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Short communication

Determination of prostaglandin E_2 by on-line solid-phase extraction–liquid chromatography with ultraviolet detection for microsomal prostaglandin E_2 synthase-1 inhibitor screening

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ABSTRACT

A rapid, robust and selective on-line solid-phase extraction–liquid chromatographic method with ultraviolet detection (on-line SPE-LC-UV) for microsomal prostaglandin E_2 synthase-1 (mPGES-1) inhibitor screening was developed and validated. Disrupted A549 cells were used as mPGES-1 source and the formation of prostaglandin E_2 (PGE_2) out of the substrate prostaglandin H_2 (PGH_2) was determined at 195 nm. Direct on-line sample clean up was achieved by automated column switch (C18 trap column) prior isocratic separation using a C18 analytical column. The on-line SPE-LC-UV method was accurate, precise and reproducible in the range of 71–1763 ng/ml for PGE_2 and met the generally accepted criteria for bioanalytical methods. The method was successfully applied to determine the IC_{50} value of the known mPGES-1 inhibitor NS-398.

1. Introduction

Prostaglandin E_2 (PGE_2) produced via cyclooxgenase-2 (COX-2) and microsomal prostaglandin E_2 synthase-1 (mPGES-1) plays an important role in the pathophysiology of inflammation, pain, fever and vascular regulation. mPGES-1 is a member of the membrane-associated proteins involved in eicosanoid and glutathione metabolism (MAPEG) superfamily [1–3]. The glutathione dependent isomerase converts the intermediate PGH_2 to PGE_2. PGE_2 exerts its action via four EP-receptor subtypes [4].

COX-2 inhibitors as a therapeutic concept for inflammation are known to increase cardiovascular adverse effects by disruption of the balance between prostacyclin and thromboxane A_2. Intriguingly, deletion of mPGES-1 in mice did not affect either thrombogenesis or blood pressure but retained the anti-inflammatory effects [5,6]. Taken together inhibition of mPGES-1 holds great potential in the treatment of inflammatory diseases.

As a detailed three-dimensional (3D) X-ray crystal structure of mPGES-1 is still missing, combined structure predicting, molecular docking, site-directed mutagenesis and enzymatic activity assays were employed to develop a 3D model of the substrate-binding domain [7–9]. Hence, until now rational design did not result in the discovery of novel mPGES-1 inhibitors. The sulfonamide NS-398 [10], fatty acid mimetica like 15-deoxy-$\Delta^{12,14}PGJ_2$ [11] and compounds derived from MK-886 [12] are literature noted inhibitors. However, these substances were found to have non-sufficient pharmacokinetic properties. Only the orally active phenanthrene imidazoles [13] show less strong protein binding and inhibition in cellular systems. Therefore the identification of new lead inhibitors is a reasonable research field and rapid, robust but selective inhibitor screening assays are necessary.

The most widely utilized methods for the detection and determination of PGE_2 in biological samples are radioimmunoassays (RIA) [14] and enzyme immunoassays (EIA) [15,16], respectively. These methods are sensitive and simple to do but show time consumption (EIA), cross-reactivity and variability in quantification on the other hand. HPLC methods offer greater flexibility and specificity but often need time consuming sample pretreatments of the biological matrices. Most of the published methods for PGE_2 determinations used off-line SPE combined with LC-UV [10,17] and ESI-MS/MS [18], respectively, or liquid–liquid extraction (LLE) with subsequent LC-MS/MS determination of PGE_2 [19]. Surprisingly a direct LC-UV determination of PGE_2 with no sample pretreatment is described [20]. On-line sample clean up is a time saving and mostly error reducing procedure. Recently an on-line sample preparation coupled with capillary LC–MS/MS for determination of prostaglandins in cell culture supernatants was published [21]. Additionally on-line-SPE-LC methods combined with UV-Flow Scintillation Analyzers (FSA) [22,23] and off-line RIA determination for prostanoids in biological matrices are known [24], respectively.

