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Research Article

Syntheses of [$^{2}H_3$, ^{15}N], [^{14}C]Nexavar^{TM} and its labeled metabolites

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Summary

Nexavar^{TM}, Sorafenib tosylate (BAY 43-9006 tosylate) is a potent small molecule Raf kinase inhibitor for the treatment of hyperproliferative disorders such as cancer. Both radiolabeled and stable isotope labeled compounds were required for drug absorption, distribution, metabolism and excretion (ADME) and quantitative mass spectrometry bio-analytical studies. Nexavar^{TM} labeled with carbon-14 in the carboxamide group was prepared in two steps in an overall radiochemical yield of 42% starting from 4-chloro-*N*-methyl-2-pyridine-[^{14}C]carboxamide. The [$^{2}H_3$,^{15}N] version of Nexavar^{TM} was prepared in 75% yield based on 4-chloro-*N*-[$^{2}H_3$]methyl-2-pyridine-[^{15}N]carboxamide. The pyridine *N*-oxide metabolite labeled with carbon-14 as well as with deuterium and nitrogen-15 and was synthesized by oxidation in yields of 59% and 87%, respectively. Starting from [$^{2}H_2$, ^{13}C]formaldehyde the *N*-hydroxymethyl metabolite was labeled with carbon-13 and deuterium in one step in a 45% overall yield.

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Key Words: Nexavar^{TM}; Raf kinase inhibitor; deuterium; carbon-13; carbon-14; nitrogen-15; synthesis

Introduction

Raf kinase, a downstream effector of ras, is a key mediator of signal transduction pathways from cell surface receptors to the cell nucleus.[1,2] Thus, small-molecule inhibitors of Raf kinase are promising agents for treatment of hyperproliferative disorders such as cancer. As part of a medicinal chemistry program directed at selective Raf kinase inhibitors, BAY 43-9006 (sorafenib, the trade name of the tosylate of BAY 43-9006 is Nexavar^{TM}, Figure 1) was

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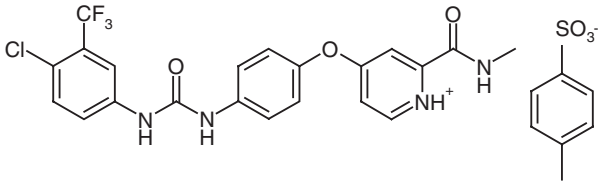

Figure 1. Nexavar™

identified as potent, orally active inhibitor of Raf kinase, and showed broad *in vivo* antitumor activity.[3,4] In support of a program to develop this new Raf kinase inhibitor syntheses of the radiolabeled and stable isotope labeled Nexavar™ and its main metabolites were required. This paper describes the synthesis of each labeled form.

Results and discussion

[^{14}C]BAY 43-9006

The approach taken for the assembly of labeled versions of Nexavar™ relies on the previously reported synthesis of BAY 43-9006.[5] The biarylether formation of 4-chloro-*N*-methyl-2-pyridine[^{14}C]carboxamide (**1**) with 4-aminophenol (**2**) in the presence of potassium tert-butylate yielded the phenyl ether (**3**). Reaction of (**3**) with 1-chloro-4-isocyanato-2-(trifluoromethyl)benzene (**4**) led to the final urea derivative [^{14}C]BAY 43-9006 (**5**) as shown in reaction Scheme 1. After purification parts of this compound were converted into the tosylate (**6**) of [^{14}C]BAY 43-9006 by dissolving equal equivalents of 4-toluenesulfonic acid and (**5**) in ethanol and subsequent crystallization yielding 65.9 mCi of [^{14}C]Nexavar™ with high purity.

[$^{2}H_3$, ^{15}N]BAY 43-9006

The stable isotope labeled compound was synthesized using the same reaction sequence as described for the carbon-14 labeled Nexavar™, shown in the reaction Scheme 2. Starting from 4-chloro-*N*-[$^{2}H_3$]methyl-2-pyridinecarbox[^{15}N]amide (**7**) the formation of phenylether (**8**) and the subsequent reaction with the isocyanate (**4**) both proceeded in high yield. Part of the raw material was directly transformed into the tosylate (**10**) in a yield of 63% using the purification effect of a crystallization. The remainder was purified by HPLC yielding [$^{2}H_3$, ^{15}N]BAY 43-9006 (**9**). A total amount of 2767 mg of [$^{2}H_3$, ^{15}N]Nexavar™ was obtained in high purity.

