Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 3621–3626

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

(2Z,4E)-5-(5,6-DICHLORO-2-INDOLYL)-2-METHOXY-N-(1,2,2,6,6-PENTAMETHYLPIPERIDIN-4-YL)-2,4-PENTADIENAMIDE, A NOVEL, POTENT AND SELECTIVE INHIBITOR OF THE OSTEOCLAST V-ATPASE.

Guy Nadler,*[a] Marcel Morvan,[a] Isabelle Delimoge,[a] Pietro Belfiore,[b] Andrea Zocchetti,[b] Ian James,[c] Denise Zembryki,[c] Elizabeth Lee-Rycakzewski,[c] Carlo Parini,[b] Emanuela Consolandi,[b] Stefania Gagliardi,[b] Carlo Farina[b]

[a]*SmithKline Beecham Research Unit, BP 58, 35762 St-Gregoire Cedex, France*
[b]*SmithKline Beecham S.p.A., Via Zambeletti, 20021 Baranzate, Milano, Italy*
[c]*SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, King of Prussia, PA 19406.*

Received 17 June 1998; accepted 2 November 1998

Abstract: Optimisation of a novel series of osteoclast ATPase inhibitors led to (2Z,4E)-5-(5,6-dichloro-2-indolyl)-2-methoxy-N-(1,2,2,6,6-pentamethylpiperidin-4-yl)-2,4-pentadienamide (**1**) that was the most potent compound in an *in vitro* osteoclast ATPase assay and in human bone resorption assays. Two of the possible geometric isomers have also been prepared and shown to be significantly less potent than **1**.

Keywords: Enzyme inhibitors.

Healthy bone results from a balance in both bone formation and bone resorption. In post-menopausal osteoporosis the resorption process exceeds formation, resulting in a net bone loss and consequently in an increased susceptibility to bone fracture. The resorption of bone requires a lowering of the pH in the extracellular microcompartments formed by the osteoclasts at their point of attachment to the bone. This acid environment is provided by the extrusion of protons which results in the dissolution of the mineralized matrix components of the bone. The exposed organic matrix components are then degraded by the action of proteolytic enzymes which function optimally at this acidic pH. The massive transcellular proton transport is accomplished by a vacuolar-type proton ATPase (V-ATPase) that is present in the osteoclast ruffled border[1] while the chloride counterions diffuse through a chloride channel.[2] Inhibition of osteoclast V-ATPase is therefore an attractive novel target for reducing osteoclast activity and subsequently bone resorption. Although the molecular structure of osteoclast vacuolar H^+-ATPase is not yet exactly known, a novel class of potent and selective inhibitors of this V-ATPase was recently identified.[3] The structure of these novel compounds, i.e. of 5-(2-indolyl)-2-methoxy-2,4-pentadienamides, was derived from the *unusual* macrolide bafilomycin A_1, a potent and specific inhibitor of all the V-ATPases.[4]

In the structure of the new inhibitors, the indole ring requires lipophilic and electron-withdrawing substituents,

Fax: + 33 299 280440; E-mail: guy_nadler@sbphrd.com

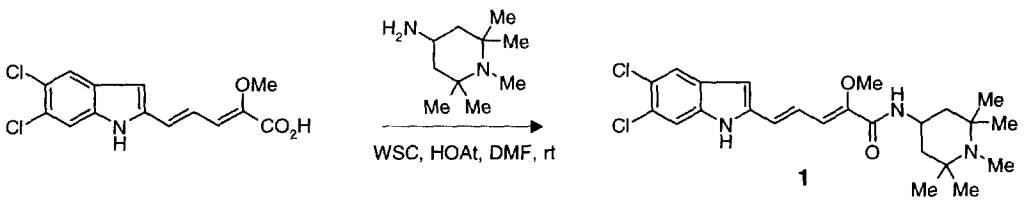
0960-894X/98/$ - see front matter
PII: S0960-894X(98)00660-X

such as chloro, at 5 and 6 position. In addition, modifications of the amino-amide moiety, in which a three carbon atom spacer is required for maximal activity, could modulate either potency or selectivity of these compounds.

In this paper we report that constraining the aminoamide moiety into a 4-aminopiperidine ring, bearing five methyl substituents, respectively at positions 1, 2 and 6, is able to maximize the inhibitory potency in both osteoclast ATPase and human osteoclast resorption assays, maintaining good selectivity against the V-ATPase of the human kidney. We also show that optimal inhibitory activity resides almost exclusively in the 2*Z*,4*E* geometric isomer.