The starting point of the present study was an A549 cell line based enzyme assay combined with a non-validated PGE_2 determi-

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nation method using off-line SPE with LC-UV [10]. Here we describe a robust mPGES-1 inhibitor-screening assay with disrupted A549 cells combined with a rapid, selective and fully validated on-line SPE-LC-UV method. PGE_2 was determined in the range from 71–1763 ng/ml. This method meets the generally accepted criteria for bioanalytical methods, is capable for higher throughput and does not require any sample pretreatment. The method was successfully applied to determine the IC_{50} value of the known mPGES-1 inhibitor NS-398.

2. Experimental

2.1. Chemicals and reagents

Prostaglandin E_1 (PGE_1), PGE_2, PGH_2, NS-398 (N-[2-(cyclohexyloxy)-4-nitrophenyl]methanesulfonamide) were obtained from Cayman Europe (Tallinn, Estonia).

L-Glutathione, reduced (GSH), Trizma®, phosphate-buffered saline (PBS) tablets for the preparation of PBS (0.1 M, pH 7.4), lipopolysaccharides (LPS) from *Escherichia coli* 0111:B4, Protease Inhibitor Cocktail for the use with mammalian cell and tissue extracts and phorbol-12-myristate-13-acetate (TPA) were purchased from Sigma–Aldrich (St. Louis, Missouri). Acetonitrile (HPLC gradient grade), potassium dihydrogen phosphate (p.A.), dipotassium hydrogen phosphate (p.A.) and dimethylsulfoxide (DMSO) (p.A.) were acquired from VWR International (Darmstadt, Germany). Trifluoroacetic acid (TFA) was obtained from Acros Organics (Geel, Belgium) and phosphoric acid (85%) (p.A.) from J.T. Baker (Deventer, Netherlands). $Fe(II)Cl_2{\cdot}4H_2O$ (p.A.) was purchased from AppliChem (Darmstadt, Germany).

DMEM high glucose (4.5 g/l) with L-glutamin was acquired from PPA laboratories (Cölbe, Germany), Gibco™ penicillin–streptomycin (10,000 U/ml penicillin G and 10,000 μg/ml streptomycin sulfate), Gibco™ trypsin–EDTA 10× (0.5% trypsin, 5.3 mM EDTA-Na_4) was bought from invitrogen life technologies (Karlsruhe, Germany). Tumor cell line CCL185 (A549, human adenocarcinoma) was kindly provided by Prof. R.M. Mesters (University of Münster, Münster, Germany). Water was purified using a Bi 18 system from Heraeus (Hanau, Germany).

2.2. Cell culture

A549 is a nonsmall lung cancer human adenocarcinoma cell line. The morphology is epithelial-like and the cells grow as an adherent monolayer. A549 cells were cultured in DMEM supplemented with heat-inactivated foetal bovine serum (10%), penicillin (166.7 U/ml) and streptomycin (166.7 μg/ml) at 37 °C in an atmosphere of 5% CO_2. Approximately 1.2×10^6 cells (improved Neubauer counting chamber) in 18 ml media were seeded in 75 cm^2 flasks. After 7 days, confluence was reached and cells were washed with 5 ml PBS buffer twice, then detached using 3 ml 0.05% trypsin (Gibco™ trypsin–EDTA 10×: 1:10 diluted with PBS) at 37 °C in an atmosphere of 5% CO_2 for 15 min. Thereafter, 10 ml medium was added to quench the trypsin and 1 ml cell suspension was reseeded.

In order to stimulate the mPGES-1 expression the cells were incubated with LPS (100 ng/ml final concentration) or TPA (1 μM final concentration) at 37 °C in an atmosphere of 5% CO_2 for 24 h. For harvest, the cells were washed with 5 ml PBS buffer twice, then trypsinated using 3 ml 0.05% trypsin solution at 37 °C in an atmosphere of 5% CO_2 for 15 min. Subsequently, 10 ml medium was added and the cells were centrifuged at 500 × *g* at room temperature for 10 min. The pellet was resuspended twice with 5 ml PBS buffer and centrifuged each time. The final cell pellet was stored at −80 °C.

2.3. Preparation of A549 cell microsomes

The cell pellets were resuspended in 1.0 ml homogenization buffer consisting of potassium phosphate buffer (100 mM, pH 7.4), protease inhibitor cocktail and sucrose (0.25 M). The samples were pulse sonicated 3 × 20 s in a −15 °C isopropanol bath. The suspension was centrifuged at 10,000 × *g* for 15 min and at 100,000 × *g* for 60 min. The microsomal fraction was resuspended in 200 μl homogenization buffer and the protein concentration was determined by modified Bradford-Assay [25] applying bovine serum albumin (BSA) as standard.

