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# Synthesis of $^{2}$H- and $^{13}$C-labelled sunitinib and its primary metabolite

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**Sunitinib (Sutent®, Pfizer) was approved in 2006 for the treatment of gastrointestinal and renal cancer. Isotope-labelled derivatives have already been prepared for PET and ADME radiography. The preparation of $^{13}$C- and $^{2}$H-labelled internal standards of sunitinib (SU11248) and its primary metabolite (SU12662) for LC-MS analysis of human blood samples is presented.**

**Keywords:** sunitinib; carbon-13; deuterium; SU11248; SU12662

## Introduction

The discovery of SU11248, later named sunitinib, was published and patented by Sugen/Pharmacia in 2001 (Figure 1).[1,2] In 2003 Pfizer acquired Sugen and by January 2006 sunitinib was approved by the US FDA for the treatment of gastrointestinal stromal tumours and advanced renal cell carcinoma.[3] Marketed as Sutent®, sunitinib is a multitargeted tyrosine kinase inhibitor with antiangiogenic and antitumour activities. It was selected from several oxindol derivatives as a selective inhibitor of the receptor tyrosine kinases VEGFR1-3, PDGFRα and β, KIT, Flt3, RET, and the CSF1 receptor.[4] Sunitinib was found to be significantly more potent against VEGFR2 and PDGFRα than other candidate drugs and exhibited superior pharmacokinetic properties.[5] Metabolism studies revealed that sunitinib is mainly metabolized by *N*-desethylation through CYP3A4 to the still active metabolite SU12662 as shown in Figure 1.[6]

Identification and quantification of drugs and metabolites by LC/MS relies very much on stable isotope-labeled analogues.[7,8] Analytical procedures to determine both compounds, SU11248 and SU12662, in biological matrices, e.g. monkey blood samples, have been published.[9] Unfortunately, the internal standard and its synthesis used in this study was not completely specified by the authors. One might conclude from the details provided ($m/z = 409$, most probably $ESI^{+}$), that a sunitinib-$d_{10}$ analogue was used. In addition, $^{18}$F-sunitinib has been prepared for PET purposes and a $^{14}$C-analogue was synthesized for ADME studies prior to FDA approval.[10,11] The aim of our work was to prepare comparable $^{13}$C/$^{2}$H-SU11248/SU12662 internal standards for LC-MS determination of sunitinib and its primary metabolite in human blood samples.

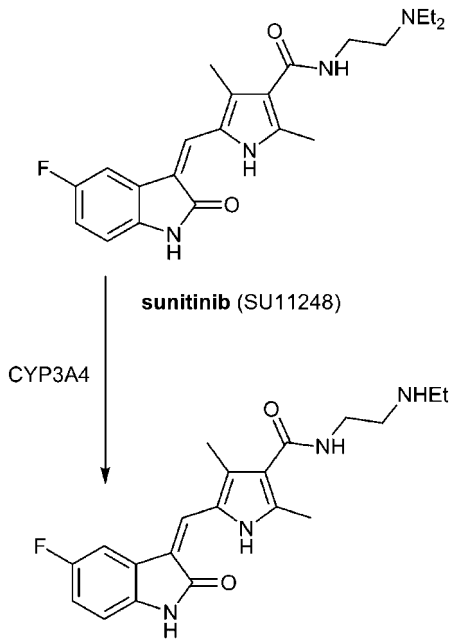


**Figure 1.** Sunitinib is mainly metabolized by CYP3A4 through desalkylation of the basic side chain to provide *N*-desethyl-sunitinib, which is still active.

## Results and discussion

*N*-Alkylation, e.g. *N*-$d_5$-ethylation, has been used to stable label a variety of drugs to obtain internal standards for biological quantification.[12–14] Between the sunitinib-$d_5$, presented herein, and sunitinib itself, neither cross-contribution to analyte ions[7] nor any difference in chromatographic behaviour,[15] e.g. lower retention times, was observed (see Supporting Information, S9, S12). Their physicochemical properties appeared therefore

