Synthesis and Biological Evaluation of Monoindolyl and Indolocarbazolyl Oxazolones and Imidazolones

Elisabète RODRIGUES PEREIRA,[a] Michelle PRUDHOMME,*,[a] Martine SANCELME,[a] Monique OLLIER,[b] Danièle SEVERE,[c] Jean François RIOU,[c] Henri CREVEL,[d] Jean Pierre SAVINEAU,[d] Doriano FABBRO,[e] and Thomas MEYER[e]

Université Blaise Pascal, Synthèse, Electrosynthèse et Etude de Systèmes à Intérêt Biologique,[a] UMR 6504 du CNRS, 63177 Aubière, France, Unité INSERM U71,[b] Rue Montalembert, 63005 Clermont-Ferrand, France, Rhône-Poulenc Rorer, 13 Quai Jules Guesde, 93403 Vitry sur Seine, France,[c] Laboratoire de Physiologie, Université Bordeaux II,[d] 146 rue Léo-Saignat, 33076 Bordeaux, France, CIBA-GEIGY Limited, K-125-409, Département d'Oncologie,[e] CH-4002 Bâle, Switzerland. Received September 13, 1996; accepted November 20, 1996

Eight compounds structurally related to protein kinase C inhibitor MDL 27032 and substituted with indole moieties were synthesized. Their activities towards protein kinase C (PKC) and protein kinase A (PKA) were determined. Their effect on PKC-mediated contraction of rat tracheal smooth muscle, their antiproliferative activity on two murine tumor cell lines, melanoma B16 and leukemia P388 and their antimicrobial activity on a gram-positive bacterium *Bacillus cereus* were also examined. The mammalian and bacterial cell antiproliferative activity, as well as vasorelaxant effect, observed for some of them could not be correlated to PKC or PKA inhibition. Only bulky bis-indolyl compounds exhibited biological activity in these experiments. Rigid indolocarbazoles had the strongest antiproliferative activity.

Key words oxazol-2-one; imidazol-2-one; protein kinase C; antiproliferation

Protein kinase C (PKC) plays a central role in carcinogenesis and is the primary receptor for tumor-promoting phorbol esters. A variety of cell functions such as cell growth and differentiation, smooth muscle contraction, gene expression and tumor promotion are mediated by PKC.[1] Some of the 12 known PKC isoforms have been shown to have a nuclear localization[2] and may be involved in DNA replication by phosphorylation of topoisomerases I and II. PKC inhibitors such as staurosporine and MDL 27,032 (Chart 1) have a smooth muscle vasorelaxant effect and antimetastatic properties.[3-5] MDL 27,032 inhibits metastatic processes, probably by PKC-mediated phosphorylation of cell surface adhesion receptors.[4] In this paper, we describe the synthesis and biological activities of compounds **1**—**8** (Chart 1), which are structurally related to MDL 27,032 and substituted with indole moieties.

Chemistry

Compound **1** was prepared from commercial oxazolidin-2-one by means of anodic oxidation followed by nucleophilic substitution with indolylmagnesium bromide.[6] Compound **2** was obtained from **1** (Chart 2) after protection of the nitrogen functions with *tert*-butyloxycarbonyl (Boc) groups, bromination using *N*-bromosuccinimide (NBS), debromination by electrochemical reduction then thermal cleavage of the remaining Boc protective group.[6] Compound **3** was prepared from commercial pyrrolidin-2-one using the same method as for **1**.[7] Compounds **4** and **5** were obtained from 2,2′-biindole by reaction with *N*-Boc-oxazol-2-one, followed for **5** by Boc protection of the three nitrogen functions then bromination of the protected intermediate with NBS, which induced concomitant dehydrobromination, aromatization and removal of the three Boc protective groups.[8] The syntheses of compounds **6**, **7** and **8** were carried out as previously described.[7]

Biological Activities

The inhibitory activities of compounds **1**—**8** towards PKC were determined. The inhibition of protein kinase A (PKA) was examined for some of them to evaluate their selectivity. The antiproliferative activities against two different murine cell lines, B16 melanoma cells and P388 leukemia cells were examined *in vitro*. Antibacterial activities were determined on a gram-positive bacterium, *Bacillus cereus*. The effects of compounds **1**—**8** on PKC-mediated contraction of rat tracheal smooth muscle were also examined. The potency of these compounds as PKC inhibitors was assessed by studying their ability to counteract the contraction induced by phorbol 12,13-dibutyrate (PDBu), a potent PKC activator in smooth muscles.[9-11] The results are presented in the table together with values for known PKC inhibitors, MDL 27,032, staurosporine, H7 and calphostin C.

