Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 19 January 2010, Revised 6 May 2010, Accepted 3 June 2010 Published online 16 September 2010 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.1812

# Synthesis of I-131 labelled 4-iodophenylacetic acid

Zoltan Szucs,[a] Mike Sathekge,[b] Biljana Marjanovic-Painter,[a] Judith Wagener,[a] Thato Sello,[a] Carl Wagener,[a] and Jan Rijn Zeevaart[c]*

**Phenylacetate has been reported to have a potent anti-proliferative and anti-differentiating effect in haematological malignancies and in solid tumours at non-toxic concentrations. This study is a preliminary investigation into the potential of 4-iodophenylacetic acid radiolabelled by $^{131}$I as a radiopharmaceutical equivalent. The radiolabelling by isotope exchange gave a radiochemical yield of 53 ± 6%, and a radiochemical purity of 97.8 ± 1.2% as qualified by HPLC. The product contained 4% ester by-product and is suitable for studies in animals.**

**Keywords:** biotracer; phenylacetic acid metabolism; prostate carcinoma; $^{131}$I

## Introduction

Phenylacetic acid (Figure 1) and its deprotonated form at physiological pH (phenylacetate) is an aromatic molecule and is present in low concentration in human serum as a product of phenylalanine metabolism. It is conjugated with glutamine in the liver by phenyl-acetyl coenzyme A to form phenyl-acetyl-glutamine, which is excreted in the urine.[1]

Phenylacetate has been reported to have a potent anti-proliferative and anti-differentiating effect in haematological malignancies and in solid tumours at non-toxic concentrations. It has been used safely to treat children with inborn errors of urea synthesis and also in patients with hyperammonaemia.[1–3]

Furthermore, it has been shown to inhibit tumor growth while sparing normal tissue and to induce phenotypic reversion and differentiation of malignant cells with some of the antiproliferative effect of phenyl-acetate in prostate carcinoma,[4] melanoma,[5] rhabdomyosarcoma,[6] breast cancer,[7] pancreatic adenocarcinoma,[8,9] ovarian carcinoma,[9] B-chronic lymphocytic leukemia [10] and medullablastoma[11] cell lines.

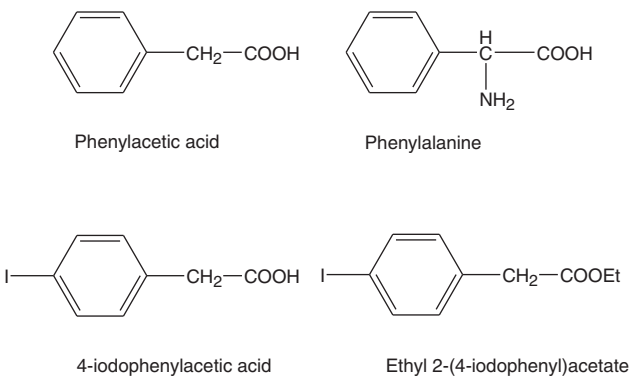


**Figure 1.** The structures of 4-iodophenylacetic acid, phenylacetic acid, phenylalanine and ethyl 2-(4-iodophenyl)acetate.

In patients with hormone-refractory prostate cancer and high-grade glioma, Phase I trials of phenylacetate showed minimal toxicity, and a partial response was observed at a serum concentration of $2–10 \times 10^{-3}$ M.[12,13]

Increasing evidence points to phenylacetate having multiple effects on gene expression and on regulatory proteins responsible for its antineoplastic effects.[14] Thus, phenylacetate has been shown to down-regulate Bcl-2 and up-regulate bax/p21 apoptosis-related genes in ovarian carcinoma cells and up-regulate p21 in K-ras mutant MCF-7 ras breast cancer cell lines.[9,15] Phenylacetate also activates the human peroxisome proliferator-activated receptors (PPAR) which belong to the superfamily of nuclear steroid receptors such as retinoids, vitamin D, and thyroid hormone receptors – all important regulators of cell growth and differentiation.[16,17] In all these studies phenylacetate exerts its effects at clinically acceptable serum concentration levels ($2.5–10 \times 10^{-3}$ M).[14]

