfinger, J.; Simon, W.-A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5374–5378.
7. (a) Senn-Bilfinger, J.; Ferguson, J. R.; Holmes, M. A.; Lumbard, K. W.; Huber, R.; Zech, K.; Hummel, R.-P.; Zimmermann, P. J. *Tetrahedron Lett.* **2006**, *47*, 3321–3323; (b) Simon, W. A.; Herrmann, M.; Klein, T.; Shin, J. M.; Huber, R.; Senn-Bilfinger, J.; Postius, S. *J. Pharmacol. Exp. Ther.* **2007**, *321*, 866–874.
8. (a) Kaminski, J. J.; Hilbert, J. M.; Pramanik, B. M.; Solomon, D. M.; Conn, D. J.; Rizvi, R. K.; Elliott, A. J.; Guzik, H.; Lovey, R. G.; Domalski, M. S.; Wong, S.-C.; Puchalski, C.; Gold, E. H.; Long, J. F.; Chiu, P. J. S.; McPhail, A. T. *J. Med. Chem.* **1987**, *30*, 2031–2046; (b) Kaminski, J. J.; Perkins, D. G.; Frantz, J. D.; Solomon, D. M.; Elliott, A. J.; Chiu, P. J. S.; Long, J. F. *J. Med. Chem.* **1987**, *30*, 2047–2051; (c) Kaminski, J. J.; Doweyko, A. M. *J. Med. Chem.* **1997**, *40*, 427–436.
9. Amin, K.; Dahlström, M.; Nordberg, P.; Starke, I. WO1999/028322.
10. Zimmermann, P. J.; Buhr, W.; Chiesa, M. V.; Palmer, A.; Brehm, C.; Grundler, G.; Senn-Bilfinger, J.; Simon, W.-A.; Postius, S.; Kromer, W. WO2004/074289.
11. For a review see: Basiuk, V. A. *Russ. Chem. Rev.* **1997**, *66*, 187–204.
12. Jiang, B.; Yang, C.-G.; Xiong, W.-N.; Wang, J. *Bioorg. Med. Chem.* **2001**, *9*, 1149–1154.
13. Vitse, O.; Laurent, F.; Pocock, T. M.; Bénézech, V.; Zanik, L.; Elliott, K. R. F.; Subra, G.; Portet, K.; Bompart, J.; Chapat, J.-P.; Small, R. C.; Michel, A.; Bonnet, P.-A. *Bioorg. Med. Chem.* **1999**, *7*, 1059–1065.
14. For related reactions of 2-amino-3,5-dibromopyrazine (**4**), see e.g., (a) Barlin, G. B.; Ireland, S. J. *Aust. J. Chem.* **1984**, *37*, 1057–1064; (b) Bonnet, P. A.; Michel, A.; Laurent, F.; Sablayrolles, C.; Rechencq, E.; Mani, J. C.; Boucard, M.; Chapat, J. P. *J. Med. Chem.* **1992**, *35*, 3353–3358.
15. (a) Horino, H.; Sakaba, H.; Arai, M. *Synthesis* **1989**, 715–718; (b) Head, R. A.; Ibbotson, A. *Tetrahedron Lett.* **1984**, *25*, 5939–5942.
16. TBTU = O-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; see e.g., Montalbetti, C. A. G. N.; Falque, V. *Tetrahedron* **2005**, *61*, 10827–10852.
17. 3-Amino-2-methyl-8-benzyloxy-imidazo[1,2-*a*]pyrazine hydrochloride (**1·HCl**) was prepared as described in Ref. 8a.
18. Yoda, A.; Hokin, L. E. *Biochem. Biophys. Res. Commun.* **1970**, *40*, 880–886.
19. Lanzetta, P. A.; Alvarez, L. J.; Reinach, P. S.; Candia, O. A. *Anal. Biochem.* **1979**, *100*, 95–97.
20. Rabon, E. C.; Im, W. B.; Sachs, G. *Methods Enzymol.* **1988**, *157*, 649–654.