Of the 8 compounds studied, only **5** exhibited a weak activity towards PKC with a stronger inhibitory effect on PKA than on PKC, while **4** had only a weak inhibitory effect against PKA. On murine tumor cell lines melanoma B16 and leukemia P388, **5** and **8** exhibited a marked antiproliferative activity, stronger against P388; **4** was only active on B16 and **7** was only active on P388. Compounds **4**, **5** and **8** were the most active against *B. cereus*. Since **4**, **7** and **8** had antiproliferative and antimicrobial properties without inhibiting PKC, we investigated their possible mode of action *via* the inhibition of topoisomerase I or II. The inhibitory potencies of **1**—**8** towards calf thymus topoisomerases I and II were examined, but all of them were inactive except **5** and **8**, which had a weak inhibitory effect against topoisomerase I (IC_{50}, 32 μM). However, this weak activity did not seem to be correlated to antiproliferation or antimicrobial activity. Moreover, since bacterial and mammalian topoisomerase I are highly divergent, it is unlikely that the antimicrobial activity on *B. cereus* was due to topoisomerase I inhibition.

* To whom correspondence should be addressed.

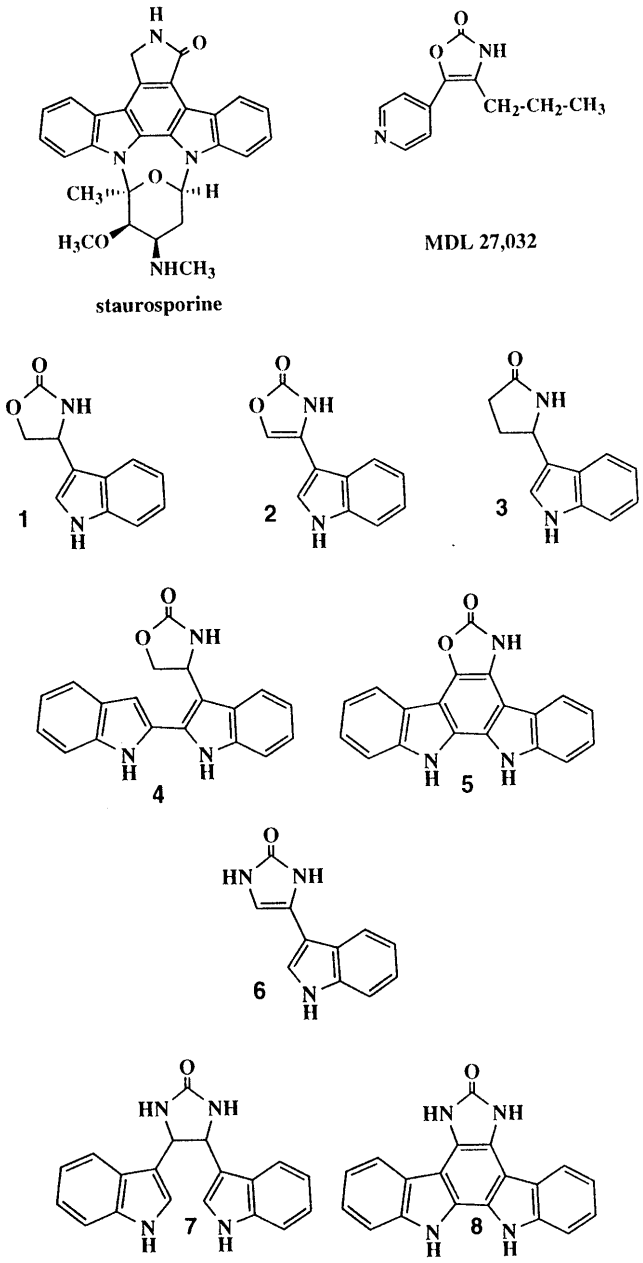


Chart 1

Compounds **4** and **5** had the strongest vasorelaxant effect, but their efficiency was weak compared to known PKC inhibitors, MDL 27,032, staurosporine, calphostin C and H7. Compound **4** was not a PKC inhibitor but exhibited a weak activity against PKA (81 μM). Compound **5** was the only product in the tested series with both PKC (89 μM) and PKA (24 μM) inhibitory effects, but since **4** had a stronger vasorelaxant effect than **5**, this biological property could not be linked to PKC or PKA inhibition. Therefore, another(s) target(s) may be involved in the mammalian and bacterial cell antiproliferative activity, as well as in the vasorelaxant potency. Of the mono-indolyl and bis-indolyl compounds, only the bulky bis-indolyl compounds **4**, **5**, **7** and **8** had antiproliferative activity on the tested cells. Rigid indolocarbazoles **5** and **8** exhibited the strongest activity against both leukemia P388 and *B. cereus*.