2.4. Incubation procedures

In precooled eppendorf caps 8 μl PGH_2 (0.28 mM in aceton), 186 μl Tris-buffer (50 mM, pH 7.4 at 0 °C) containing reduced GSH (2.5 mM) and 2 μl DMSO were pipetted and stored at 0 °C. In case of inhibitor screening 2 μl of inhibitor DMSO solutions at different concentrations were added instead. After 2 min the reaction was initiated by the addition of 4 μl enzyme solution (3.5 mg/ml protein) and the mixture was processed at 0 °C for 5 min. The reaction was quenched by the addition of 100 μl stop solution (60 mM $Fe(II)Cl_2$ in 0.05 M HCl) and 100 μl internal standard (ISTD) solution (PGE_1, 20 μM in MeCN with 0.1% TFA). Two hundred twenty five microliters of this solution was injected onto the HPLC column. Reference incubations were performed without enzyme solution in the same way.

2.5. On-line SPE-LC-UV-analysis

A schematic presentation of the on-line SPE-LC-UV system 1 with a 10-port 2-position valve from Rheodyne (Waters, EV700-102-WA) is given in Fig. 1. A Knauer HPLC Pump 64 (Berlin, Germany, pump 1) was coupled to a Waters autosampler model 717 plus (Milford, USA) and connected to a Kromasil 100-5-C18 (40 mm × 4 mm) trap column (CS-Chromatographie, Germany). A Waters 515 gradient pump system (Milford, USA, pump 2) was connected to a Kromasil 100-5-C18 (250 mm × 3 mm) analytical column, which was protected by a C18 (4 mm × 3 mm) SecurityGuard™ Cartidge (Phenomenex, Germany) and temperature controlled at 30 °C by a jetstream plus column oven (Waters, Milford). A Waters UV–vis-detector model 2487 was used for detection at 195 nm and system control was performed by Millennium^{32} software. The temperature of the autosampler was kept at 10 °C. The mobile phase for the first dimension was MeCN-Aqua bidest.-TFA (8:92:0.1, v/v/v) and 225 μl of each sample was stacked at a flow of 0.5 ml/min on the trap column. After 5 min the 10-port 2-position valve was switched to position 2. An isocratic separation was conducted with MeCN-Aqua bidest.-H_3PO_4 (35:65:0.025, v/v/v) (A) and MeCN-Aqua bidest.-H_3PO_4 (90:10:0.025, v/v/v) (B) with a flow rate of 0.6 ml/min. The initial composition of 100% A was retained after injection for 19 min. A linear washing gradient with 0% A was programmed over 3 min. After valve switch to position 1 at 22 min reequilibration was performed at 100% A for 6 min.

Alternatively, the SPE-LC-UV system 2 (Fig. 1) can be employed for chromatographic determination of PGE_2. As two trap columns are used for sample clean-up, the analysis time can be reduced significantly. The solvents for system 2 and flow rates were kept the same. In detail, before injection the 10-port 2-position valve was switched on position 1. 225 μl of an injected sample probe was stacked on trap column 2 with MeCN-Aqua bidest.-TFA (8:92:0.1, v/v/v). At the same time the isocratic separation of a probe stacked on trap column 1 was conducted with 100% of solvent A for 6 min. Then a linear washing gradient with 0% A was programmed over 3 min. After 13 min the 10-port 2-position valve was switched to