*Supporting information may be found in the online version of this article.*

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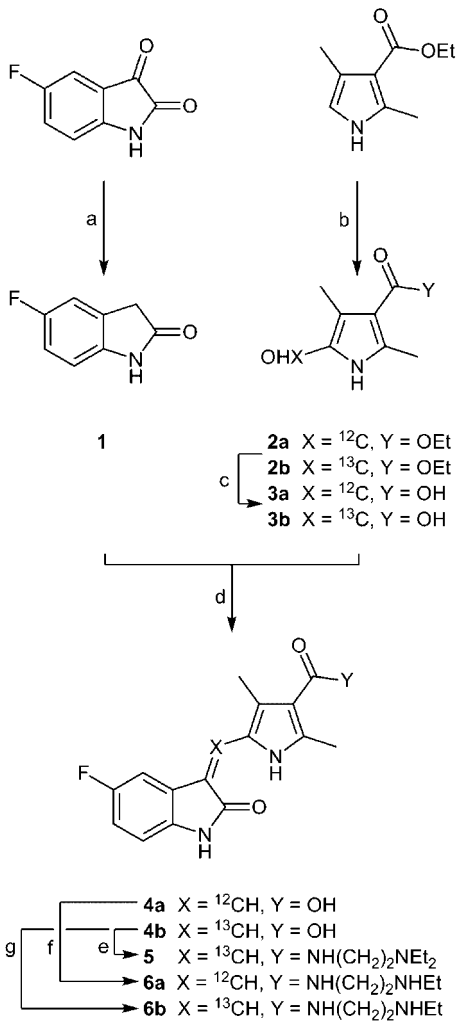
very similar and the internal standard was considered suitable for the determination of sunitinib. Next to the presented synthesis of a sunitinib-$d_5$ internal standard, an analogously $d_5$-deuterized metabolite, requiring mono-alkylation of ethylenediamine, posed a major chemical challenge, and was therefore not addressed. Nevertheless, also SU12662 was successfully analysed using the sunitinib-$d_5$ internal standard. Structural elucidation of the sunitinib scaffold was based particularly on NMR experiments exploiting the unique $^{1}H$, $^{13}C$, and $^{19}F$ coupling patterns observed with the prepared $^{13}C$-isotopomers.

The convergent synthesis of sunitinib and related indolin-2-one derived structures started from 5-fluoroindolin-2-one (**1**), which was readily obtained by a Wolff–Kishner-type reduction of the corresponding 5-fluoroisatin (Scheme 1). The pyrrole-containing building block was prepared from commercially available ethyl 2,4-dimethyl-1*H*-pyrrole-3-carboxylate (Ace Synthesis, Woburn, USA). A Vilsmeier reaction, introducing a $^{12}C$- or $^{13}C$-formyl function into the pyrrole, was carried out affording compounds **2a** and **2b**. Subsequent alkaline hydrolysis of the ethyl ester moiety produced **3a** and **3b**, which where joined with **1** in a pyrrolidine-catalyzed aldol reaction to obtain key intermediates **4a** and **4b**. Reacting ethyl esters **2** with **1** prior to alkaline hydrolysis is not advisable, as the poor solubility of 3-substituted indolin-2-ones, e.g. **4**, impairs following reaction steps. Compounds **4a** and **4b** were obtained exclusively in *Z*-configuration, a fact that has already been investigated in detail.[16] The mono- or dialkylaminoethylcarboxamide side chains of sunitinib ($^{13}C$: **5**) and its primary metabolite ($^{12}C$: **6a**, $^{13}C$: **6b**) where introduced in a final step through an EDC/HOBT coupling procedure applying careful temperature control, i.e. lowered reaction temperature in case of *N*-ethylethylenediamine to receive regioselectivity in favour of the primary amino function. No side chain precursor was commercially available for the synthesis of the sunitinib-$d_5$ internal standard. Therefore, *N*-ethylethylenediamine was reacted in a Gabriel-like synthesis to the phthalimide **7**, which was subsequently alkylated with [$^{2}H_5$]ethyl iodide (Scheme 2). Final hydrazinolysis provided the deuterized side chain precursor *N,N*-[$^{2}H_5$]diethylethylenediamine *in situ*, which was reacted with **4a** to afford sunitinib-$d_5$ (**8**). Purification of **5**, **6a**, **6b**, and **8** was achieved by preparative column chromatography. The corresponding salts, i.e. **5** and **6b**, were obtained by lyophilization from dilute hydrochloric acid.




**Scheme 1.** Synthesis of $^{12/13}C$-labelled precursors, sunitinib and its primary metabolite. (a) $N_2H_4$, $Et_3N$, *n*-BuOH, 100°C, 16 h; (b) 1. [$^{12}C/^{13}C$]DMF, $POCl_3$, $CH_2Cl_2$, 4°C, 15 min to reflux, 1 h; 2. HCl, $H_2O$, RT, 30 min; (c) KOH, $H_2O$, reflux, 5 h; (d) EtOH, pyrrolidine (cat.), reflux, 3 h; (e) $H_2N(CH_2)_2NEt_2$, HOBT, EDC, $Et_3N$, DMF, RT, 48 h; (f, g) $H_2N(CH_2)_2NHEt$, HOBT, EDC, $Et_3N$, DMF, −60°C, 6 h to RT, 18 h.