Experimental

Chemistry IR spectra were recorded on a Perkin-Elmer 881 spectrometer (ν in cm^{-1}), and NMR spectra on a Bruker AC 400 (^1H, 400 MHz; ^{13}C, 100 MHz) (chemical shifts δ in ppm).The following abbreviations are used: singlet (s), doublet (d), triplet (t), multiplet (m), tertiary carbons (C *tert.*), quaternary carbons (C *quat.*). Mass spectra electron impact (EI) were determined at Cesamo (Talence) on a high-resolution Fisons Autospec-Q spectrometer. Chromatographic purifications were performed with a flash Geduran SI 60 (Merck) 0.040—0.063 mm column.

4-(3-Indolyl)oxazolidin-2-one (1) Ethyl bromide (6.01 g) was added dropwise to magnesium (1.32 g) in tetrahydrofuran (THF) (30 ml) and the mixture was warmed to start the reaction, then indole (6.3 g, 53.8 mmol) in THF (50 ml) was added. The whole was warmed at 45 °C for 45 min. After cooling to −30 °C, 4-methoxyoxazolidin-2-one (1.605 g, 13.7 mmol) was added, followed by BF_3–Et_2O (3.3 ml) and the mixture was stirred at −30 °C for 12 h. After quenching with saturated aqueous NH_4Cl, and extraction with AcOEt, the organic phase was dried over $MgSO_4$. After removal of the solvent, the residue was purified by flash chromatography (eluent AcOEt) to give **1** (1.878 g, 9.30 mmol, 68% yield) as a white solid.

4-(3-*N*-*tert*-Butyloxycarbonylindolyl)-*N*-*tert*-butyloxycarbonyloxazolidin-2-one Compound **1** (1.870 g, 9.26 mmol) was dissolved in CH_2Cl_2 (5 ml), then Et_3N (1.5 ml), $(Boc)_2O$ (5.046 g, 23.1 mmol, 2.5 eq) and dimethyl aminopyridine (1.12 g) were added. The mixture was stirred at room temperature overnight. After removal of the solvent, the residue was purified by flash chromatography (eluent, cyclohexane–AcOEt, 7 : 3) to give the di-*N*-protected oxazolidin-2-one (3.50 g, 8.75 mmol, 94% yield) as a white solid.

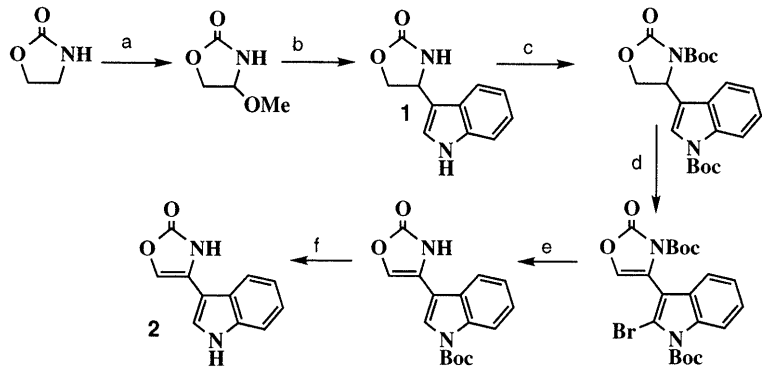
4-[3-(2-Bromo-*N*-*tert*-butyloxycarbonyl)indolyl]-*N*-*tert*-butyloxycarbonyloxazol-2-one A mixture of the previous compound (456 mg, 1.13 mmol) in CCl_4 (20 ml), NBS (444 mg, 2.49 mmol) and a catalytic amount of azobisisobutyronitrile (AIBN) was refluxed for 6 h. After removal of the solvent, the residue was purified by flash chromatography (eluent, cyclohexane–AcOEt, 5 : 5) to give the brominated oxazol-2-one (310 mg, 65 mmol, 58% yield) as an off-white solid.