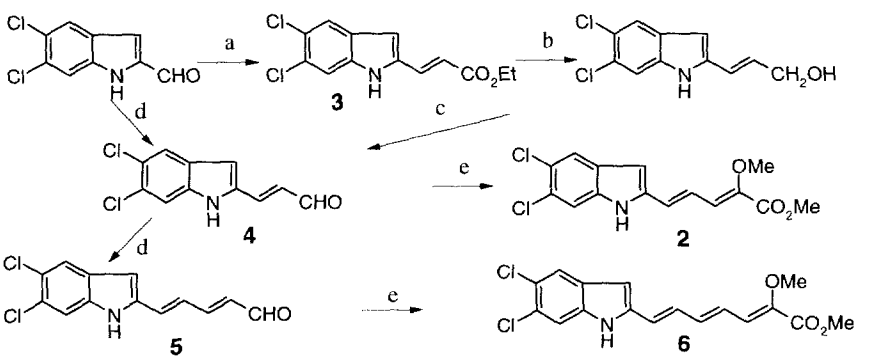
Chemistry

The synthesis of (2*Z*,4*E*)-5-(5,6-dichloro-2-indolyl)-2-methoxy-N-(1,2,2,6,6-pentamethylpiperidin-4-yl)-2,4-pentadienamide (SB 242784)[5] (**1**) is outlined in Scheme 1. The acid has been obtained from the corresponding ester by saponification with KOH in ethanol.



Scheme 1. *WSC: 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride, HOAt: 1-hydroxy-7-azabenzotriazole.*

Preparation of the corresponding ester (**2**) was already described[3] and entailed nine steps from the commercially available 3,4-dichlorotoluene. For the construction of the dienic system, Wittig reaction with ethyl triphenylphosphoranylideneacetate and DBU minimized but did not avoid the formation of cis double bond isomer. On the contrary Horner-Emmons reaction with triethyl phosphonoacetate and NaH gave ethyl (*E*) 3-(5,6-dichloro-2-indolyl)-2-propenoate (**3**) (Scheme 2) as a single reaction product. Reduction with DIBAH and subsequent oxidation with MnO_2 afforded (*E*)-3-(5,6-dichloro-2-indolyl)-2-propenaldehyde (**4**).

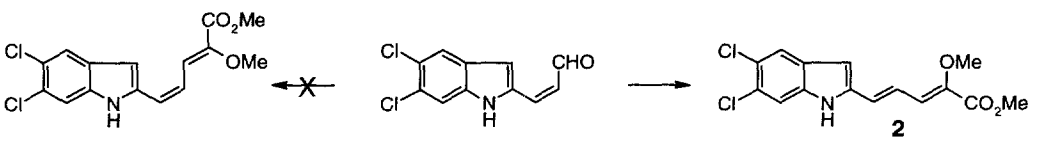


Scheme 2. *a) triethylphosphonoacetate, NaH in THF; b) DIBAH; c) MnO_2 in AcOEt; d) formylmethylene triphenylphosphorane, dichloromethane; e) methyl 2-methoxy-2-triphenylphosphoniumacetate bromide.*

To avoid these two last steps, the Wittig reaction of 5,6-dichloroindole-2-carboxaldehyde with formylmethylene triphenylphosphorane has been performed, however the desired (*E*) 3-(5,6-dichloro-2-indolyl)-2-propenaldehyde (**4**) was obtained along with at least 10% of the homologated aldehyde **5**. This dienic aldehyde could not be removed from the reaction mixture and the final ester **2** was contaminated with approximately the same percentage of the trienic ester **6** and could not be easily purified.

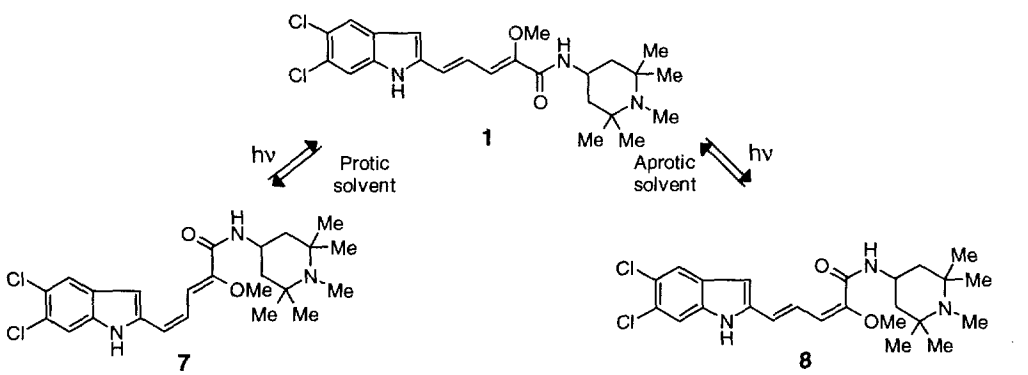
The key amine, 4-amino-1,2,2,6,6-pentamethylpiperidine, has been synthesised by N-methylation of commercially available tetramethyl-4-piperidone followed by the formation of the oxime and catalytic reduction over Rh/C. The use of a chemical reductions (LAH or sodium/amyl alcohol) in the last step led to a significant reduction in yield.