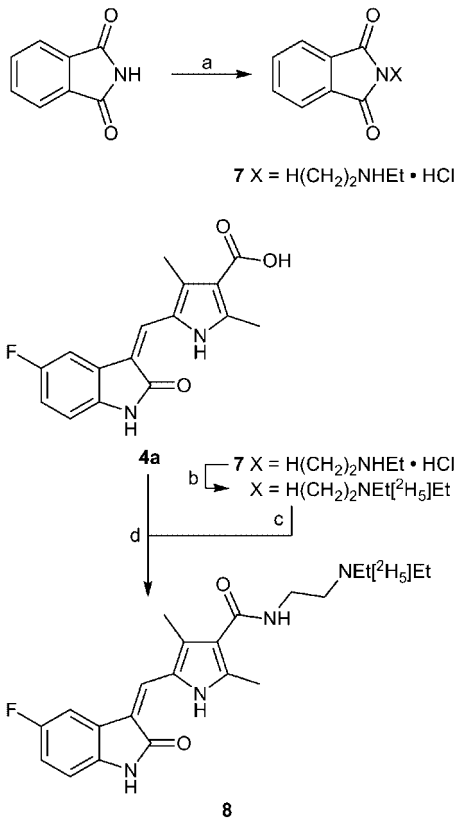
## Experimental section

### General methods and materials

Melting points were determined on a Boëtius melting point apparatus (VEB Wägetechnik Rapido PHMK) and are uncorrected. Thin-layer chromatography was performed on aluminium sheets coated with silica gel 60 $F_{254}$, preparative column chromatography using silica gel 60, 70–230 mesh (Merck, Darmstadt, Germany) or a $C_{18}$ column (Knauer Eurospher 100, 10 µm, 250 mm × 20 mm). LC-MS analysis was performed on an API 2000 mass spectrometer (ESI, Applied Biosystems, Darmstadt, Germany), coupled with an HPLC system (Agilent 1100) using a $C_{18}$ column (Phenomenex Luna, 3 µm, 50 × 2 mm, Aschaffenburg, Germany) and a gradient of MeOH/$H_2O$. Additional $MS^2$ experiments were carried out to acquire the corresponding fragmentation patterns. HPLC purity assessment was carried out using a $C_{18}$ column (Ziemer Hypersil-ODS, 3 µm, 125 × 4.6 mm, Langerwehe, Germany) attached to a UV detector (Jasco UV-2075Plus, 220 nm) under isocratic conditions (MeCN/$H_2O$, 65/35 v/v). $^{13}C$ NMR (125 MHz) and $^{1}H$ NMR spectra (500 MHz) were recorded on a Avance DRX 500 spectrometer (Bruker BioSpin, Rheinstetten, Germany) and chemical shifts δ are given in ppm referring to the signal centre using the solvent peaks for reference (DMSO-$d_6$: 2.49/39.7). To characterize the spin multiplicity the following abbreviations are used: s singlet, bs broad singlet, d doublet, dd doublet of doublets, ddd doublet of doublet of doublets, t triplet, q quartet, dq doublet of quartets, m multiplet. Apparent spin multiplicity is denoted by a preceding ‚app'. $^{13}C$ NMR signals were assigned on the basis of $^{13}C/^{1}H$, $^{13}C/^{19}F$, and $^{13}C/^{13}C$ coupling patterns.

### 5-Fluoro-1,3-dihydro-2*H*-indol-2-one (1)

5-Fluoroisatin (30.0 mmol, 4.95 g) and hydrazine (61.8 mmol, 3.0 mL hydrazine hydrate, 100%) were suspended in *n*-butanol (50 mL) and stirred at room temperature for 30 min.[17] After heating to 80°C for 3 h, triethylamine (5 mL) was added and the suspension was stirred further 12 h at 100°C. Upon cooling to room temperature and solvent removal *in vacuo* the crude product was dissolved in ethyl acetate (100 mL) and washed




**Scheme 2.** Synthesis of $^{2}$H-labelled sunitinib. (a) $H_2N(CH_2)_2NHEt$, 100°C, 3 h; (b) [$^{2}H_5$]EtI, $K_2CO_3$, MeCN, RT, 16 h; (c) $N_2H_4$, EtOH, reflux, 1 h; (d) HOBT, EDC, $Et_3N$, DMF, RT, 18 h.