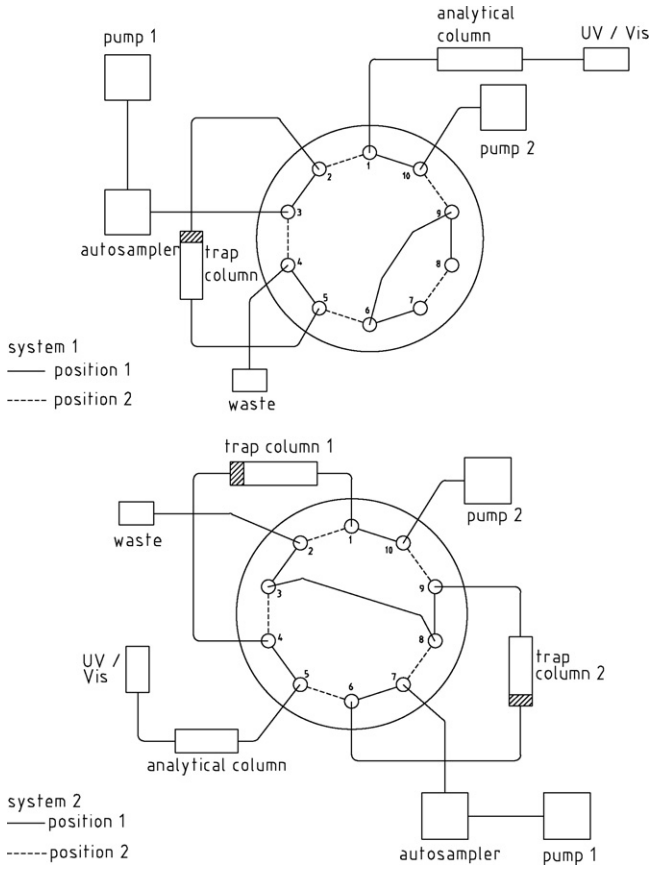

Fig. 1. Schematic drawing of separation systems 1 and 2.

position 2 and the isocratic separation of the stacked probe on trap column 2 was started. Simultaneously trap column 1 was reequilibrated for 6 min with MeCN-Aqua bidest.-TFA (8:92:0.1, v/v/v). After 19 min a new injection was started with the 10-port 2-position valve at position 2.

2.6. Method validation

Linearity was proven by PGE_2 spiked matrix samples at nine concentration levels in the range of 71–1763 ng/ml. Within a day, six replicates ($n = 6$) of spiked matrix samples at three concentration levels (176, 588 and 1322 ng/ml) were analyzed for intraday precision and accuracy. Interday-precision was investigated with independent matrix samples at 5 consecutive days ($n = 5$) at three concentration levels (176, 588 and 1322 ng/ml).

Recovery was calculated for three different concentration levels (176, 588 and 1322 ng/ml) of spiked matrix samples against standard solutions. Latter were injected directly via a sample loop on the analytical column. The limit of detection (LOD) and limit of quantification (LOQ) were determined based on a signal-to-noise of 3 and 10, respectively. Blank and zero samples were investigated.

Stability investigations (days 0, 12, 17 and 26) were made at −20 °C at two concentration levels (441 and 1763 ng/ml). Stability at 4 °C was determined for 24 h.

The variability of the enzyme assay was investigated by replicate determinations of incubations with the same A549 cell line preparation as well as by the exploration of the IC_{50} value of NS-398 with different A549 cell line preparations. Kinetic investigations were performed.

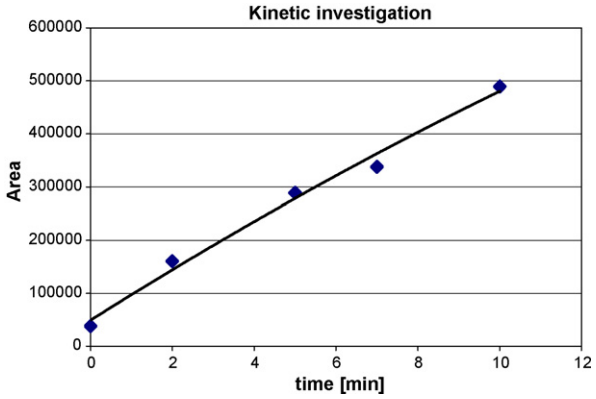

Fig. 2. Reaction progress curve of the mPGES-1 catalyzed isomerisation of PGH_2 to PGE_2 in Tris-buffer (50 mM, pH 7.4 at 0 °C) in presence of GSH (2.5 mM).

