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Authors
Tarzia, Giorgio
Antonietti, Francesca
Duranti, Andrea
et al.

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IDENTIFICATION OF A BIOACTIVE IMPURITY IN A COMMERCIAL SAMPLE OF 6-METHYL-2-p-TOLYLAMINOBENZO[d][1,3]OXAZIN-4-ONE (URB754)

Giorgio TARZIA(°)1, Francesca ANTONIETTI1, Andrea DURANTI1, Andrea TONTINI1, Marco MOR2, Silvia RIVARA2, Pietro TRALDI3, Giuseppe ASTARITA4, Alvin KING4, Jason R. CLAPPER4 and Daniele PIOMELLI4

1 Istituto di Chimica Farmaceutica e Tossicologica, Università degli Studi di Urbino “Carlo Bo”, Piazza del Rinascimento 6, 61029 Urbino, Italy
2 Dipartimento Farmaceutico, Università degli Studi di Parma, Viale G.P. Usberti 27/A, 43100 Parma, Italy
3 CNR, Istituto di Scienze e Tecnologie Molecolari, Corso Stati Uniti, 4, 35127 Padova, Italy
4 Department of Pharmacology and Center for Drug Discovery, University of California, Irvine, 92697-4625 USA

Summary - The compound URB754 was recently identified as a potent inhibitor of the endocannabinoid-deactivating enzyme monoacylglycerol lipase (MGL) by screening of a commercial chemical library. Based on HPLC/MS, NMR and EI/MS analyses, the present paper shows that the MGL-inhibitory activity attributed to URB754 is in fact due to a chemical impurity present in the commercial sample, identified as bis(methylthio)mercurane. Although this organomercurial compound is highly potent at inhibiting MGL (IC₅₀ = 11.9±1.1 nM), its biological use is prohibited by its toxicity and target promiscuity.

INTRODUCTION

2-Arachidonoylglycerol (2-AG) and anandamide are the most abundant endocannabinoids in the brain, with 2-AG¹-³ involved in significant physiological processes, e.g. stress-induced analgesia (SIA)⁴ and synaptic plasticity⁵. These actions are proven by the inhibition of monoacylglycerol lipase (MGL), a serine hydrolase responsible for the inactivation of 2-AG.⁶,⁷ In recent years a few novel MGL inhibitors were discovered.⁴,⁸-¹¹ Until recently, the only selective small-molecule inhibitor of MGL was URB602 (FIG. 1), a carbamate derivative possessing a moderate activity (half-maximal inhibitory concentration, IC₅₀ = 75±7 µM in rat brain MGL expressed in HeLa cells and 28±4 µM in native rat brain MGL).⁵ With the aim to find potent and selective MGL inhibitors,

(°) Corresponding author; fax: (+39)-0722-303313; e-mail: gat@uniurb.it
we screened a collection of commercial compounds having in their structure carbonyl groups activated towards nucleophilic attack. It turned out that one of these products (6-methyl-2-p-tolylaminobenzo[d][1,3]oxazin-4-one, SPECS lot ID N° AO-095/41416985, purity declared >95%), renamed by us URB754 (FIG. 1), inhibited MGL activity in vitro with an IC\textsubscript{50} of 200±16 nM.\textsuperscript{5} In order to further characterize the pharmacological profile of this substance we prepared URB754 in our laboratories according to Papadopoulos et al.’s procedure.\textsuperscript{12} Surprisingly, it did not prove to be effective as an MGL inhibitor. Because physicochemical analyses confirmed the correct structure of the synthesized compound,\textsuperscript{13} we concluded that the activity previously attributed to URB754 was due to an impurity in the commercial sample. In the present communication we report on the analysis of the commercial sample (henceforth referred to as “SPECS”) used in our tests and on the identification of its bioactive component.

![Chemical structures of URB602, URB754, 6-methyl-3-p-tolyl-1H-quinazoline-2,4-dione (1), 6-methyl-2-thioxo-3-p-tolyl-2,3-dihydro-1H-quinazolin-4-one (2), 6-methyl-2-p-tolylaminobenzo[d][1,3]thiazin-4-one (3), and bis(methylthio)mercure (4).](image-url)

**FIGURE 1.** - Chemical structures of URB602, URB754, 6-methyl-3-p-tolyl-1H-quinazoline-2,4-dione (1), 6-methyl-2-thioxo-3-p-tolyl-2,3-dihydro-1H-quinazolin-4-one (2), 6-methyl-2-p-tolylaminobenzo[d][1,3]thiazin-4-one (3), and bis(methylthio)mercure (4).