[$^{2}H_3$, ^{15}N] and [^{14}C]BAY 67-3472, metabolite M-2 of BAY 43-9006

The stable isotope and carbon-14 labeling of BAY 67-3472, the *N*-oxide and metabolite M-2 of BAY 43-9006 was performed by oxidation of

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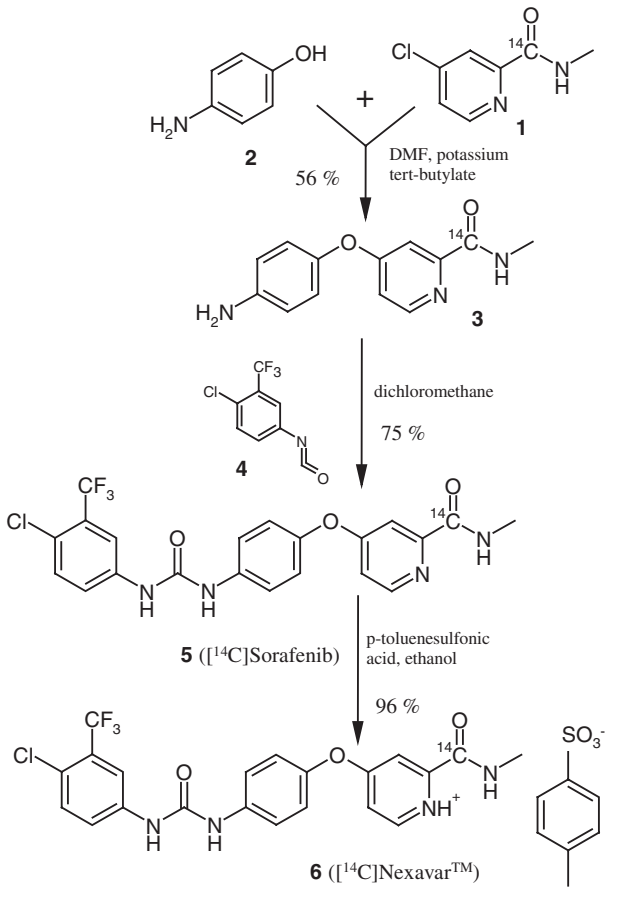

Scheme 1. Synthesis of [^{14}C]BAY 43-9006 ([^{14}C]Sorafenib) and [^{14}C]Nexavar™

[$^{2}H_3$, ^{15}N]BAY 43-9006 (**9**) and [^{14}C]BAY 43-9006 (**5**), respectively, using 3-chloroperbenzoic acid as shown in reaction Scheme 3. After work-up and chromatographic purification a yield of 89% of [$^{2}H_3$, ^{15}N]BAY 67-3472 (**11**) was achieved and in case of [^{14}C]BAY 67-3472 (**12**) a 59% yield. Both compounds were obtained in high purity.

[$^{2}H_2$, ^{13}C]BAY 72-1973, metabolite M-3 of BAY 43-9006

The stable labeled BAY 72-1973 (**14**) was synthesized starting from the *N*-desmethyl BAY 43-9006 (**13**) and [$^{2}H_2$, ^{13}C]formaldehyde as labeling source in a one-step reaction depicted in the reaction Scheme 4. To avoid H/D exchange at the labeled formaldehyde the reaction was carried out in deuterium water. The reaction yield was 45%.