3. Discussion and results

3.1. Assay and sample pretreatment

Disrupted cells as enzyme source and incubation solution are often incompatible with direct LC-UV investigation at 195 nm. Thus, a sample treatment is necessary prior chromatographic analysis to remove interfering components. Most of the mPGES-1 functional assays with chromatographic determination use off-line SPE for sample purification as a time consuming, expensive and quite susceptible step. Therefore, an on-line sample pretreatment was pursued, minimizing sample loss and time consuming operations at high sensitivity. The on-line backflush RP trap column removed both salts and proteins and increased sensitivity by stacking effects. To prevent the trap column from damage the enzyme assay constitution had to be modified significantly. In literature described assays often showed precipitations, which were not removable by centrifugation [10,26]. Different buffers, acids and auxiliary salts were investigated and precipitation could be avoided. More than a hundred injections were done without any significant pressure enhancement. The large injection volume of 225 μl did not decrease selectivity, showed symmetric peaks and can be even amplified.

An incubation time of 5 min at 0 °C was derived from kinetic investigations (Fig. 2). It was considerably longer than the short incubation times of 60 s often applied in the literature. Therefore, inaccuracies based on incubation time variability were insignificantly. Precooled buffer, tips and eppendorf caps were obligate for reproducible results. PGH_2 has to be stored on dry ice during the experiments.

The mPGES-1 expression in A549 cells was stimulated by a supplement of TPA and LPS, respectively. Latter was about two times more effective than TPA.

3.2. Validation

Blank and zero sample chromatograms did not show any interference with the analyte PGE_2 and ISTD PGE_1 (data not shown). Both peaks were base line separated in the incubation chromatogram (Fig. 3). The calibration curve showed a non-weighted linear regression with a coefficient of determination (R^2) of 0.999. No calibration standards deviated more than ±8% from the nominal concentration.

Intraday-precision, accuracy, interday-precision and recovery met the accepted criterias for bioanalytical methods (Table 1). Also the results without ISTD are in accordance with the terms (Table 2). The LOQ and LOD were 57 and 17 ng/ml, respectively. Stability investigations at −20 °C revealed that the probe solution was not

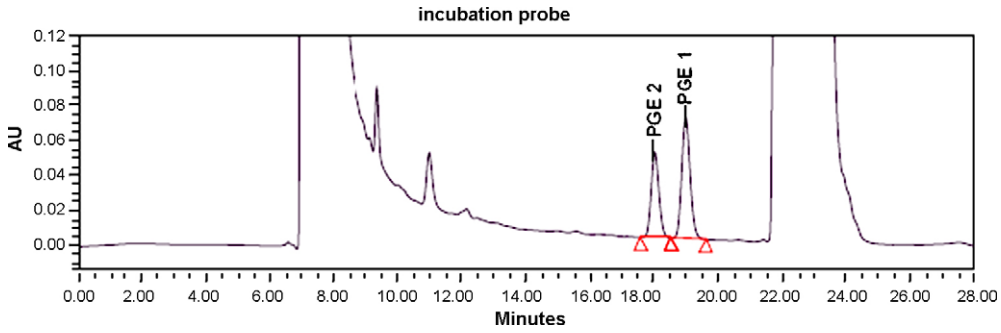

Fig. 3. Sample chromatogram of an incubation probe showing PGE_2 with ISTD (PGE_1). Eight microliters PGH_2 (0.28 mM in aceton), 186 μl Tris-buffer (50 mM, pH 7.4 at 0 °C) containing reduced GSH (2.5 mM) and 2 μl DMSO (without inhibitor) were mixed and after 2 min the reaction was initiated by the addition of 4 μl solution of LPS-stimulated A549 cell microsomes. The reaction was quenched after 5 min by the addition of 100 μl stop solution (60 mM $Fe(II)Cl_2$ in 0.05 M HCl) and 100 μl internal standard (ISTD) solution (PGE_1, 20 μM in MeCN with 0.1% TFA). The sample was injected directly on a C18 trap column for sample clean up with MeCN-Aqua bidest.-TFA (8:92:0.1, v/v/v) as mobile phase. Subsequent isocratic chromatographic separation was performed on a Kromasil 100-5-C18 (250 mm × 3 mm) with MeCN-Aqua bidest.-H_3PO_4 (35:65:0.025, v/v/v) as mobile phase at a detection wavelength of 195 nm. A linear washing gradient with MeCN-Aqua bidest.-H_3PO_4 (90:10:0.025, v/v/v) followed up. The position of the switching valve was changed at 5 and 22 min.