In recent years, the quest for an agent that can be used for both the diagnosis and therapy of a disease is being addressed world-wide. Most notable is the application of the synergism between the chemistry of the isotopes of technetium ($^{99m}$Tc) and rhenium ($^{186}$Re and $^{188}$Re), in which the former isotope is used for diagnosis and the latter for therapy of the disease.[18,19] $^{131}$I-labelled compounds fulfil the criteria of being both a diagnostic and/or therapeutic agent. Although the $\gamma$-energy of $^{131}$I is not ideal ($t_{1/2}$ = 8.02 d; $\gamma$-energy = 364 keV; β-energy = 0.6 + 0.8 MeV,[19] it is adequate for use in diagnostic studies using a high-energy

[a]*Necsa, Radiochemistry, Pretoria, South Africa*

[b]*University of Pretoria/Pretoria Academic Hospital Pretoria, Pretoria, South Africa*

[c]*CARST, North West University, Mafikeng Campus, PO Box 582, Pretoria 0001, Mmabatho, South Africa*

**Correspondence to: Jan Rijn Zeevaart, CARST, PO Box 582, Pretoria 0001, South Africa.*
*E-mail: janrijn.zeevaart@necsa.co.za*

collimator. The β-energy is sufficient to deliver an internal therapeutic radiation dose to the target organ or carcinoma. A further advantage of $^{131}$I is that the biodistribution of the labelled phenylacetic acid and radiation dose of a therapeutic dose to the target organ or carcinoma can be evaluated by imaging the emitted γ-radiation. These diagnostic and therapeutic effects are obtained by using extremely small amounts of the agent, as the effects observed are determined by the radioactivity of the compound and not by the mass of material used. Radiolabelling with iodine also affords the options of $^{123}$I (SPECT imaging with $t_{1/2} = 13.3$ h; γ-energy = 159 keV), $^{124}$I (PET imaging with $t_{1/2} = 4.2$ d; $\beta^+$-energy = 1.5 MeV) and therapeutic $^{125}$I ($t_{1/2} = 59.4$ d; Auger electrons).

To our knowledge radioiodinated 4-iodophenylacetic acid has never been synthesized before and the biodistribution of $^{131}$I labelled phenylacetic acid has never been evaluated in experimental animals or humans. This novel synthesis attempt was based on the general reaction parameters for Cu(I) assisted radiohalogenation as proposed in the literature.[20] Because of the closeness of the structure of 4-iodophenylacetic to that of phenylacetic acid, as well as the reported biological activity of phenylacetic acid, it is hypothesized that useful information of the biodistribution of the $^{131}$I labelled agent can be obtained from experimental animals, especially with regards to its uptake in neoplastic versus normal tissue. The results presented here describe the synthesis of 4-[$^{131}$I] iodophenylacetic acid as a first step in assessing its potential as diagnostic imaging and/or therapeutic agent for the treatment of neoplastic conditions.

## Experimental

### General

All chemicals and solvents used were of AR or high performance liquid chromatography (HPLC) grade purity (Merck, Darmstad, Germany, Sigma-Aldrich). 4-Iodophenylacetic acid was purchased from Alfa Aesar L13345 with a certified purity of 97%. The $^{131}$I solution was supplied by NTP Radioisotopes Pty. Ltd, Pretoria, South Africa, in a 0.05 M sodium hydroxide solution without thiosulphate or buffer solution. The fission $^{131}$I had a radiochemical purity of >95%, a radionuclidic purity of 99.9% and a specific activity of 294 TBq $^{131}$I/mmol I (62 Ci/mg). The AG-MP-1M Biorad anion exchanger resin was treated with 1 M sodium hydroxide, washed with water and packed in the column one day before the synthesis. The solution of ascorbic acid was freshly prepared just before the synthesis.

Two HPLC instruments (a Varian and an Agilent coupled to an MS detector) were used for separation and identification of components. The Varian HPLC system consisted of a Zorbax Eclipse XDB C-18 4.6 × 150 mm, 5 μm particle size column, Varian ProStar model 230 dual pumps, a synchronized Rheodine 7725i injector, an in-line Varian ProStar model 325 UV and VIS detector, and a single sodium iodide crystal flow radioactivity detector (Raytest Gabi.). Varian HPLC chromatograms were recorded by a Varian Star 800 channel control/interface module connected to a computer running Galaxie Chromatographic Data System software. The radioactive chromatograms were obtained using the Gina Star 4.07 version of software. The radioactivity was measured by a Capintec CRC-15 βETA Radioisotope Dose calibrator meter and by γ-spectrometry with the Genie-2000 3.1 Gamma Analyzer Software, Canberra.