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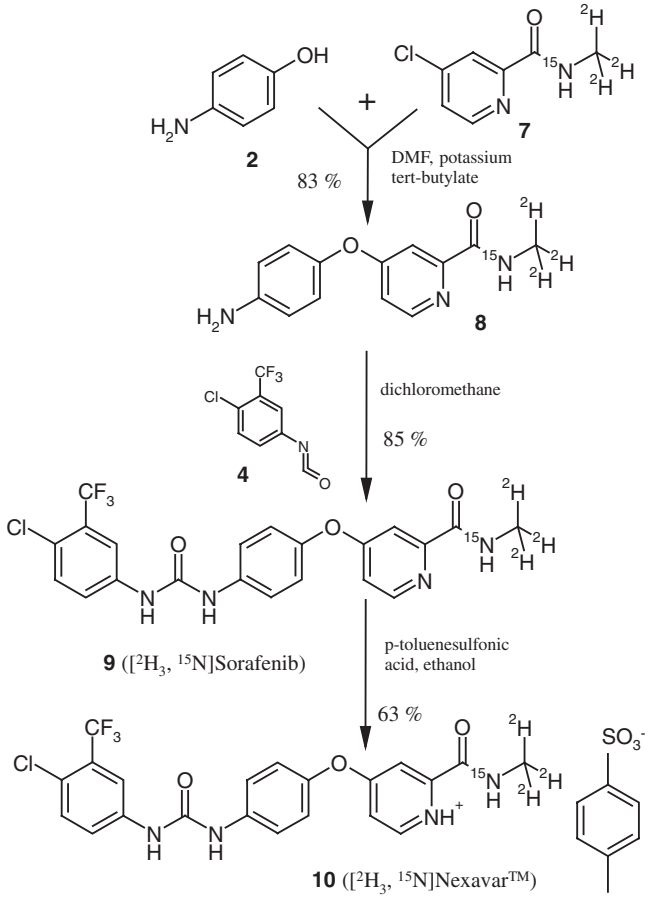

Scheme 2. Synthesis of [$^2H_3$, ^{15}N]BAY 43-9006 ([$^2H_3$, ^{15}N]Sorafenib) and [$^2H_3$, ^{15}N]Nexavar™

Experimental

Materials

4-Chloro-*N*-methyl-2-pyridine[^{14}C]carboxamide was purchased from American Radiolabeled Compounds, Inc., St. Louis, USA, 4-chloro-*N*-[$^2H_3$]methyl-2-pyridinecarbox[^{15}N]amide was purchased from Witega Laboratorien GmbH, Berlin, Germany and the [$^2H_2$, ^{13}C]formaldehyde was delivered by Cambridge Isotope Laboratories, Inc., Andover, USA. Compounds **4** and **13** were obtained from the Chemical Development or Chemical Research Departments, Bayer HealthCare AG. All remaining reagents and solvents were purchased from commercial suppliers and were used as received.

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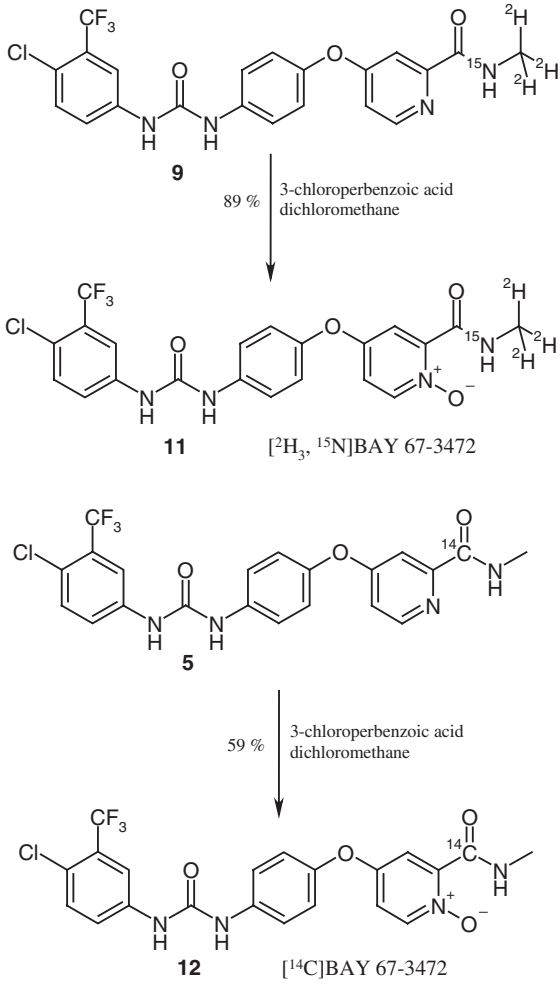

Scheme 3. Synthesis of [$^2H_3$, ^{15}N] and [^{14}C]BAY 67-3472, metabolite M-2 of BAY 43-9006

Liquid scintillation counting

Quantification of radioactivity was performed using a PerkinElmer TRI-CARB® 2500 TR liquid scintillation analyzer, with Ultima Gold™ cocktail used throughout.