with 10% potassium hydrogen sulphate solution. The aqueous layer was extracted with ethyl acetate (1 × 100 mL), the combined organic phases were washed with brine (1 × 50 mL) and evaporated *in vacuo*. The residue was dissolved in hot ethyl acetate (50 mL) and petroleum ether was added until the solution turned slightly cloudy. After filtration and cooling to room temperature, **1** was separated by suction filtration to afford light brown crystals (3.12 g, 69%), mp 128°C, lit.[18] 121–134°C. $^{1}$H NMR (DMSO-$d_6$) δ: 3.47 (s, 2H, 3-H), 6.76 (dd, 1H, $^{3}J$ (H,H) = 8.5 Hz, $^{4}J$ (H,F) = 4.7 Hz, 7-H), 6.97 (ddd, 1H, $^{3}J$ (H,F) = 9.1 Hz, $^{3}J$ (H,H) = 8.3 Hz, $^{4}J$ (H,H) = 2.9 Hz, 6-H), 7.07 (dd, 1H, $^{3}J$ (H,F) = 8.6 Hz, $^{4}J$ (H,H) = 2.8 Hz, 4-H), 10.32 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$) δ: 36.32 (C-3), 109.70 (d, 1C, $^{3}J$ (C,F) = 8.4 Hz, C-7), 112.32 (d, 1C, $^{2}J$ (C,F) = 24.5 Hz, C-6), 113.67 (d, 1C, $^{2}J$ (C,F) = 23.1 Hz, C-4), 127.81 (d, 1C, $^{3}J$ (C,F) = 8.9 Hz, C-3a), 140.05 (C-7a), 157.87 (d, 1C, $^{1}J$ (C,F) = 234 Hz, C-5), 176.32 (C-2). HPLC purity: 99.9%.

### Ethyl 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylate (2a)

A solution of *N,N*-dimethyl-formamide (11.0 mmol, 0.80 g) and phosphorus(V) oxychloride (11.0 mmol, 1.69 g) in dichloromethane (10 mL) was cooled to 4°C and solid ethyl 2,4-dimethyl-1*H*-pyrrole-3-carboxylate (10.0 mmol, 1.67 g) was added slowly (maximum temperature 10°C). The reaction mixture was stirred 15 min and subsequently heated to reflux for 1 h. Upon cooling to 10°C water (5 mL), followed by hydrochloric acid (10 M, 5 mL) was added with vigorous stirring. The layers were allowed to separate and the organic phase was extracted with hydrochloric acid (10 M, 2 × 10 mL). The combined aqueous extracts were washed with dichloromethane (1 × 20 mL), and sodium hydroxide (10 M, 25 mL) was added to afford **2a** as a yellow precipitate (1.89 g, 97%), mp 167°C, lit.[19] 163–164°C. $^{1}$H NMR (DMSO-$d_6$) δ: 1.26 (t, 3H, $^{3}J$ (H,H) = 7.1 Hz, $CH_2\underline{CH_3}$), 2.41 (s, 3H, 2-$CH_3$), 2.45 (s, 3H, 4-$CH_3$), 4.18 (q, 2H, $^{3}J$ (H,H) = 7.2 Hz, $CH_2$), 9.60 (s, 1H, CHO), 12.13 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$) δ: 10.48 (4-$CH_3$), 13.66 (2-$CH_3$), 14.38 ($CH_2\underline{CH_3}$), 59.18 ($CH_2$), 112.81 (C-3), 128.41 (C-5), 133.80 (C-4), 142.73 (C-2), 164.45 ($\underline{C}O_2CH_2CH_3$), 178.01 (CHO). HPLC purity: 98.9%.

### Ethyl 5-[$^{13}$C]formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylate (2b)

*N,N*-dimethyl-[$^{13}$C]formamide (11.0 mmol, 0.82 g) was reacted as described above to afford **2b** as a yellow precipitate (1.78 g, 91%), mp 158°C. $^{1}$H NMR (DMSO-$d_6$) δ: 1.27 (t, 3H, $^{3}J$ (H,H) = 7.1 Hz, $CH_2\underline{CH_3}$), 2.41 (s, 3H, 2-$CH_3$), 2.45 (s, 3H, 4-$CH_3$), 4.18 (q, 2H, $^{3}J$ (H,H) = 7.1 Hz, $CH_2$), 9.60 (d, 1H, $^{1}J$ (H,C) = 174 Hz, CHO), 12.12 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$) δ: 10.48 (4-$CH_3$), 13.66 (2-$CH_3$), 14.38 ($CH_2\underline{CH_3}$), 59.19 ($CH_2$), 112.81 (d, 1C, $^{3}J$ (C,C) = 3.7 Hz, C-3), 128.39 (d, 1C, $^{1}J$ (C,C) = 65.7 Hz, C-5), 133.81 (d, 1C, $^{2}J$ (C,C) = 5.2 Hz, C-4), 142.72 (C-2), 164.45 ($\underline{C}O_2CH_2CH_3$), 178.02 (CHO). HPLC purity: 100.0%.

### 5-Formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3a)

A suspension of ethyl 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylate (**2a**; 8.0 mmol, 1.56 g) in a solution of potassium hydroxide (90%, 16.0 mmol, 1.0 g) in water (18 mL) was heated to reflux for 5 h. After cooling to room temperature the clear solution was diluted with water (30 mL) and washed with dichloromethane (1 × 40 mL). Subsequently, the pH was adjusted to 4 using concentrated hydrochloric acid to recover **3a** as a yellow precipitate (1.19 g, 89%), mp 286°C (decomposition). $^{1}$H NMR (DMSO-$d_6$) δ: 2.41 (s, 3H, 2-$CH_3$), 2.44 (s, 3H, 4-$CH_3$), 9.59 (s, 1H, CHO), 12.04 (s, 2H, $CO_2H$, NH); $^{13}$C NMR (DMSO-$d_6$) δ: 10.53 (4-$CH_3$), 13.72 (2-$CH_3$), 113.51 (C-3), 128.37 (C-5), 134.21 (C-4), 142.89 (C-2), 166.12 ($CO_2H$), 177.89 (CHO). HPLC purity: 99.4%.