In order to obtain the geometric isomers of **1**, a stereospecific synthesis of the *Z,Z* analogue **7** was attempted starting from (*Z*) 3-(4,5-dichloroindolyl)propenaldehyde, obtained as a by-product of the first Wittig reaction, with methyl 2-methoxy-2-(triphenylphosphonium)acetate bromide. The reaction was very slow and no complete conversion was observed even after 24 h. The only isolable compound was the 2*Z*,4*E* ester (**2**), obtained in very low yield (Scheme 3).



Scheme 3. *Wittig reaction on (Z) 3-(4,5-dichloroindolyl)propenaldehyde.*

Therefore direct isomerization of SB 242784 by irradiation with UV light was attempted. A solution of **1** in MeOH/H_2O was irradiated at 366 nm (125 W) for 1.5 h and a mixture of **1** with two geometric isomers was obtained. Purification on column chromatography eluting with CH_2Cl_2 and MeOH (max. 1%) afforded the 2*Z*,4*Z* isomer (**7**)[6] in 7% yield.



Scheme 4. *Geometric isomers of 1: Z,Z (7) and E,E (8)*

When a solution of SB 242784 (**1**) in THF was exposed to the sunlight for 5 days, the 2*E*,4*E* isomer (**8**)[7] was obtained in 21% yield, after purification by column chromatography.

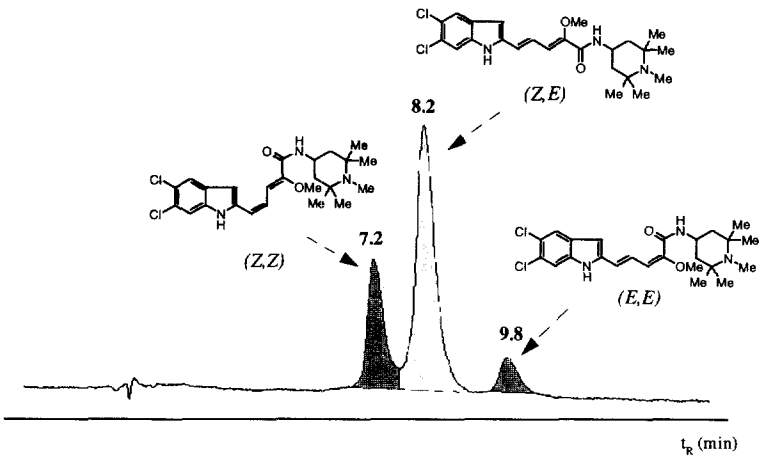


Figure 1. *Chromatogram of the mixture of isomers Z,Z (7), Z,E (1) and E,E (8).*[8]

The choice of the solvent was crucial because it is able to change the amount of each isomer in the mixture at the equilibrium point, even if 2*Z*,4*E* isomer (**1**) remains preponderant.

Both isomers were thermodynamically less stable than the 2*Z*,4*E* isomer and both of them quickly re-isomerised to SB 242784 when their solutions were exposed to the sunlight.

Pharmacology

The inhibition of bafilomycin-sensitive ATPase activity was measured in partially purified membrane vesicle preparations from chicken osteoclasts (cOC) obtained from the medullary bone of calcium starved egg-laying hens using a colourimetric method.[9] Selectivity was assessed using V-ATPase from chicken adrenal glands (cAG)[10] since this enzyme is phylogenetically related to the cOc proton pump. In human tissues the selectivity was preliminarily assessed using membranes from human kidney cortex (hK).[11] All the ATPase assays were performed in the presence of oligomycin (5 μg/ml) and vanadate (1 mM), as inhibitors of F-and P-ATPases, respectively. As can be observed in Figure 2, ATPase activity was about 40% bafilomycin sensitive in chicken osteoclasts and 85-90% in chicken adrenals and human kidney. Micromolar concentrations of SB 242784 (**1**) showed maximal inhibitory effect in all preparations. Since no additive inhibitory effect was observed when the compound was assayed in presence of 10 nM bafilomycin A_1, it can be concluded that SB 242784 inhibits bafilomycin-sensitive (V-type) ATPase activity selectively and has no effect against other contaminating ATPases.