High-performance liquid chromatography

All final compounds were analyzed by HPLC using a HP 1050 system Series II (Hewlett-Packard, Waldbronn, Germany) with a Ramona® Cell 2270 (Raytest, Straubenharst, Germany) for radioactivity detection. The LC–MS analytics were obtained at a PE 7 Sciex/API III with MacIntosh Quadra® 900

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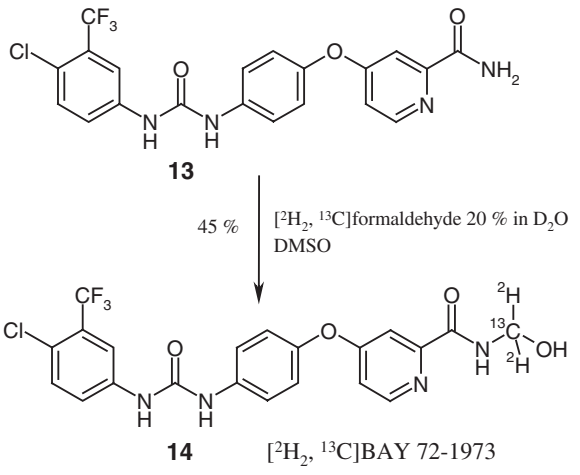

Scheme 4. Synthesis of [$^{2}H_2$, ^{13}C]BAY 72-1973, metabolite M-3 of BAY 43-9006

(PerkinElmer Sciex Instruments, Thornhill, Canada). The following HPLC system was used for the purity check:

(1) Nucleosil® 100 AB, 5 µm, 250 × 4 mm (Macherey & Nagel, Berlin Germany), column temperature: 45°C, flow rate: 1 ml/min, gradient: A: water + 0.5 ml H_3PO_4 per liter, B: acetonitrile, 0 min: 0% B, 0–25 min: 90% B, 25–35 min 90% B, detection: UV 220 nm.

NMR spectra

The NMR spectra were recorded at a Bruker DRX 400 spectrometer (Bruker, Rheinstetten, Germany).

*Synthesis of 4-(4-aminophenoxy)-N-methyl-2-pyridine[^{14}C]carboxamide (**3**)*

A mixture of 4-aminophenol (**2**) (556 mg, 5.1 mmol) and potassium tert-butylate (590 mg, 5.25 mmol) in 5 ml dimethyl formamide was stirred for two hours at room temperature. To the brown suspension 4-chloro-*N*-methyl-2-pyridine[^{14}C]carboxamide (863 mg, 5.0 mmol, total radioactivity: 285.2 mCi, specific activity: 57.0 mCi/mmol, radiochemical purity: 87%) dissolved in 1 ml dimethyl formamide, and potassium carbonate (374 mg, 2.7 mmol) were added and stirred for 8 h at 80°C and further 15 h at room temperature. This mixture was treated with 10 ml brine and extracted three times with each 15 ml ethyl acetate. The organic phases were combined, washed with brine and water, dried over sodium sulfate and evaporated to dryness.

DOI: 10.1002/jlcr

The raw material was purified by flash chromatography using the following conditions: column 2.5 cm × 35 cm filled with 100 g silica gel 60, eluent: dichloromethane/methanol 9:1, fraction volume: 60–70 ml. The eluent was fractionated and monitored by TLC ($R_f = 0.65$, silica gel dichloromethane/methanol 9:1). The compound (**3**) containing fractions were combined and evaporated to dryness yielding 703 mg (2.8 mmol) of compound (**3**) (56%), which was directly used in the next step.

*Synthesis of 4-[4-({[4-chloro-3-(trifluoromethyl)anilino]carbonyl}amino)-phenoxy]-N-methyl-2-pyridine[^{14}C]carboxamide (**5**)*

To a solution of compound (**3**) (703 mg, 2.8 mmol) in 5 ml dichloromethane 4-chloro-3-trifluoromethylphenylisocyanate (**4**) (635 mg, 2.8 mmol), dissolved in 5 ml dichloromethane was slowly added under an argon atmosphere within 5 min at a temperature of 0°C. This mixture was warmed to room temperature and stirred for further 21 h. The formed precipitate was filtered off, washed twice with dichloromethane and dried. The filter cake (1240 mg) was dissolved in 1.65 ml dimethyl formamide and purified in 16 equal portions under the following conditions: column: Lichrosorb® RP 18, 7 µm, 250 × 25 mm, (Merck, Darmstadt, Germany); eluent: acetonitrile/water 65:35 (v + v); flow rate: 10 ml/min, detection: UV 220 nm, retention time: 24–28 min. The compound (**5**) containing fractions were combined and evaporated to dryness yielding 1061 mg of compound (**5**) with a total radioactivity of 120.7 mCi corresponding to 75% yield. The UV purity was 98.6% and the radiochemical purity was 99.3% by reversed phase HPLC (HPLC system 1).