### 5-[$^{13}$C]Formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3b)

A suspension of ethyl 5-[$^{13}$C]formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylate (**2b**; 8.0 mmol, 1.57 g) was treated as described above to recover **3b** as a yellow precipitate (1.11 g, 83%), mp 285°C (decomposition). $^{1}$H NMR (DMSO-$d_6$) δ: 2.41 (s, 3H, 2–$CH_3$), 2.44 (s, 3H, 4–$CH_3$), 9.59 (d, 1H, $^{1}J$ (H,C) = 174 Hz, CHO), 12.04 (s, 2H, $CO_2H$, NH); $^{13}$C NMR (DMSO-$d_6$) δ: 10.52 (4-$CH_3$), 13.70 (2-$CH_3$), 113.51 (d, 1C, $^{3}J$ (C,C) = 3.5 Hz, C-3), 128.34 (d, 1C, $^{1}J$ (C,C) = 65.9 Hz, C-5), 134.19 (d, 1C, $^{2}J$ (C,C) = 5.2 Hz, C-4), 142.87 (d, 1C, $^{3}J$ (C,C) = 2.5 Hz, C-2), 166.11 ($CO_2H$), 177.89 (CHO). HPLC purity: 99.9%.

### 5-((5-Fluoro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)methyl)-2,4-dimethyl-1*H*-pyrrole-3- carboxylic acid (4a)

A solution of 5-fluoro-1,3-dihydro-2*H*-indol-2-one (**1**; 8.8 mmol, 1.33 g), 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (**3a**; 8.8 mmol, 1.47 g), and pyrrolidine (18.0 mmol, 1.5 mL) in ethanol (120 mL) was heated to reflux for 3 h. Upon cooling to room temperature hydrochloric acid (2 M, 15 mL) was added to the suspension, a crude precipitate was recovered by suction filtration and washed with ethanol (20 mL) followed by petroleum ether (20 mL) to afford **4a** as a yellow powder



(2.59 g, 98%), mp 320°C (decomposition). $^1$H NMR (DMSO-$d_6$) δ: 2.49 (s, 3H, 2-$CH_3$), 2.52 (s, 3H, 4-$CH_3$), 6.83 (dd, 1H, $^3J$ (H,H) = 8.5 Hz, $^4J$ (H,F) = 4.8 Hz, 7′-H), 6.92 (ddd, 1H, $^3J$ (H,F) = 9.5 Hz, $^3J$ (H,H) = 8.5 Hz, $^4J$ (H,H) = 2.5 Hz, 6′-H), 7.72 (s, 1H, 3′-CH-5), 7.74 (dd, 1H, $^3J$ (H,F) = 9.5 Hz, $^4J$ (H,H) = 2.5 Hz, 4′-H), 10.88 (s, 1H, $NH_{ind}$), 12.07 (s, 1H, $CO_2H$), 13.84 (s, 1H, $NH_{pyr}$); $^{13}$C NMR (DMSO-$d_6$) δ: 11.59 (4-$CH_3$), 14.62 (2-$CH_3$), 106.32 (d, 1C, $^2J$ (C,F) = 25.5 Hz, C-4′), 110.24 (d, 1C, $^3J$ (C,F) = 8.4 Hz, C-7′), 112.85 (d, 1C, $^2J$ (C,F) = 24.0 Hz, C-6′), 114.53 (C-3), 115.83 (d, 1C, $^4J$ (C,F) = 3.0 Hz, C-3′), 124.90 (3′-CH-5), 126.21 (C-5), 127.15 (d, 1C, $^3J$ (C,F) = 9.4 Hz, C-3a′), 133.58 (C-4), 134.89 (C-7a′), 141.02 (C-2), 158.42 (d, 1C, $^1J$ (C,F) = 233 Hz, C-5′), 166.06 ($CO_2H$), 169.76 (C-2′). HPLC purity: 97.3%.