**EXPERIMENTAL**

**Sample analyses and chemistry**

LC/MS analyses were performed using an Agilent 1100 series system equipped with a Zorbax SB-CN (2.1 mm x 150 mm, 5 µm particle size) under gradient conditions; mobile phase: A = 40 mM acetic acid and 5 mM ammonium carbonate in water; B = 40 mM acetic acid and 5 mM ammonium carbonate in methanol; 40-100% B on 6 min, 100% B 8 min, re-equilibration time 8 min; flow rate: 1.5 mL min\textsuperscript{-1}; 1 µL volume injected (about 500 pM); column temperature was 30 °C. MS analyses were performed with an electrospray ion source in the positive ionization mode. Capillary voltage was 3000 kV, N\textsubscript{2} gas flow was 13 L min\textsuperscript{-1} at 350 °C and nebulizer pressure was 60 psi. El-MS spectra were recorded with a Fisons Trio 1000 (70 eV) and Micromass VG Autospec spectrometers. NMR spectra were recorded on an AVANCE Bruker 200 spectrometer and analyzed using the WIN-NMR software package; chemical shifts were measured by using the central peak of the solvent; coupling constants (\textit{J} values) are given in hertz (Hz). IR spectra were obtained with a Nicolet Avatar 360 spectrometer. All reagents were purchased from Aldrich in the highest quality commercially available. Solvents were RP grade. Chromatographic separations were performed on silica gel columns by flash chromatography (Kieselgel 60, 0.040-0.063 mm, Merck). TLC analyses were performed on silica gel on aluminium sheets (Kieselgel 60 F\textsubscript{254}, Merck). Melting points were determined on a Büchi SMP-510 capillary apparatus.
Identification of a bioactive impurity in a commercial sample of URB754

6-Methyl-2-p-tolylaminobenzod[f]1,3]oxazin-4-one (URB754, synthesized according to literature).\(^{12}\)

Off-white needles. Mp: 230-233 °C (acetone). MS (EI) m/z 266 (M\(^+\)), 222, 160 (100), 133, 104, 77. \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) 10.07 (s, 1H), 7.76 (m, 1H), 7.65-7.61 (m, 2H), 7.59-7.54 (m, 1H), 7.26 (d, 1H, \(J = 8.3\) Hz), 7.15 (d, 2H, \(J = 8.5\) Hz), 2.36 (s, 3H), 2.27 (s, 3H) ppm; \(^{13}\)C NMR (DMSO-d\(_6\)) \(\delta\) 159.71, 150.76, 147.81, 138.43, 136.02, 134.04, 129.61, 127.88, 124.87, 119.98, 113.88, 20.85, 20.77 ppm. IR (nujol) 3282, 1740, 1638, 1609 cm\(^{-1}\).

6-Methyl-3-p-tolyl-1H-quinazoline-2,4-dione (I, FIG. 1).\(^{13}\)

To a hot suspension of 6-methyl-2-p-tolylaminobenzod[f]1,3]oxazin-4-one (0.35 g, 1.3 mmol) in EtOH (15 mL), 2N NaOH (10 mL) was added. The solution was refluxed for 10 min, cooled at room temperature, acidified with HCl 20%, and the precipitate filtered. Purification of the solid by column chromatography (cyclohexane/EtOAc 6:4) and recrystallization gave I as white solid. Yield: 35% (0.122 g). Mp: 284-286 °C (EtOH) [lit. 286-288 °C (EtOH)].\(^{13}\) MS (EI) m/z 266 (M\(^+\)), 265, 160, 133 (100), 104, 77. \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) 11.47 (s, 1H), 7.72 (s, 1H), 7.54-7.49 (m, 1H), 7.27 (d, 2H, \(J = 8.1\) Hz), 7.17-7.10 (m, 3H), 2.36 (s, 3H), 2.34 (s, 3H) ppm; \(^{13}\)C NMR (DMSO-d\(_6\)) \(\delta\) 162.68, 150.64, 138.09, 137.82, 136.59, 133.63, 132.15, 129.70, 129.21, 127.46, 115.61, 114.58, 21.17, 20.68 ppm. IR (nujol) 3216, 1731, 1714, 1650, 1514 cm\(^{-1}\).