LC–MS: $m/z = 465$ $[^{35}Cl-M+H]^+$, 467 $[^{14}C\text{-}^{35}Cl-M+H]^+$, 469 $[(^{14}C\text{-}^{37}Cl-M+H]^+$.

*Synthesis of 4-[4-({[4-chloro-3-(trifluoromethyl)anilino]carbonyl}amino)-phenoxy]-N-methyl-2-pyridine[^{14}C]carboxamide tosylate (**6**)*

To compound **5** (558 mg, 1.19 mmol), dissolved in 9 ml ethanol, 3 ml of a solution of 4-toluenesulfonic acid monohydrate (227 mg, 1.19 mmol) in ethanol were added and warmed under stirring to 60°C. Further 6 ml ethanol were added in order to obtain a clear solution. After cooling to room temperature the crystallization began. The mixture was evaporated to dryness and the residue was dried under high vacuum giving 757 mg of compound **6** with a total radioactivity of 65.9 mCi showing a chemical purity of 99.9% and a radiochemical purity of 99.4% by HPLC (HPLC system 1).

^1H NMR (400 MHz, D_6-DMSO) d: 11.40 (m, 1H), 9.25 (s, 1H), 9.07 (s, 1H), 8.85 (d, 1H) 8.52 (d, 1H) 8.12 (s, 1H), 7.68–7.56 (m, 4H), 7.51–7.42 (m, 3H), 7.32–7.08 (m, 5H), 2.80 (d, 3H), 2.49 (s, 3H) ppm.

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*Synthesis of 4-(4-aminophenoxy)-N-[$^2H_3$]methyl-2-pyridinecarbox[^{15}N]amide (**8**)*

A mixture of 4-aminophenol (**2**) (1147 mg, 10.5 mmol) and potassium tert-butylate (1215 mg, 10.8 mmol) in 10 ml dimethyl formamide was stirred for two hours at room temperature. To the brown suspension 4-chloro-*N*-[$^2H_3$]methyl-2-pyridinecarbox[^{15}N]amide (**7**) (1803 mg, 5.0 mmol), dissolved in 0.75 ml dimethyl formamide, and potassium carbonate (770 mg, 5.6 mmol) were added and stirred for 8 h at 80°C and further 15 h at room temperature. This mixture was treated with 30 ml brine and extracted three times with each 20 ml ethyl acetate. The organic phases were combined, washed four times with 5 ml brine and subsequently with water, dried over sodium sulfate and evaporated to dryness.

To the oily residue 15 ml diisopropyl ether were added resulting in crystallization of the desired product (**8**). The crystallization was completed overnight. The crystals were filtered off and dried under high vacuum yielding 2152 mg of compound **8** (83% yield) which was directly used in the next step.

*Synthesis of 4-[4-({[4-chloro-3-(trifluoromethyl)anilino]carbonyl}amino)-phenoxy]-N-[$^2H_3$]methyl-2-pyridinecarbox[^{15}N]amide (**9**)*

To a solution of compound **8** (2148 mg, 8.7 mmol) in 9 ml dichloromethane 1-chloro-4-isocyanato-2-(trifluoromethyl)benzene (**4**) (1927 mg, 8.7 mmol), dissolved in 8 ml dichloromethane was slowly added under an argon atmosphere within 5 min at a temperature of 0°C. This mixture was warmed up to room temperature and stirred for further 21 h. The precipitate was filtered off, washed four times with each 1–2 ml dichloromethane and dried. The filter cake (3957 mg) was divided in two parts; about 2900 mg were transferred into the tosylate (**10**) and about 1000 mg were dissolved in 1.2 ml dimethyl formamide and purified in 11 portions under the following conditions: column: Lichrosorb® RP 18, 7 µm, 250 × 25 mm, (Merck, Darmstadt, Germany); eluent: acetonitrile/water 65:35 (v + v); flow rate: 10 ml/min, detection: UV 220 nm, retention time: 24–28 min. The compound (**9**) containing fractions were combined and evaporated to dryness to give 873 mg of compound (**9**) (85%). The chemical purity by HPLC was 99.5% (HPLC system 1).