The Agilent HPLC-MS instrument was equipped with a 1200 series quaternary and an isocratic pump, a single quad mass spectrometer, a 1200 series diode array and multiple wavelength detector and connected to gamma and beta detectors (Raytest, Germany). The column was the same as for the Varian instrument.

### Radiosynthesis of 4-[$^{131}$I] iodophenylacetic acid

About 0.3 mg (1 μmol) cold 4-iodophenylacetic acid was dissolved in 10 μl ethanol in a 1 ml vial. Freshly prepared ascorbic acid (0.5 mg, 3 μmol) in 20 μl water, 0.6 μmol of $CuCO_3$ catalyst and 2.1 mg (11 μmol) citric acid was dissolved in 20 μl water and transferred to the 1 ml vial. The reaction mixture was diluted with 50 μl water and 40 μl ethanol. The reaction contents were purged with argon gas, a micro-magnetic stirrer bar was introduced and 100 μl (>150 MBq) of the radioactive $^{131}$I was added. The reaction vial was hermetically closed and the isotope exchange reaction was carried out at 150°C for one hour with vigorous stirring. The reaction mixture was left to cool to room temperature and the reaction contents were transferred to the AG-MP-1M column. The reaction vial was washed with 2 × 100 μl ethanol, which was also pushed through the AG-MP-1M column. The column was washed additionally with 1 ml of a 1:1 mixture of saline and ethanol. The eluates were collected and gently evaporated under argon gas flow down to 1 ml. The residue was diluted by saline to 3 ml and filtered through a 0.22 μm Millex GV (Millipore) sterile filter into a sterile, pyrogen-free bottle with a silicon-based septum. A polyethylene film was placed between the septum and the solution. Quality control was carried out by the Varian HPLC equipped with the Agilent Eclipse XDB-C18 column. Separation was carried out by a gradient method, using ethanol and 0.01 M ortho-phosphoric acid in water as solvents. The ratio of the solvents was 70/30% (ethanol/water) for the first 4 min, then it was decreased to 40/60% over a 5 min period, kept at 40/60% for another 5 min, after which it was increased back to 70/30% over a 1 min period and kept at this level until the end of the chromatographic elution. The flow rate was 1.0 ml/min. About 20 μl of the final product solution was injected. The analysis was done at room temperature. The total time of analysis was 25 min. The radioactivity of the sample was detected by a 30 μl flow-cell and the chemical composition by the UV-detector at 210 nm.

### Radiosynthesis of ethyl 2-(4-[$^{131}$I] iodophenyl)acetate

The possibility exists that the ester of 4-iodophenylacetic acid will form as a by-product during the above synthesis. To establish the radiochemical purity of the 4-[$^{131}$I] iodophenylacetic acid by HPLC analysis, the retention time of the ester (ethyl 2-(4-[$^{131}$I] iodophenylacetate) had to be determined. Therefore, ethyl 2-(4-[$^{131}$] iodophenyl)acetate was synthesized as follows.

About 100 μl of $^{131}$I labelled 4-iodophenylacetic acid solution was introduced into a solution of ethanol (400 μl) and concentrated sulphuric acid (10 μl) in a reaction vial. The reaction mixture was heated in a pre-heated (100°C) lead pot for 1.5 h. The reaction mixture was left to cool to room temperature and injected in the HPLC-MS for separation and identification. The same HPLC separation method and conditions as described in the earlier section were used.

## Results

### Radiosynthesis of 4-[$^{131}$I] iodophenylacetic acid

During the development phase of the labelling process, it was found that an inert atmosphere is key to the success of the synthesis. When oxygen is allowed in the reaction vial, the yield drops to less than 20%. Furthermore, the temperature should be kept between 145 and 155°C. If the temperature is lower, the yield drops, while a too high temperature causes decomposition of the 4-iodophenylacetic acid. A freshly prepared solution of ascorbic acid is also essential. When the ascorbic acid was prepared the day before, a lower yield was recorded. The reaction time could be reduced from the 12 h initially used to 1 h, without changing the yield. The ratio of substrate to catalyst was investigated, but no detrimental effect was found when a different ratio is used than reported herein. The precursor 4-iodophenylacetic acid was added to the reaction mixture in solid form taken directly from the