4-(3-*N*-*tert*-Butyloxycarbonylindolyl)oxazol-2-one Electrochemical reduction of the previous brominated oxazol-2-one (216 mg, 0.45 mmol) was performed according to the reported method[7] to give, after purification by flash chromatography (eluent, cyclohexane–AcOEt, 5 : 5), 4-(3-*N*-*tert*-butyloxycarbonylindolyl)oxazol-2-one (134 mg, 0.335 mmol, 75% yield) as a white solid.

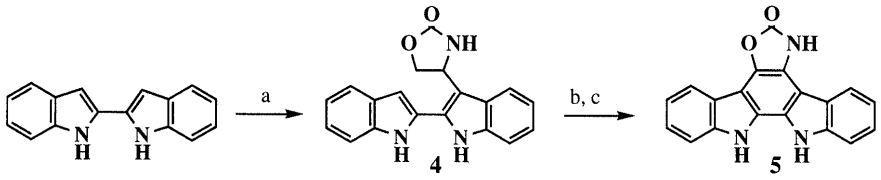
4-(3-Indolyl)oxazol-2-one (2) The previous debrominated oxazolone (23 mg, 0.077 mmol) was heated at 150 °C under argon with no solvent for 30 min to give, after purification by flash chromatography (eluent, cyclohexane–AcOEt, 5 : 5), **2** (14.6 mg, 0.073 mmol, 95% yield) as a yellowish solid. Spectroscopic data for these compounds were previously described.[6]

4-(2,2′-Bis-indol-3-yl)oxazolidin-2-one (4) 2,2′-Biindole (100 mg, 0.43 mmol) and *N*-Boc oxazol-2-one (79 mg, 0.43 mmol) were refluxed in toluene (20 ml) for 48 h. After removal of the solvent, purification of the residue by flash chromatography (eluent, cyclohexane–AcOEt, 5 : 5) yielded **4** (55 mg, 0.17 mmol, 40% yield) as a white solid.

4-(2,2′-Bis-*N*-*tert*-butyloxycarbonylindol-3-yl)-*N*-*tert*-butyloxycarbonyloxazolidin-2-one Compound **4** (150 mg, 0.473 mmol) was dissolved in CH_2Cl_2 (2.5 ml), then Et_3N (0.2 ml), 4-dimethylaminopyridine (DMAP) (173 mg, 1.42 mmol) and $(Boc)_2O$ (618 mg, 2.83 mmol) were added. The mixture was stirred at room temperature for 19 h. After removal of the solvent, the residue was purified by flash chromatography (eluent, cyclohexane–AcOEt, 5 : 5) to give 4-(2,2′-bis-*N*-*tert*-butyloxycarbonylindol-3-yl)-*N*-*tert*-butyloxycarbonyloxazolidin-2-one (291 mg, 0.471 mmol, 100% yield) as an off-white solid. This was a mixture of 2 diastereomers in equal proportions as determined from the ^1H-NMR spectrum. mp 118—120 °C. IR (KBr): ν_{CO} 1730 and 1800 cm^{-1}. HRMS (EI) Calcd for $C_{34}H_{39}N_3O_8$: 617.2737. Found: 617.2797. ^1H-NMR (400 MHz, acetone-d_6) δ: 1.00—1.45 (27H, several s, CH_3 of Boc groups), 4.16 and 4.33 (1H, 2dd, J=5, 8 Hz), 4.60 and 4.75 (1H, 2t, J=8 Hz), 5.46 and 5.64 (1H, 2dd, J=5, 8 Hz), 6.77 and 7.07 (1H, 2s), 7.20—7.42 (4H, m), 7.50—7.68 (2H, m), 8.25—8.40 (2H, m). ^{13}C-NMR (100 MHz, acetone-d_6) δ: 26.8, 26.9, 27.0, 27.1, 27.2, 27.3 (CH_3 of Boc groups), 51.9, 52.7 (CH), 66.6, 67.3 (CH_2), 82.5, 82.7, 83.2, 83.4 (2C), 83.6 (C *quat.* of Boc), 111.8, 112.9, 115.6, 115.7, 115.8, 116.0, 118.7 (2C), 120.9, 121.0, 122.8, 122.9, 123.0, 123.3, 124.8, 125.0, 125.3, 125.4 (C *tert.* arom.), 118.6, 119.7, 125.7, 126.0, 128.8, 128.9 (2C), 129.0, 129.7, 129.8, 136.7, 136.8, 137.2, 137.4 (C *quat.* arom.), 149.4, 149.5, 149.6 (4C), 151.5, 151.7



a) -e, MeOH, 71%; b) indolyl-MgBr, BF_3-Et_2O, THF, -30°C, 68%; c) $(Boc)_2O$, DMAP, CH_2Cl_2, Et_3N, 94% ; d) NBS, AIBN, CCl_4, 58%; e) electrochemical reduction, 72%; f) 160°C, 30 min., 95%.