LC–MS: $m/z = 469$ [$^2H_3$-^{15}N-^{35}Cl−M + H]$^+$, 471 [$^2H_3$-^{15}N-^{37}Cl−M + H]$^+$.

*Synthesis of 4-[4-({[4-chloro-3-(trifluoromethyl)anilino]carbonyl}amino)-phenoxy]-N-[$^2H_3$]methyl-2-pyridinecarbox[^{15}N]amide tosylate (**10**)*

To compound (**9**) (2949 mg, 6.2 mmol), dissolved in 9 ml ethanol, 4.5 ml of a solution of 4-toluenesulfonic acid-monohydrate (1470 mg, 7.7 mmol) in

DOI: 10.1002/jlcr

ethanol were added. Further 2 ml ethanol were dropped in and the crystals were filtered off, washed with cold ethanol and dried under high vacuum. The raw material was re-crystallized from 158 ml ethanol giving 2767 mg of compound (**10**) (63%). The UV purity was 99.0% by HPLC using HPLC system 1.

^{1}H NMR (400 MHz, D_6-DMSO) d: 9.31 (s, 1H), 9.10 (s, 1H), 9.10 (s, 1H), 8.81 (d, 1H) 8.55 (d, 1H) 8.14 (s, 1H), 7.69–7.57 (m, 4H), 7.53–7.42 (m, 3H), 7.32–7.10 (m, 5H), 2.28 (s, 3H) ppm.

*Synthesis of 4-[4-({[4-chloro-3-(trifluoromethyl)anilino]carbonyl}amino)-phenoxy]-N-[$^2H_3$]methyl-2-pyridinecarbox[^{15}N]amide 1-oxide (**11**)*

A mixture of compound (**9**) (150 mg, 0.32 mmol), dissolved in 0.66 ml dichloromethane and 0.66 ml tetrahydrofurane, and 3-chloroperbenzoic acid (323 mg, 1.44 mmol, substance content maximum 77%) was stirred for 6.5 h at 40°C. The mixture was evaporated and subsequently stirred with 2 ml of a 15% potassium hydroxide solution for 30 min at 40°C. The precipitate was filtered off, washed with 1 ml water and dried in a desiccator. The raw material was purified under the following conditions: column: Lobar® LiChroprep®, Si60, 40–63 µm, size B 310 × 25 mm, (Merck, Darmstadt, Germany); eluent: dichloromethane/methanol 92:8 (v + v); flow rate: 10 ml/min, detection: UV 230 nm, retention time: about 16 min. The compound **11** containing fractions were combined and evaporated to dryness to give 138 mg of compound **11** (89%). The chemical purity by HPLC was 98.6% (HPLC system 1).

LC–MS: $m/z = 485$ [$^2H_3$-^{15}N-^{35}Cl−M + H]$^+$, 487 [$^2H_3$-^{15}N-^{37}Cl−M + H]$^+$.

^{1}H NMR (400 MHz, D_6-DMSO) d: 11.39 (d, 1H), 9.22 (s, 1H), 9.03 (s, 1H), 8.13 (s, 1H) 8.39 (d, 1H) 8.14 (s, 1H), 7.70–7.55 (m, 4H), 7.33–7.25 (m, 1H), 7.22–7.14 (m, 2H) ppm.

*Synthesis of 4-[4-({[4-chloro-3-(trifluoromethyl)anilino]carbonyl}amino)-phenoxy]-N-methyl-2-pyridine[^{14}C]carboxamide 1-oxide (**12**)*