Potency in human tissue was assessed by the ability of the compound to inhibit bone resorption by isolated human osteoclasts, obtained from giant cell tumors of bone.[12] In this assay the osteoclasts are settled on bone slices (8 bone slices for each concentration) and incubated for 48 h in a standard culture medium. Cellular activity is measured by counting the number of pits formed during the resorption using a light microscope. In these conditions, 53 ± 13 pits are observed in the control slices, while no excavations were observed in the

presence of 300 nM **1** or 1 nM bafilomycin A_1. In the pit assay, the osteoclast activity has been further evaluated using an in vitro ELISA Kit for quantification of type I collagen fragments released into the culture medium.[13]

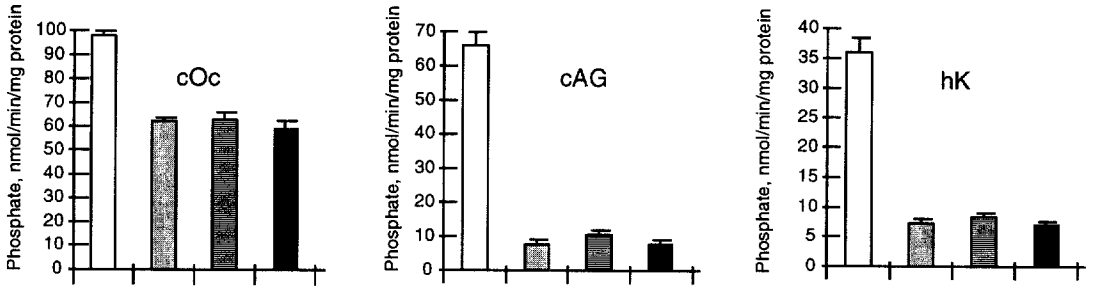


Figure 2. *ATPase activity in membranes prepared from chicken osteoclasts (cOc), chicken adrenal glands (cAG) and human kidney (hK); □: basal; □: bafilomycin A1 10 nM; ▤: compound 1 (1 μM in cOc or 3 μM in cAG or hK); ■: compound 1 (1 or 3 μM) + bafilomycin A_1 (10 nM)*

Values for half maximal inhibition by SB 242784 are reported in Table 1. This compound is a potent V-ATPase inhibitor with an IC_{50} of 26 nM in cOc and with about 5-fold selectivity against the vacuolar enzyme in chicken adrenal glands. SB 242784 was also very potent as an inhibitor of *in vitro* bone resorption by human osteoclasts as assessed either by counting the number of pits/bone slice or by the ELISA assay. Compound **1** showed an IC_{50} of 3.4 nM in the latter assay, with a selectivity of about 40-fold if compared with its potency in the human kidney V-ATPase assay. The geometry of the dienic chain was also crucial for potency as both the 2*Z*,4*Z* (**7**) and the 2*E*,4*E* (**8**) geometric isomers were much less potent than **1**.

Table 1. In vitro *activity of SB-242784 (1) and its geometric isomers (IC_{50} nM[a]).*

	V-ATPase assay			Human osteoclast resorption assay	
Comp	cOc	cAG	hK	pits/bone slices	ELISA
1	26.5 ± 1.5	125 ± 15	158 ± 12	13.5 ± 2.0	3.4 ± 1.9
7	663 ± 46	nt	2504 ± 106	nt	nt
8	2363 ± 163	nt	6334 ± 228	nt	nt

[a] Reported values represent the mean ± SE of triplicate experiments

Conclusion

The data reported in this communication provide a further insight into the structural requirements of 5-(2-indolyl)-2-methoxy-2,4-pentadienamides for V-ATPase inhibitory activity. In particular, the geometric isomerism appeared to be crucial for potency and a constrained and lipophilic amino-amide moiety increases both potency and selectivity. SB-242784 (**1**) is a novel potent and selective inhibitor of human osteoclast V-ATPase and bone resorption in vitro by human osteoclasts. This compound is, therefore, a very useful tool to prove that the inhibition of the osteoclast proton pump is a valid approach to novel drugs for treating osteopenic diseases such as osteoporosis.