a) N-Boc-oxazol-2-one, reflux 48h, 40%; b) $(tBuO_2C)_2O$ 6 eq., DMAP, Et_3N, CH_2Cl_2, 98%; c) NBS 4 eq., AIBN, CCl_4, reflux 18h, 32%.

Chart 2

Table 1. Inhibition of PKC and PKA. Antiproliferative Activities against Murine Tumor Cell Lines Melanoma B16 and Leukemia P388, Antibacterial Activities against *B. cereus* (ATCC 14579) and Vasorelaxant Effect on PKC-Mediated Contraction of Rat Tracheal Smooth Muscle Induced by PDBu

Compound	PKC IC_{50} (μM)	PKA IC_{50} (μM)	B16 IC_{50} (μM)	P388 IC_{50} (μM)	*B. cereus* MIC (μg/ml)	Vasorelaxant effect IC_{50} (μM)
1	>100[a]	>100	>150	>10	>50	n.d.
2	>150[a]	>150	>150	>10	50	255
3	>150[a]	>150	>100	>10	>50	n.d.
4	>150[a]	81	41.8	>10	12.5	38
5	89[a]	24	7	3.5	3.1	180
6	>100[b]	n.d.	>150	>10	25	265
7	>150[a]	>150	>150	9	50	225
8	>100[b]	n.d.	46	3.5	3.1	250
MDL 27,032[12]	46.5	67				1[c]
Staurosporine[13,14]	0.003	0.008				0.012[d]
H7[13,14]	6	3				13[d]
Calphostin C[5,13]	0.05	>50				1[e]

a) PKC and PKA inhibition measured according to procedure a mentioned in the experimental protocols. *b*) PKC inhibition measured according to procedure b. *c*) Relaxation of dog artery strips contracted by phorbol 12,13-dibutyrate (PDBu). *d*) Relaxation of rat portal vein contracted by phorbol 12-myristate 13-acetate (PMA). *e*) Relaxation of rat aortic rings contracted by PDBu.

(C=O).

7*H*-Indolo[2,3-*a*]oxazolo[4,5-*c*]carbazol-6-one (5) A mixture of the previous compound (150 mg, 0.243 mmol), NBS (172 mg, 0.966 mmol), CCl_4 (10 ml) and a catalytic amount of AIBN was refluxed for 18 h. After removal of the solvent, the residue was purified by flash chromatography (eluent, cyclohexane–AcOEt, 5 : 5) to yield **5** (24 mg, 0.078 mmol, 32% yield) as an off-white solid. Spectroscopic data for **4** and **5** were previously described.[8]

Biological Tests Protein Kinase Inhibition: Procedure a: PKC and PKA phosphorylation assays were performed respectively on histones IIIs and IIa as previously described.[7] Procedure b: Expression and partial purification of PKCs, and measurements of activities were carried out as previously described.[15,16] Compounds were tested on PKC-α in two independent experiments.

Growth Inhibition Assay: P388 murine leukemia cells: P388 murine leukemia cells were incubated at 37 °C for 96 h in the presence of various concentrations of a drug and evaluated for viability by neutral red staining according to a published procedure.[17] The concentrations of drugs giving 50% growth inhibition (IC_{50}) were determined. B16 murine melanoma cells: B16 culture and cytotoxicity assay were carried out as previously described.[18] The antiproliferative activity was expressed as IC_{50}, the drug concentration giving a 50% cloning efficiency compared to untreated cells.

Antimicrobial Activities: The minimum inhibitory concentrations (*MIC*s) of **1**—**8** were determined on *B. cereus* ATCC 14579 in Mueller–Hilton broth, pH 7.4 (Difco), after 24 h incubation at 27 °C. The compounds diluted in dimethyl sulfoxide (DMSO) were added to 12 tubes; the concentration range was from 100 to 0.05 μg/ml.