6-Methyl-2-thioxo-3-p-tolyl-2,3-dihydro-1H-quinazolin-4-one (2, FIG. 1).\(^{14}\)

Compound 2 was synthesized according to Muthusamy et al.’s procedure.\(^{15}\) Off-white solid. Yield: 36%. Mp: 349-351 °C (dec. from 310 °C) (EtOH) [lit. 335 °C (EtOH)].\(^{14}\) MS (EI) m/z 284, 283, 282 (M\(^+\)), 281 (100). \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) 12.94 (s, 1H), 7.74 (br s, 1H), 7.60 (dd, 1H, \(J = 8.4\) and 1.9 Hz), 7.35 (d, 1H, \(J = 8.4\) Hz), 7.26 (d, 2H, \(J = 8.3\) Hz), 7.11 (br d, 2H, \(J = 8.3\) Hz), 2.37 (s, 6H) ppm; \(^{13}\)C NMR (DMSO-d\(_6\)) \(\delta\) 176.13, 160.28, 138.03, 137.79, 137.24, 137.06, 134.35, 129.87, 129.14, 127.21, 116.45, 116.13, 21.24, 20.88 ppm. IR (nujol) 3246, 1655, 1522, 1206 cm\(^{-1}\).

6-Methyl-2-p-tolylaminobenzod[f][1,3]thiazin-4-one (3, FIG. 1).

To a solution of 2-amino-5-methylbenzoic acid (0.5 g, 3.31 mmol) in THF (2 mL), \(p\)-tolylisothiocyanate (0.494 g, 3.31 mmol) was added. The mixture was stirred at room temperature for 4 h, then petroleum ether was added and the precipitate was filtered. Purification of solid by column chromatography (EtOAc, then EtOAc/MeOH 9:1) gave 2-[3-(4-methylphenyl)thioureido]-5-methylbenzoic acid which was solubilized in H\(_2\)SO\(_4\) 96%. The solution was stirred at room temperature for 1 h, diluted with ice water, neutralized with solid NaHCO\(_3\) and the precipitate filtered. Purification of the solid by column chromatography (cyclohexane/EtOAc 65:35) and recrystallization gave 3 as yellow crystals. Yield: 18% (0.168 g). Mp: 147-150 °C (acetone/petroleum ether). MS (EI) m/z 282, 281, 176, 133 (100), 104, 77. \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) 10.02 (br s, 1H), 7.76 (br s, 1H), 7.67 (br d, 2H, \(J = 8.5\) Hz), 7.57 (dd, 1H, \(J = 8.3\) and 2.2 Hz), 7.38 (d, 1H, \(J = 8.3\) Hz), 7.16 (d, 2H, \(J = 8.5\) Hz), 2.36 (s, 3H), 2.28 (s, 3H) ppm; \(^{13}\)C NMR (DMSO-d\(_6\)) \(\delta\) 183.99, 151.48, 148.18, 137.86, 137.39, 134.44, 132.98, 129.68, 128.70, 124.20, 120.90, 117.43, 20.91, 20.80 ppm. IR (nujol) 3327, 1709, 1640, 1580, 1538 cm\(^{-1}\).

Bis(methylthio)mercurane (4, FIG. 1).\(^{16}\)

4 was synthesized as described.\(^{16}\) MS (EI) m/z 296 (M\(^+\)), 249 ([M-SCH\(_3\)]\(^+\)), 202 ([M-2SCH\(_3\)]\(^+\)), 94 ([CH\(_3\)-S-S-CH\(_3\)]\(^+\)), 47 ([SCH\(_3\)]\(^+\)).\(^{17}\) Other physico-chemical data are in agreement...
with those reported in the literature: mp 145-150 °C (EtOH) (dec.); \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) 2.32 (s) ppm; \(^{13}\)C NMR (DMSO-\(d_6\)) \(\delta\) 10.60 ppm.\(^{16}\)