### 5-((5-Fluoro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-[$^{13}$C]methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (4b)

A solution of 5-fluoro-1,3-dihydro-2*H*-indol-2-one (**1**; 3.0 mmol, 0.45 g), 5-[$^{13}$C]formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (**3b**; 3.0 mmol, 0.59 g), and pyrrolidine (6.0 mmol, 0.5 mL) in ethanol (20 mL) was reacted as described before to obtain **4b** as a yellow powder (0.86 g, 95%), mp 316°C (decomposition). $^1$H NMR (DMSO-$d_6$) δ: 2.49 (s, 3H, 2-$CH_3$), 2.52 (s, 3H, 4-$CH_3$), 6.83 (dd, 1H, $^3J$ (H,H) = 8.2 Hz, $^4J$ (H,F) = 4.4 Hz, 7′-H), 6.91 (ddd, 1H, $^3J$ (H,F) = 9.0 Hz, $^3J$ (H,H) = 9.0 Hz, $^4J$ (H,H) = 2.3 Hz, 6′-H), 7.70 (d, 1H, $^1J$ (H,C) = 153 Hz, 3′-CH-5), 7.73 (dd, 1H, $^3J$ (H,F) = 9.2 Hz, $^4J$ (H,H) = 2.2 Hz, 4′-H), 10.89 (s, 1H, $NH_{ind}$), 13.82 (s, 1H, $NH_{pyr}$); $^{13}$C NMR (DMSO-$d_6$) δ: 11.59 (4-$CH_3$), 14.62 (2-$CH_3$), 106.26 (d, 1C, $^2J$ (C,F) = 25.3 Hz, C-4′), 110.22 (d, 1C, $^3J$ (C,F) = 8.4 Hz, C-7′), 112.78 (d, 1C, $^2J$ (C,F) = 24.0 Hz, C-6′), 114.98 (C-3), 115.63 (dd, 1C, $^1J$ (C,C) = 71.7 Hz, $^4J$ (C,F) = 2.5 Hz, C-3′), 124.90 (3′-CH-5), 126.24 (d, 1C, $^1J$ (C,C) = 68.9 Hz, C-5), 127.18 (d, 1C, $^3J$ (C,F) = 8.7 Hz, C-3a′), 133.60 (d, 1C, $^2J$ (C,C) = 4.5 Hz, C-4), 134.87 (d, 1C, $^4J$ (C,F) = 4.5 Hz, C-7a′), 140.97 (C-2), 158.41 (d, 1C, $^1J$ (C,F) = 233 Hz, C-5′), 166.25 ($CO_2H$), 169.76 (d, 1C, $^2J$ (C,C) = 2.5 Hz, C-2′). HPLC purity: 97.9%.

### *N*-(2-(Diethylamino)ethyl)-5-((5-fluoro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-[$^{13}$C]methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxamide hydrochloride (5, $^{13}$C-SU11248)