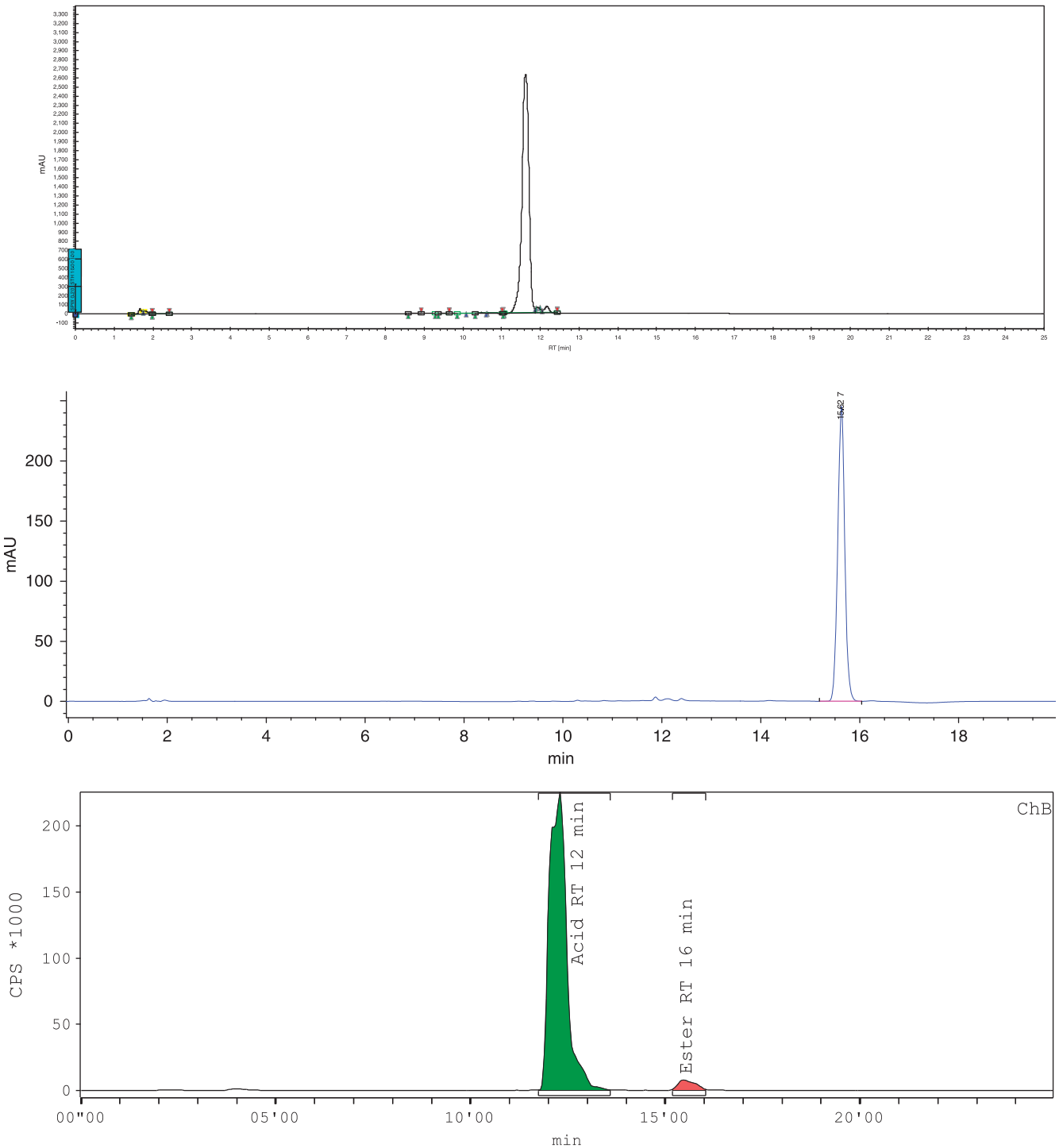


**Figure 2.** UV chromatograms of 4-iodophenylacetic acid (retention time ~12 min, top), ethyl 2-(4-iodophenyl)acetate (retention time ~16 min, middle) and radiochromatogram of radiolabelled 4-iodophenylacetic acid and ethyl 2-(4-Iodophenyl)acetate.