Table 1
Validation data calculated on the base of an internal standard.

Concentration level (ng/ml)	Precision ($n = 6$) RSD (%)	Accuracy RE (%)	Interday-precision ($n = 5$) RSD (%)	Rel. recovery ($n = 6$) (%) ± SD
176	3.8	−2.4	5.1	96.7 ± 2.8
588	0.6	2.0	4.1	91.1 ± 1.7
1322	1.2	1.1	2.6	94.4 ± 2.0

Table 2
Validation data calculated without an internal standard.

Concentration level (ng/ml)	Precision ($n = 6$) RSD (%)	Accuracy RE (%)	Interday-precision ($n = 5$) RSD (%)	Abs. recovery ($n = 6$) (%) ± SD
176	3.9	−2.7	4.1	105.0 ± 2.8
588	1.1	1.6	2.4	99.0 ± 1.7
1322	0.8	−0.1	1.4	103.2 ± 2.9

affected by freeze–thaw cycles. Stability probe solutions with a concentration of 441 and 1763 ng/ml PGE_2 possessed 1.1 and 0.3% RSD for the period of investigation, respectively. Furthermore no significant loss of PGE_2 could be found at 4 °C. The dissolved $Fe(II)Cl_2$ might function as a stabilizer for the probe solution.

Investigations of the variability of the enzyme assay showed a good reproducibility for the PGE_2 formation in incubation probes of the same cell line preparation (Table 3) The overall precision for the formation and determination of PGE_2 varies between 5.1 and 9.7% depending on the specific concentration level. As the intraday-precision for the PGE_2 determination of the chromatographic method itself varies between 0.6 and 3.8% (Table 1) the robustness of the enzyme assay within the same cell line preparation can be assumed. The literature describes NS-398 as mPGES-1 inhibitor with an IC_{50} value of 20 μM [10]. In our inquiries NS-398 exhibited an IC_{50} value between 16 ± 1.6–35 ± 4.9 μM ($n = 3$) (Table 4). The acceptable variation can be explained by the independent cell line preparations. As noted before, the precision within the same preparations is high; the enzyme assay can be used for reliable inhibitor screening.

Table 3
Investigation of comparability of separation system 1 and system 2. Each 225 μl of double sized incubations probes (800 μl total volume) were investigated on system 1 and system 2, respectively. A low level PGE_2 content was achieved by addition of inhibitor NS-398 (100 μM final concentration).

	System 1 Precision ($n = 5$) RSD (%)	System 2 Precision ($n = 5$) RSD (%)	Rel. accuracy ratio: mean system 1/mean system 2 (%)
Incubation probe without inhibitor	5.1	6.7	103.5
Incubation probe with NS-398	9.7	9.9	102.6

Table 4
A549 cell line microsomes were independently prepared according to described procedures. IC_{50} determinations were carried out at 3 different days each.

Different LPS-stimulated A549 cell line preparations	IC_{50} value (μM ± SD)
Preparation 1 ($n = 3$)	16 ± 1.6
Preparation 2 ($n = 3$)	35 ± 4.9
Preparation 3 ($n = 3$)	30 ± 3.3

4. Conclusions

A fast and validated mPGES-1 inhibitor screening method using on-line SPE for sample pretreatment and RP-LC-UV for PGE_2 determination with disrupted A549 cells as enzyme source was developed. Both, LPS or TPA pretreated A549 cells can be used. The incubation protocol allows direct probe measurement with no necessary off-line sample pretreatment. Because of the fast and simple incubation procedure, an ISTD even can be omitted. The reliability of chromatographic method as well as the enzyme assay is shown by the validation values, assay investigations and IC_{50} determination of NS-398.