A solution of 5-((5-fluoro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-[$^{13}$C]methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (**4**; 1.0 mmol, 0.30 g), *N,N*-diethylethylenediamine (1.2 mmol, 0.14 g), 1-hydroxybenzotriazole (1.5 mmol, 0.20 g), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.0 mmol, 0.38 g), and triethylamine (2.0 mmol, 0.20 g) in anhydrous *N,N*-dimethylformamide (10 mL) was stirred at room temperature for 48 h. The reaction mixture was subsequently evaporated *in vacuo* and the crude residue subjected to preparative HPLC on $C_{18}$-silica using a gradient of methanol–water containing 1.5% triethylamine. The product fractions were combined, evaporated *in vacuo* and lyophilized from hydrochloric acid (0.1 M, 25 mL) to obtain **5** as an orange powder (0.40 g, 91%), mp 274°C (decomposition). $^1$H NMR (DMSO-$d_6$) δ: 1.25 (t, 6H, $^3J$ (H,H) = 7.3 Hz, $CH_2\underline{CH_3}$), 2.45 (s, 3H, 2-$CH_3$), 2.47 (s, 3H, 4-$CH_3$), 3.15 (dq, 4H, $^3J$ (H,H) = 4.8 Hz, $^3J$ (H,H) = 7.3 Hz, $\underline{CH_2}CH_3$), 3.20 (app q, 2H, $^3J$ (H,H) = 6.1 Hz, $CONHCH_2\underline{CH_2}$), 3.61 (app q, 2H, $^3J$ (H,H) = 6.3 Hz, $CONH\underline{CH_2}CH_2$), 6.84 (dd, 1H, $^3J$ (H,H) = 8.5 Hz, $^4J$ (H,F) = 4.7 Hz, 7′-H), 6.91 (ddd, 1H, $^3J$ (H,F) = 9.5 Hz, $^3J$ (H,H) = 8.5 Hz, $^4J$ (H,H) = 2.6 Hz, 6′-H), 7.70 (d, 1H, $^1J$ (H,C) = 153 Hz, 3′-CH-5), 7.74 (dd, 1H, $^3J$ (H,F) = 9.5 Hz, $^4J$ (H,H) = 2.5 Hz, 4′-H), 8.02 (t, 1H, $^3J$ (H,H) = 5.7 Hz, $CONHCH_2CH_2$), 8.03 (bs, 1H, $N^+H$), 10.92 (s, 1H, $NH_{ind}$), 13.72 (s, 1H, $NH_{pyr}$); $^{13}$C NMR (DMSO-$d_6$) δ: 8.61 ($CH_2\underline{CH_3}$), 10.92 (4-$CH_3$), 13.74 (2-$CH_3$), 34.12 ($CONH\underline{CH_2}CH_2$), 47.03 ($\underline{CH_2}CH_3$), 50.37 ($CONHCH_2\underline{CH_2}$), 106.15 (d, 1C, $^2J$ (C,F) = 25.3 Hz, C-4′), 110.23 (d, 1C, $^3J$ (C,F) = 8.4 Hz, C-7′), 112.62 (d, 1C, $^2J$ (C,F) = 24.3 Hz, C-6′), 115.11 (d, 1C, $^1J$ (C,C) = 71.3 Hz, $^4J$ (C,F) = 2.5 Hz, C-3′), 119.84 (d, 1C, $^3J$ (C,C) = 3.0 Hz, C-3), 124.98 (3′-CH-5), 126.04 (d, 1C, $^1J$ (C,C) = 68.7 Hz, C-5), 127.26 (d, 1C, $^3J$ (C,F) = 8.7 Hz, C-3a′), 130.58 (d, 1C, $^2J$ (C,C) = 4.7 Hz, C-4), 134.78 (d, 1C, $^4J$ (C,F) = 4.5 Hz, C-7a′), 137.16 (C-2), 158.40 (d, 1C, $^1J$ (C,F) = 233 Hz, C-5′), 165.37 ($\underline{C}ONHCH_2CH_2$), 169.72 (d, 1C, $^2J$ (C,C) = 2.7 Hz, C-2′). LC-MS: purity 99.7%. $MS^2$: $ESI^+$ 400.1 ($[C_{21}^{13}CH_{27}FN_4O_2+H]^+$, 40%), 327.1 ($[C_{21}^{13}CH_{27}FN_4O_2–C_4H_{10}N]^+$, 52%), 283.9 ($[C_{21}^{13}CH_{27}FN_4O_2–C_6H_{15}N_2]^+$, 100%); $ESI^-$ 398.1 ($[C_{21}^{13}CH_{27}FN_4O_2–H]^-$, 100%), 256.1 ($[C_{21}\,^{13}CH_{27}FN_4O_2–C_7H_{15}N_2O]^-$, 34%).

### *N*-(2-(Ethylamino)ethyl)-5-((5-fluoro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxamide (6a, SU12662)

A suspension of 5-((5-fluoro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (**4a**; 2.0 mmol, 0.60 g), 1-hydroxybenzotriazole (3.0 mmol, 0.40 g), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (4.0 mmol, 0.76 g), and triethylamine (6.0 mmol, 0.60 g) in *N,N*-dimethylformamide (20 mL) was stirred at room temperature for 2 h. Upon cooling to −60°C *N*-ethylethylenediamine (2.4 mmol, 0.22 g) in *N,N*-dimethylformamide (1 mL) was added, the solution was stirred 6 h at −60°C and warmed to room temperature within 18 h. Solvent removal provided a crude residue that was subjected to column chromatography on silica using a mixture of ethyl acetate, methanol, and triethylamine (8:2:1). Product fractions were combined, evaporated, dissolved in hydrochloric acid (1.0 M, 10 mL), and subsequently filtered. **6a** was precipitated as a yellow powder (0.55 g, 74%) after addition of sodium hydroxide solution (2.0 M, 5 mL), mp 280°C (decomposition). $^1$H NMR (DMSO-$d_6$) δ: 1.11 (t, 3H, $^3J$ (H,H) = 7.1 Hz, $CH_2\underline{CH_3}$), 2.43 (s, 3H, 2-$CH_3$), 2.45 (s, 3H, 4-$CH_3$), 2.76 (app q, 2H, $^3J$ (H,H) = 7.2 Hz, $\underline{CH_2}CH_3$), 2.85 (app t, 2H, $^3J$ (H,H) = 6.5 Hz, $CONHCH_2\underline{CH_2}$), 3.40 (bs, 1H, NH), 3.41 (app q, 2H, $^3J$ (H,H) = 6.1 Hz, $CONH\underline{CH_2}CH_2$), 6.84 (dd, 1H, $^3J$ (H,H) = 8.4 Hz, $^4J$ (H,F) = 4.8 Hz, 7′-H), 6.91 (ddd, 1H, $^3J$ (H,F) = 9.5 Hz, $^3J$ (H,H) = 8.5 Hz, $^4J$ (H,H) = 2.5 Hz, 6′-H), 7.70 (s, 1H, 3′-CH-5), 7.71 (t, 1H, $^3J$ (H,H) = 5.7 Hz, $CONHCH_2CH_2$), 7.74 (dd, 1H, $^3J$ (H,F) = 9.5 Hz, $^4J$ (H,H) = 2.5 Hz, 4′-H), 10.90 (s, 1H, $NH_{ind}$), 13.69 (s, 1H, $NH_{pyr}$); $^{13}$C NMR (DMSO-$d_6$) δ: 10.78 (4-$CH_3$), 13.26 ($CH_2\underline{CH_3}$), 13.58 (2-$CH_3$), 37.40 ($CONH\underline{CH_2}CH_2$), 42.74 ($\underline{CH_2}CH_3$), 47.53 ($CONHCH_2\underline{CH_2}$), 106.07 (d, 1C, $^2J$ (C,F) = 26.5 Hz, C-4′), 110.18 (d, 1C, $^3J$ (C,F) = 8.7 Hz, C-7′), 112.53 (d, 1C, $^2J$ (C,F) = 23.8 Hz, C-6′), 114.86 (d, 1C, $^4J$ (C,F) = 2.7 Hz, C-3′), 120.54 (C-3), 124.99 (3′-CH-5), 125.95 (C-5), 127.28 (d, 1C, $^3J$ (C,F) = 9.4 Hz, C-3a′), 130.50 (C-4), 134.69 (C-7a′), 136.88 (C-2), 158.38 (d, 1C, $^1J$ (C,F) = 233 Hz, C-5′), 165.12 ($\underline{C}ONHCH_2CH_2$), 169.70 (C-2′). LC-MS: purity 99.2%. $MS^2$: $ESI^+$ 371.1 ($[C_{20}H_{23}FN_4O_2+H]^+$, 17%), 325.9 ($[C_{20}H_{23}FN_4O_2–C_2H_6N]^+$, 10%), 282.9 ($[C_{20}H_{23}FN_4O_2–C_4H_{11}N_2]^+$, 100%); $ESI^-$ 368.9 ($[C_{20}H_{23}FN_4O_2–H]^-$, 100%), 254.9 ($[C_{20}H_{23}FN_4O_2–C_5H_{11}N_2O]^-$, 34%).