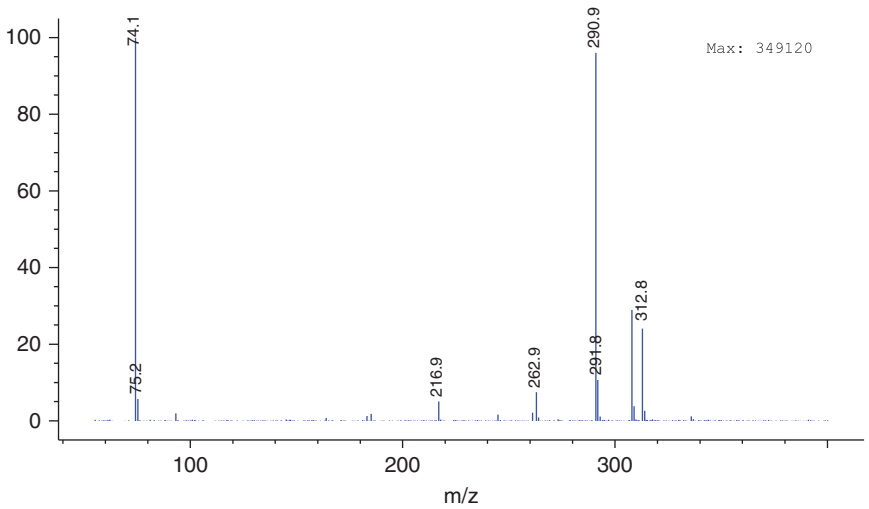


**Figure 3.** Mass spectrum of ethyl 2-(4-iodophenyl)acetate.

refrigerator. The ratio of the reaction constituents was chosen according to Gysemans *et al.*[20] The reaction mixture was pushed through an AG-MP-1M anion exchanger column to separate the un-reacted anionic $^{131}$I. Six labelling procedures were performed with an average radiochemical purity of 97.8 ± 1.2% and yield of 53 ± 6%. The radiochemical purity of the run in which the HPLC-MS analysis was used (using only 150 MBq of $^{131}$I) was 96% (Figure 2). The results presented herein further refer to this particular run, which had a lower yield and radiochemical purity, probably due to the lower activity of $^{131}$I used. The activity of the product was 64 MBq. The radiochemical yield was 43% (decay corrected). The calculated specific activity (from HPLC analysis) was 63.7 MBq/μmol (6.6 Ci/g).

### Radiosynthesis of ethyl 2-(4-[$^{131}$I] iodophenyl) acetate

After the synthesis step, 20 μl of product solution was injected into the HPLC-MS. The separation conditions as described in the previous section were used, except for orthophosphoric acid, which was exchanged with the same concentration of formic acid to avoid undue suppression of the MS signal. The retention time of ethyl 2-(4-[$^{131}$I] iodophenyl) acetate was approximately 16 min, compared to the retention time of the 4-[$^{131}$I] iodophenylacetic acid of approximately 12 min (Figure 2). (Please note the different total analysis times for the three chromatograms.) The positive ES mass spectrum of the cold compounds shows ions at $[M+H]^+$ *m/z* 290.1, $[M+Na]^+$ *m/z* 312.8, $[M+H\text{-}COOCH_2CH_3]^+$ *m/z* 216.9 and $[COOCH_2CH_3]^+$ *m/z* 74.1. The MS spectrum (Figure 3) confirmed that the product was the ester.

## Discussion

The labelling via isotopic exchange did yield a low specific activity of the tracer, which meant that a substantial amount of 4-iodophenylacetic acid will be injected. This would amount to 30 μg per rat, or 75 μg/kg, or 1.1 μg/ml blood (mass of rats ~400 g, injection of 7.4 MBq and assuming that 7% of body weight is blood). The $LD_{50}$ value for phenylacetic acid is 1600 mg/kg for intraperitoneal injection in rats,[21] which is orders of magnitude higher than the amount that will be injected and one can therefore assume that the 0,075 mg/kg injected 4-iodophenylacetic acid will not adversely interfere with the biological processes in the rat. The amount of phenylacetic acid in normal tissue is 16.8 μg/ml,[22] which is also an order of magnitude higher than the amount of 4-iodophenylacetic acid that will be injected. This implies that the biodistribution of the tracer will not be influenced by its metabolic product, due to equilibrium with phenylacetic acid already present in the body. For therapeutic doses, the preparation method will have to be adapted to make use of a direct labelling method, such as that described by Machulla *et al.*,[23] to increase the specific activity. For the preparations used for animal studies, the percentage ethanol (that is higher than 10% in the current labelling procedure) will be lowered by reducing the volume further during the evaporation step using Ar. This has been demonstrated in one of the runs, where the volume was reduced to 100 μl without causing a lower radiochemical purity or yield.

## Conclusions

4-[$^{131}$I]-Iodophenylacetic acid was successfully prepared, although 4% of the ester by-product was present. These data indicate that 4-[$^{131}$I] iodophenylacetic acid shows promise for *in vivo* investigations and therapy.