*Product purchased from SPECS (Delft, The Netherlands; ID N° AO-095/41416985, bar code 1238040000601).*

White solid. MS (El) (direct introduction and sample heating by a slow temperature ramp) \(m/z\) 296, 281, 249, 202 (isotopic clusters typical of mercury-containing compounds), 94, 79, 47 (single peaks, presumably 4); 266 (M\(^+\)), 160 (100), 133, 104, 77 (URB754); 266 (M\(^+\), 100), 265, 160, 133, 104, 77 (presumably 1); 284, 283, 282 (M\(^+\)), 281 (100) (sulfur-containing impurity, presumably 2 or 3). \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) 10.07 (s, 1H), 7.76-7.75 (m, 1H), 7.74-7.72 (m, 1H), 7.64-7.60 (m, 2H), 7.59-7.54 (m, 1H), 7.53-7.48 (m, 1H), 7.36 (s, benzene), 7.34 (br s, unidentified impurity), 7.30 (br s, 1H), 7.26 (d, 1H, \(J = 8.3\) Hz), 7.21-7.20 (m, unidentified impurity), 7.15 (d, 2H, \(J = 8.5\) Hz), 7.11 (br s, 1H), 2.39 (br s, unidentified impurity), 2.36 (br s, 3H, partially overlapping with the 2.35 signal), 2.35 (s, partially overlapping with the 2.36 signal, 4), 2.34 (br s, 1H), 2.27 (br s, 3H) ppm. \(^{13}\)C NMR (DMSO-\(d_6\)) \(\delta\) 159.71, 150.77, 147.82, 138.44, 136.03, 134.04, 132.42, 129.62, 127.89, 124.88, 119.97, 113.91, 20.86, 20.78 ppm.

**Pharmacology**

MGL (purified rat recombinant, expressed in *E. coli*) and FAAH (rat brain membranes) assays were conducted as described.\(^6\) The expression in *E. coli* and purification of rat recombinant MGL will be described elsewhere (A. King *et al.*, manuscript in preparation).

**RESULTS AND DISCUSSION**

**Analysis of SPECS product lot ID N° AO-095/41416985**

First, the sample was subjected to HPLC/MS analysis. The chromatogram revealed that it contained the title compound URB754 (molecular mass 266) as the main peak, together with two other peaks at a smaller retention time. The first of the two (henceforth referred to as “A”) was given by a compound of molecular mass 266; the second (“B”) by one of molecular mass 282 (FIG. 2).

**FIGURE 2.** - HPLC/ESI(+) of SPECS and URB754 products.
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Besides the signals attributable to URB754, which were prevailing, the $^1$H NMR spectrum of the sample showed two further sets of peaks, distributed between the aromatic and the aliphatic regions. These were likely due to components structurally similar to URB754. A sharp singlet was also present at 2.35 ppm (FIG. 3), which an HMQC (Heteronuclear Multiple Quantum Correlation) experiment showed to be coupled with a carbon resonating at 10.60 ppm, a chemical shift consistent with a thiomethyl group.

![FIGURE 3. - Superposition of $^1$H NMR spectra related to SPECS (top) and URB754 (bottom) products.](image)

Lastly, we examined the mixture by EI-MS. Specifically, the sample was directly introduced and the inlet temperature raised at a rate of 10 °C/min; this caused fractional evaporation of the sample components, allowing the detection of a third impurity ("C"). This was identified as bis(methylthio)mercurane [Hg(SCH$_3$)$_2$] on the basis of the mercury typical isotopic pattern and MS fragment experiments (FIG. 4).

![FIGURE 4. - 70 eV EI mass spectrum of the bioactive component bis(methylthio)mercurane.](image)
The detection of Hg(SCH$_3$)$_2$ in the mixture permitted us to hypothesize that the SPECS 6-methyl-2-p-tolylaminobenzo[d][1,3]oxazin-4-one specimen had been obtained by means of Garin et al.'s procedure, employing HgO. In addition to the desired product, this method would have plausibly led to 6-methyl-3-p-tolyl-1H-quinazoline-2,4-dione (1, MW 266 as A), 6-methyl-2-thi oxo-3-p-tolyl-2,3-dihydro-1H-quinazolin-4-one (2, MW 282 as B), 6-methyl-2-p-tolylaminobenzo[d][1,3]thiazin-4-one (3, MW 282 as B) and Hg(SCH$_3$)$_2$ (4, MW 296 as C) (FIG. 1).