Acknowledgements

The authors are grateful to Alberto Cerri and Isabelle Léger for the recording of NMR-spectra.

References and Notes

1 Baron, R.; Neff, L.; Louvard, D.; Courtoy, P.J. *J. Cell Biol.* **1985**, *101*, 2210.

2 Schlesinger, P.H.; Blair, H.C.; Teitelbaum, S.; Edwards, J.C. *J. Biol. Chem.* **1997**, *272*, 18636.

3 Gagliardi, S.; Nadler, G.; Consolandi, E.; Parini, C.; Morvan, M.; Legave, M-N.; Belfiore, P.; Zocchetti, A.; Clarke, G.D.; James, I.; Nambi, P.; Gowen, M.; Farina, C. *J. Med. Chem.* **1998**, *41*, 1568.

4 Bowman, E.M.; Siebers, A., Altendorf, K. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7972.

5 ^{1}H-NMR (200MHz, DMSO- d_6): 11.74 (s, 1H); 7.91 (d, 1H); 7.75 (s, 1H); 7.51 (s, 1H); 7.14 (dd, 1H); 6.84 (d, 1H); 6.60 (m, 2H); 4.07 (m, 1H); 3.70 (s, 3H); 2.18 (s, 3H); 1.62 (m, 2H); 1.44 (dd, 2H); 1.08 (s, 6H); 1.02 (s, 6H).
Mass spectra recorded on a Finningan MAT TSQ-700 spectrometer: m/z=
A) 464 $[M+H]^+$ (ESI POS, solvent: methanol, spray 4.5 kV, skimmer: 60 V, capillary temperature 220 C).
B) 464; 433; 377; 294; 266; 262; 123; 72 (CID, Offset= -34V)
Elemental analysis ($C_{24}H_{31}Cl_2N_3O_2$.HCl): Calculated C 57.55% H 6.44% N 8.39% Cl (tot) 21.23% Cl (ion) 7.08% Found C 57.02% H 6.56% N 8.23% Cl (tot) 20.83% Cl (ion) 7.17%

6 ^{1}H-NMR (300MHz, THF-d_8) δ: 10.48 (s, 1H); 7.58 (s, 1H); 7.44 (s, 1H); 7.22 (d, 1H); 6.99 (d, 1H); 6.61 (s, 1H); 6.59 (dd, 1H); 6.42 (d, 1H); 4.25-4.12 (m, 1H); 3.75 (s, 3H); 2.28 (s, 3H); 1.72 (m, 2H); 1.33 (dd, 2H); 1.11 (s, 12H).

7 ^{1}H-NMR (300MHz, THF-d_8) δ: 10.76 (s, 1H); 8.19 (dd, 1H); 7.54 (s, 1H); 7.40 (s, 1H); 6.93 (d, 1H); 6.51 (d, 1H); 6.32 (d, 1H); 5.92 (d, 1H); 4.25-4.12 (m, 1H); 3.70 (s, 3H); 2.25 (s, 3H); 1.72 (m, 2H); 1.32 (dd, 2H); 1.10 (s,12H).

8 HPLC conditions: Column: Lichrosorb RPSelect B 5μ, 250 × 4.1 mm (Merck); 40% B isocratic; Flux: 1 ml/min; Detection: 340 nm. Eluent A: H_2O / CH_3CN / TFA = 900 / 100 / 0.5. Eluent B: H_2O / CH_3CN / TFA = 100 / 900 / 0.5

9 Chan, K-M.; Delfert, D., Junger, K.D. *Anal. Biochem.* **1986**, *157*, 375.

10 The whole adrenal glands from male chicken were used, obtaining a membrane preparation similar to that described in literature for bovine chromaffin granules: Cidon, S.; Nelson, N. J. *Biol. Chem.* **1983**, *258*, 2892.

11 Wang, Z-Q.; Gluck, S. *J. Biol. Chem.*, **1990**, *265*, 21957.

12 Votta, B.J., Levy, M.A.; Badger, A.; Bradbeer, J.; Dodds, R.A.; James, I.E.; Thompson, S.; Bossard, M.J., Carr, T.; Connor, J.R.; Tomaszek, T.A.; Szewezuck, L.; Drake, F.H.; Veber, D.F.; Gowen, M. *J. Bone Miner.Res.* **1997**, *12*, 1396.

13 Foged, N.T.; Delaisse, J-M.; Hou, P.; Lou, H.; Sato, T.; Winding, B.; Bonde, M. J. *Bone Miner. Res.* **1996**, *11*, 226.