A mixture of compound **5** (75 mg, 0.16 mmol) and non-labeled BAY 43-9006 (52 mg, 0.11 mmol), dissolved in 1.0 ml dichloromethane and 1.0 ml tetrahydrofurane, and 3-chloroperbenzoic acid (488 mg, 2.18 mmol, substance content maximum 77%) was stirred for 4 h at 40°C. After adding 1 ml dichloromethane and stirring for 30 min at 0°C the formed microcrystalline solid was filtered off and washed with 1 ml dichloromethane. The crude product was dissolved in 4 ml acetonitrile and purified in 5 portions under the following conditions: column: Lobar® LiChroprep®, RP-18, 40–63 µm, size B 310 × 25 mm, (Merck, Darmstadt, Germany); eluent: acetonitrile/water 50:50 (v + v) for 40 min followed by acetonitrile/water 80:20 (v + v); flow rate: 15 ml/min, detection: UV 210 nm. Under these conditions the product **12**

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eluted between 25 and 37 min. The combined fractions were concentrated under reduced pressure at 40°C and passed through a reversed phase cartridge C-18 (Varian 1225-6031, 60 ml, 10 g). The product was washed out with neat acetonitrile, evaporated to dryness to give 78 mg (5.3 mCi) of compound **12** (59%). The UV purity by HPLC was 99.3% and the radiochemical purity was 98.5% (HPLC system 1).

LC–MS: $m/z = 480$ [^{14}C-^{35}Cl−M + Hl]$^+$, 482 [^{14}C-^{37}Cl−M + H]$^+$.

^{1}H NMR (400 MHz, D_6-DMSO) d 11.41 (d, 1H), 9.25 (s, 1H), 9.02 (s, 1H), 8.40 (d, 1H) 8.12 (s, 1H), 7.68–7.54 (m, 5H), 7.28 (m, 1H), 7.20 (m, 2H), 2.88 (d, 3H).

*Synthesis of 4-[4-({[4-chloro-3-(trifluoromethyl)anilino]carbonyl}amino)-phenoxy]-N-hydroxy[$^2H_2$, ^{13}C]methyl-2-pyridine[^{14}C]carboxamide, (***14***)*

A mixture of the desmethyl derivative of BAY 43-9006 **13** (250 mg, 0.55 mmol), potassium carbonate (5 mg, 0.05 mmol) and [$^2H_2$, ^{13}C]formaldehyde (6.06 mmol, 20% solution in deuterium water, cat. No. CDLM-4599) in 1.0 ml dimethyl sulfoxide was stirred at 100°C for two hours. After addition of 4 ml acetonitrile the raw material was purified in three portions under the following conditions: column: Nucleosil® 100-7 C-18, 7 µm, 250 × 20 mm, (Macherey & Nagel, Berlin, Germany); eluent: acetonitrile/water 40:60 (v + v); flow rate: 20 ml/min, detection: UV 260 nm, retention time: about 75 min. The compound **14** containing fractions were combined and evaporated to dryness to give 122 mg of compound **14** (45%, based on compound **13**). The UV purity by HPLC was 98.6% (HPLC system 1).

LC–MS: $m/z = 485$ [^{14}C-^{35}Cl−M + H]$^+$, 487 [^{14}C-^{37}Cl−M + H]$^+$.

^{1}H NMR (400 MHz, D_6-DMSO) d: 9.28 (s, 1H), 9.05 (s, 1H), 9.02 (s, 1H), 8.53 (d, 1H) 8.12 (s, 1H), 7.68–7.55 (m, 4H), 7.40 (s, 1H), 7.22–7.14 (m, 2H) 5.61 (s, 1H) ppm.

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References

1. Moelling K, Heinemann B, Beimling P, Rapp U. *Nature* 1984; **312**: 558–561.
2. Monia B, Johnston J, Geiger T, Muller M, Fabbro D. *Nat Med* 1996; **2**: 668–675.
3. Riedl B, Lowinger T, Bankston D, Barbosa J, Brittelli D, Carlson R, Dumas J, Hibner B, Kadono H, Katz, M, Kennure M, Khire U, Lee W, Monahan M, Natero R, Renick J, Rong H, Scott W, Sibley R, Smith R. In *Book of Abstracts of*

the 92nd Meeting of the American Association for Cancer Research, New Orleans, 24–28 March 2001 (Abstract 4956).

4. Riedl B, Dumas J, Khire U, Lowinger T, Scott W, Smith R, Wood J, Monahan M, Natero R, Renick J, Sibley R. PCT Int Appl WO0042012, 20 July 2000.
5. Bankston D, Dumas J, Natero R, Riedl B. Monahan M-K, Sibley R. *Org Proc Res Dev* 2002; **6**: 777–781.

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