Authentic synthesis of all four supposed by-products and comparison of their $^1$H-NMR and EI-MS spectra with those of SPECS mixture allowed us to confirm that the contaminants of SPECS lot ID N° AO-095/41416985 are 1 (~10%), 2 (~4%) and 4 (~8%) and that Hg(SCH$_3$)$_2$ was not an accidental impurity in SPECS, but a side product of the Garin's et al. procedure, as demonstrated by its presence in a sample of URB754 prepared in our laboratory according to Garin et al. This procedure repeatedly gave in our hands compounds 1, 2 and 4 in addition to the desired compound URB754. Compounds 1 and 2 were obtained in variable amounts depending on the reaction conditions (temperature, duration, work up procedure) and could be completely eliminated by column chromatography and recrystallization of URB754. Chromatography is ineffective in eliminating compound 4 that is a practically unavoidable impurity with Garin's et al. procedure. Pure URB754 obtained by a procedure alternative to that by Garin et al. is stable when stored at room temperature and in the solid state for at least 15 months (TLC and $^1$H NMR).

**Pharmacological tests**

Standard samples of 1-4 were assayed for their ability to inhibit rat recombinant MGL. The results showed that Hg(SCH$_3$)$_2$ (4) is highly potent at inhibiting MGL activity (IC$_{50}$ = 11.9±1.1 nM; n = 3, FIG. 5), whereas compounds 1-3 inhibited MGL activity with IC$_{50}$ values greater than 10 µM (data not shown). Thus, the levels of Hg(SCH$_3$)$_2$ present in the commercial sample of URB754 (~8%) satisfactorily account for the MGL-inhibitory activity of the sample (IC$_{50}$ = 200±16 nM). Moreover, as previously described for commercial URB754, Hg(SCH$_3$)$_2$ did not inhibit fatty-acid amide hydrolase activity at concentrations as high as 1 µM (data not shown).

**FIGURE 5.** Effect of URB754 and other compounds on the activity of recombinant rat MGL expressed in *E. coli*. Open circles, SPECS product; closed circles, URB754; triangles, bis(methylthio)mercurane.
CONCLUSIONS

This paper demonstrates that the bioactive impurity responsible of MGL-inhibitory action exhibited by the commercial specimen analyzed is bis(methylthio)mercurane, an organomercurial compound showing toxicity and target promiscuity.\(^\text{18}\) This explains the inactivity of URB754 observed by Saario \textit{et al.}\(^\text{19}\) In addition, the present study highlights all the side products deriving from Garin \textit{et al.}'s procedure thus giving some hints for its mechanistic aspects and the inconveniences that might result from its use. In view of the biological relevance of benzo[d]oxazin-4-ones (see for example references 20-24) and of the activity of Hg(SCH\(_3\))\(_2\), the presence of this impurity should be monitored with great care.