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References

[1] B. Samuelsson, R. Morgenstern, P.J. Jakobsson, Pharmacol. Rev. 59 (2007) 207.
[2] S. Thoren, R. Weinander, S. Saha, C. Jegerschold, P.L. Pettersson, B. Samuelsson, H. Hebert, M. Hamberg, R. Morgenstern, P.J. Jakobsson, J. Biol. Chem. 278 (2003) 22199.
[3] M. Ouellet, J.P. Falgueyret, P.H. Ear, A. Pen, J.A. Mancini, D. Riendeau, M.D. Percival, Protein Exp. Purif. 26 (2002) 489.
[4] S. Narumiya, Y. Sugimoto, F. Ushikubi, Physiol. Rev. 79 (1999) 1193.
[5] T. Grosser, S. Fries, G.A. FitzGerald, J. Clin. Invest. 116 (2006) 4.
[6] Y. Cheng, M. Wang, Y. Yu, J. Lawson, C.D. Funk, G.A. Fitzgerald, J. Clin. Invest. 116 (2006) 1391.
[7] M.D. AbdulHameed, A. Hamza, J. Liu, X. Huang, C.G. Zhan, J. Chem. Inf. Model 48 (2008) 179.
[8] X. Huang, W. Yan, D. Gao, M. Tong, H.H. Tai, C.G. Zhan, Bioorg. Med. Chem. 14 (2006) 3553.
[9] A.A. San Juan, S.J. Cho, J. Mol. Model 13 (2007) 601.
[10] S. Thoren, P.J. Jakobsson, Eur. J. Biochem. 267 (2000) 6428.
[11] O. Quraishi, J.A. Mancini, D. Riendeau, Biochem. Pharmacol. 63 (2002) 1183.
[12] D. Riendeau, R. Aspiotis, D. Ethier, Y. Gareau, E.L. Grimm, J. Guay, S. Guiral, H. Juteau, J.A. Mancini, N. Methot, J. Rubin, R.W. Friesen, Bioorg. Med. Chem. Lett. 15 (2005) 3352.
[13] B. Cote, L. Boulet, C. Brideau, D. Claveau, D. Ethier, R. Frenette, M. Gagnon, A. Giroux, J. Guay, S. Guiral, J. Mancini, E. Martins, F. Masse, N. Methot, D. Riendeau, J. Rubin, D. Xu, H. Yu, Y. Ducharme, R.W. Friesen, Bioorg. Med. Chem. Lett. 17 (2007) 6816.
[14] M.C. Catley, J.E. Chivers, L.M. Cambridge, N. Holden, D.M. Slater, K.J. Staples, M.W. Bergmann, P. Loser, P.J. Barnes, R. Newton, FEBS Lett. 547 (2003) 75.
[15] Y. Ikeda-Matsuo, Y. Ikegaya, N. Matsuki, S. Uematsu, S. Akira, Y. Sasaki, J. Neurochem. 94 (2005) 1546.
[16] F. Masse, S. Guiral, L.J. Fortin, E. Cauchon, D. Ethier, J. Guay, C. Brideau, J. Biomol. Screen. 10 (2005) 599.
[17] G. Dannhardt, M. Lehr, J. Pharm. Pharmacol. 44 (1992) 419.
[18] A. Margalit, K.L. Duffin, P.C. Isakson, Anal. Biochem. 235 (1996) 73.
[19] R. Schmidt, O. Coste, G. Geisslinger, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 826 (2005) 188.
[20] K. Olofsson, E. Suna, B. Pelcman, V. Ozola, M. Katkevics, I. Kalvins, WIPO (2005) WO 2005/123673.
[21] S. Rinne, C. Ramstad Kleiveland, M. Kassem, T. Lea, E. Lundanes, T. Greibrokk, J. Sep. Sci. 30 (2007) 1860.
[22] W.S. Powell, Anal. Biochem. 164 (1987) 117.
[23] W. Schafer, C. Strandberg, T. Gaillard, H.P. Zahradnik, Eicosanoids 1 (1988) 101.
[24] G. Hotter, I. Ramis, G. Bioque, C. Sarmiento, J.M. Fernandez, J. Rosello-Catafau, E. Gelpi, Chromatographia 36 (1993) 33.
[25] C.M. Stoscheck, Anal. Biochem. 184 (1990) 111.
[26] F.J. Sweeney, T.S. Wachtmann, J.D. Eskra, K.A. Verdries, R.H. Lambalot, T.J. Carty, J.R. Perez, L.P. Audoly, Mol. Cell. Endocrinol. 205 (2003) 151.