## Acknowledgements

The authors thank Prof. B. J. Meyer for suggesting this study before his untimely death and dedicate this paper to his memory. Thanks to Dr Knoesen for the initial exploratory work. The authors thank Necsa for permission to publish this work.

## References

[1] S. W. Brusilow, M. Danney, L. J. Waber, M. Batshaw, B. Burton, L. Levitsky, K. Roth, C. McKeethren, J. Ward, *N. Encl. J. Med.* **1984**, *310*, 1630–1634.
[2] C. L. Mendenhall, S. Rouster, L. Marshall, *Am. J. Gastroenterol.* **1986**, *81*, 540–543.
[3] M. Del Rosario, S. L. Werlin, S. J. Lauer, *J. Clin. Gastroenterol.* **1997**, *25*, 682–684.
[4] D. Samid, S. Shack, C. E. Myers, *J. Clin. Invest.* **1993**, *91*, 2288–2295.

[5] L. Liu, S. Shack, W. Stetler-Stevenson, W. Hudgins, D. Samid, *J. Invest. Dermatol.* **1994**, *103*, 335–340.
[6] J. Cinatl Jr, J. Cinatl, P. Hernáiz Driever, H. Rabenau, M. Novak, R. Benda, H. O. Gümbel, B. Kornhuber, H. W. Doerr, *Cancer Lett.* **1994**, *78*, 41–48.
[7] L. Adam, M. Crepin, L. Israel, *Cancer Res.* **1997**, *57*, 1023–1029.
[8] L. E. Harrison, D. C. Wojciechowicz, M. F. Brennan, P. B. Paty, *Surgery* **1998**, *124*, 541–550.
[9] G. Ferrandina, B. Melichar, A. Loercher, C. F. Verschraegen, A. P. Kudelka, C. L. Edwards, G. Scambia, J. J. Kavanagh, J. L. Abbruzzese, R. S. Freedman, *Cancer Res.* **1997**, *57*, 4309–4315.
[10] C. D. R. T. Call, M. J. Stenson, T. E. Witzig, *Leuk. Lymphoma* **1994**, *14*, 145–149.
[11] G. Stockhammer, G. T. Manley, R. Johnson, M. Rosenblum, D. Samid, F. Lieberman, *J. Neurosurgery* **1995**, *83*, 672–681.
[12] A. Thibault, D. Samid, M. R. Cooper, W. D. Figg, A. C. Tomkins, N. Patronas, D. J. Headley, D. R. Kohler, D. J. Venzon, C. E. Myers, *Cancer* **1995**, *75*, 2932–2938.
[13] A. Thibault, M. Cooper, W. Figg, D. Venzon, *Cancer Res.* **1994**, *54*, 1690–1694.
[14] E. Kebebew, M. G. Wong, A. E. Siperstein, Q.-Y. Duh, O. H. Clark, *J. Clin. Endocrin. Metab.* **1999**, *84*, 2840–2847.
[15] M. Gorospe, S. Shack, K. Z. Guyton, D. Samid, N. J. Holbrook, *Cell Growth Differ.* **1996**, *7*, 1609–1615.
[16] I. Issemann, S. Green, *Nature* **1990**, *347*, 645–650.
[17] R. Apfel, D. M. Benbrook, E. Lernhardt, M. A. Ortiz, M. A. Pfahl, *Mol. Cell. Biol.* **1994**, *14*, 7015–7035.
[18] E. Deutsch, K. Libson, J.-L. Vanderheyden, A. Ketring, H. R. Maxon, *Nucl. Med. Biol.* **1986**, *13*, 465–477.
[19] Th. M. Behr, M. Gotthardt, W. Becker, M. Béhé, *Nuklearmedizin* **2002**, *2*, 71–79.
[20] M. Gysemans, J. Mertens, *J. Label. Compd. Radiopharm.* **1993**, *32*, 195.
[21] CHEMTOX® online 2005 Atrion International Inc.
[22] S. Hoda, K. Yamamoto, M. Sekizuka, Y. Oshima, K. Nagai, G. Hashimoto, H. Kaneko, T. Tomomasa, Y. Konno, R. Horiuchi, *Biol. Pharm. Bull.* **2002**, *25*, 1244–1246.
[23] H.-J. Machulla, M. Masrsmann, K. Dutschka, *Eur. J. Nucl. Med.* **1980**, *5*, 171–173.



  