Effect on PKC-Mediated Contraction of Rat Tracheal Smooth Muscle: Tissue preparation: Isolated tracheal rings were obtained from male Wistar rats, 10 to 15 weeks old, weighing 300 to 400 g, and were prepared as previously described.[19] Protocol: At the beginning of each experiment, a supramaximal bolus of acetylcholine (AcCh, 10^{-3} M final concentration in the bath) was administered to each ring to elicit a control contractile response. After washing out the AcCh, resting tension was reestablished, and the rings were then precontracted with 30 mM KCl[20] before addition of PDBu (3.10^{-7} M, a concentration close to the concentration producing half of the maximum PDBu-induced contraction determined in control

experiments). After the PDBu-induced contraction had reached its steady state (generally 35—40 min), increasing concentrations (1—500 μM) of one of the compounds were added cumulatively in 2 baths; the 2 others received the equivalent concentration of vehicle (DMSO) and served as paired temporal controls. A concentration increment of the compound was made once the maximal relaxant effect of the preceding concentration had been recorded (usually 20—25 min). It was verified that the PDBu-induced contractile tone in paired control rings remained constant throughout the experiment (*i.e.* 130—150 min). Data were expressed as IC_{50}, the concentration of the compound producing 50% of its maximal effect.

Topoisomerase Inhibition: Topoisomerases I and II were prepared from calf thymus. Topoisomerase I or II inhibition was evaluated by using the DNA cleavage assay carried out according to the procedure previously described.[18]

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References

1) Nishizuka Y., *Science*, **33**, 305—312 (1986).
2) Buchner K., *Eur. J. Biochem.* (Tokyo), **228**, 211—221 (1995).
3) Herbert J. M., *Biochem. Pharmacol.*, **45**, 527—537 (1993).
4) Dumont J. A., Jones W. D., Bitonti A. J., *Cancer Res.*, **52**, 1195—1200 (1992).
5) Shimamoto Y., Shimamoto H., Kwan C. Y., Daniel E. E., *Am. J. Physiol.*, **264**, H1300—H1306 (1993).
6) Rodrigues Pereira E., Spessel V., Prudhomme M., Martre A. M., Mousset G., *Tetrahedron Lett.*, **36**, 2479—2482 (1995).
7) Rodrigues Pereira E., Sancelme M., Towa J. J., Prudhomme M., Martre A. M., Mousset G., Rapp M., *J. Antibiot.*, **49**, 380—385 (1996).
8) Rodrigues Pereira E., Prudhomme M., *Tetrahedron Lett.*, **36**, 2477—2478 (1995).
9) Savineau J. P., Marthan R., Crevel H., *Br. J. Pharmacol.*, **104**, 639—644 (1991).
10) Rossetti M., Savineau J. P., Crevel H., Marthan R., *Am. J. Physiol.*, **268**, L966—L971 (1995).
11) Lee M. K., Severson D. L., *Am. J. Physiol.*, **267**, C659—C678 (1994).
12) Cheng H. C., Robinson P. J., Dage R. C., Jones W. D., *J. Cardiovasc. Pharmacol.*, **17**, 445—455 (1991).
13) Schramm C. M., Grunstein M. M., *Am. J. Physiol.*, **262**, L119—L139 (1992).
14) Silva A. M., Brum R. L., Calixto J. B., *Life Sci.*, **57**, 863—871 (1995).
15) Marte B. M., Meyer T., Stabel S., Gesche J. R., Jaken S., Fabbro D., Hynes N. E., *Cell Growth & Diff.*, **5**, 239—247 (1994).
16) McGlynn E., Liebetanz J., Reutener S., Wood J., Lydon N. B., Hofstetter H., Vanek M., Meyer T., Fabbro D., *J. Cell. Biochem.*, **49**, 239—250 (1992).
17) Riou J. F., Naudin A., Lavalle F., *Biochem. Biophys. Res. Commun.*, **187**, 164—170 (1992).
18) Rodrigues Pereira E., Belin L., Sancelme M., Prudhomme M., Ollier M., Rapp M., Sevère D., Riou J. F., Fabbro D., Meyer T., *J. Med. Chem.*, **39**, 4471—4477 (1996).
19) Ben Jebria A., Marthan R., Rossetti M, Savineau J. P., Ultman J. S., *Respir. Physiol.*, **93**, 111—123 (1993).
20) Ozaki H., Kwon S. C., Tajimi M., Karaki H., *Pflügers Archiv.*, **416**, 351—359 (1990).