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REFERENCES

1) R. Mechoulam, S. Ben-Shabat, L. Hanuš, M. Ligumsky, N.E. Kaminski, A.R. Schatz, A. Gopher, S. Almog, B.R. Martin, D.R. Compton, R.G. Pertwee, G. Griffin, M. Bayewitch, J. Barg, Z. Vogel, \textit{Biochem. Pharmacol.}, \textbf{50}, 83 (1995)
2) T. Sugiura, S. Kondo, A. Sukagawa, S. Nakane, A. Shinoda, K. Itoh, A. Yamashita, K. Waku, \textit{Biochem. Biophys. Res. Commun.}, \textbf{215}, 89 (1995)
3) N. Stella, P. Schweitzer, D. Piomelli, \textit{Nature (London)}, \textbf{388}, 773 (1997)
4) A.G. Hohmann, R.L. Suplita, N.M. Bolton, M.H. Neely, D. Fegley, R. Mangieri, J.F. Krey, J.M. Walker, P.V. Holmes, J.D. Crystal, A. Duranti, A. Tontini, M. Mor, G. Tarzia, D. Piomelli, \textit{Nature (London)}, \textbf{435}, 1108 (2005)
5) J.K. Makara, M. Mor, D. Fegley, S.I. Szabó, S. Kathuria, G. Astarita, A. Duranti, A. Tontini, G. Tarzia, S. Rivara, T.F. Freund, D. Piomelli, \textit{Nat. Neurosci.}, \textbf{8}, 1139 (2005) and \textbf{10}, 134 (2007)
6) T.P. Dinh, D. Carpenter, F.M. Leslie, T.F. Freund, I. Katona, S.L. Sensi, S. Kathuria, D. Piomelli, \textit{Proc. Natl. Acad. Sci. U. S. A.}, \textbf{99}, 10819 (2002)
7) S.M. Saario, J.R. Savinainen, J.T. Laitinen, T. Järvinen, R. Niemi, \textit{Biochem. Pharmacol.}, \textbf{67}, 1381 (2004)
8) S.M. Saario, O.M.H. Salo, T. Nevalainen, A. Poso, J.T. Laitinen, T. Järvinen, R. Niemi, \textit{Chem. Biol.}, \textbf{12}, 649 (2005)
9) K. Nithipatikom, M.P. Endsley, M.A. Isbell, C.E. Wheelock, B.D. Hammock, W.B. Campbell, \textit{Biochem. Biophys. Res. Commun.}, \textbf{332}, 1028 (2005)
10) G.B. Quistad, R. Klintenberg, P. Caboni, S.N. Liang, J.E. Casida, \textit{Toxicol. Appl. Pharmacol.}, \textbf{211}, 78 (2006)
11) T. Bisogno, M.G. Cascio, B. Saha, A. Mahadevan, P. Urbani, A. Minassi, G. Appendino, C. Saturnino, B. Martin, R. Razdan, V. Di Marzo, \textit{Biochim. Biophys. Acta}, \textbf{1761}, 205 (2006)
12) E.P. Papadopoulos, C.D. Torres, \textit{J. Heterocycl. Chem.}, \textbf{19}, 269 (1982)
13) J. Garin, E. Melendez, F.L. Merchán, T. Tejero, E. Villarroya, \textit{Synthesis}, 406 (1983)
14) P.D. Kennewell, R.M. Scrowston, I.G. Shenouda, W.R. Tully, R. Westwood, *J. Chem. Res., Miniprint*, 7, 2001 (1986)

15) S. Muthusamy, V.T. Ramakrishnan, *Synth. Commun.*, 22, 519 (1992)

16) I. Steinfatt, G.G. Hoffmann, *Z. Naturforsch. B: Chem. Sci.*, 49, 1507 (1994)

17) G.G. Hoffmann, W. Brockner, I. Steinfatt, *Inorg. Chem.*, 40, 977 (2001)

18) E. Dopp, L.M. Hartmann, A.-M. Florea, A.W. Rettenmeier, A.V. Hirner, *Crit. Rev. Toxicol.*, 34, 301 (2004)

19) S.M. Saario, V. Palomäki, M. Lehtonen, T. Nevalainen, T. Järvinen, J.T. Laitinen, *Chem. Biol.*, 13, 811 (2006)

20) H. Kakuta, A. Tanatani, K. Nagasawa, Y. Hashimoto, *Chem. Pharm. Bull.*, 51, 1273 (2003)

21) P.-W. Hsieh, F.-R. Chang, C.-H. Chang, P.-W. Cheng, L.-C. Chiang, F.-L. Zeng, K.-H. Lin, Y.-C. Wu, *Bioorg. Med. Chem. Lett.*, 14, 4751 (2004)

22) P.-W. Hsieh, T.-L. Hwang, C.-C. Wu, F.-R. Chang, T.-W. Wang, Y.-C. Wu, *Bioorg. Med. Chem. Lett.*, 15, 2786 (2005)

23) J.C.G. Halford, *Curr. Opin. Invest. Drugs*, 7, 312 (2006)

24) H.-P. Kley, G. Hanauer, D. Hauser, B. Schmidt, D. Bredenbröker, W. Wurst, J. Kemkowski, WO 2006094942