### *N*-(2-(Ethylamino)ethyl)-5-((5-fluoro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-[$^{13}$C]methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxamide hydrochloride (6b, $^{13}$C-SU12662)

A suspension of 5-((5-fluoro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-[$^{13}$C]methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (**4b**; 1.0 mmol, 0.30 g), 1-hydroxybenzotriazole (1.5 mmol, 0.20 g),

1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.0 mmol, 0.38 g), and triethylamine (3.0 mmol, 0.30 g) in *N,N*-dimethylformamide (20 mL) was stirred at room temperature for 2 h. Upon cooling to −60°C *N*-ethylethylenediamine (1.2 mmol, 0.11 g) in *N,N*-dimethylformamide (1 mL) was added, the solution was stirred 6 h at −60°C and warmed to room temperature within 18 h. Solvent removal provided a crude residue that was subjected to preparative HPLC on $C_{18}$-silica using a gradient of methanol–water containing 1.5% triethylamine. Product fractions were combined, evaporated *in vacuo* and dissolved in a mixture of hydrochloric acid (0.1 M, 100 mL) and methanol (400 mL). Evaporation *in vacuo* afforded **6b** as a yellow powder (0.33 g, 81%), mp 306°C (decomposition). $^1$H NMR (DMSO-$d_6$) δ: 1.21 (t, 3H, $^3J$ (H,H) = 7.3 Hz, $CH_2\underline{CH_3}$), 2.46 (s, 3H, 2-$CH_3$), 2.48 (s, 3H, 4-$CH_3$), 2.97 (app q, 2H, $^3J$ (H,H) = 7.1 Hz, $\underline{CH_2}CH_3$), 3.05 (app t, 2H, $^3J$ (H,H) = 6.2 Hz, $CONHCH_2\underline{CH_2}$), 3.55 (app q, 2H, $^3J$ (H,H) = 6.1 Hz, $CONH\underline{CH_2}CH_2$), 6.84 (dd, 1H, $^3J$ (H,H) = 8.5 Hz, $^4J$ (H,F) = 4.8 Hz, 7′-H), 6.92 (ddd, 1H, $^3J$ (H,F) = 9.6 Hz, $^3J$ (H,H) = 8.4 Hz, $^4J$ (H,H) = 2.6 Hz, 6′-H), 7.71(d, 1H, $^1J$ (H,C) = 152 Hz, 3′-CH-5), 7.75 (dd, 1H, $^3J$ (H,F) = 9.5 Hz, $^4J$ (H,H) = 2.5 Hz, 4′-H), 7.88 (t, 1H, $^3J$ (H,H) = 5.7 Hz, $CONHCH_2CH_2$), 8.98 (bs, 2H, $N^+H_2$), 10.90 (s, 1H, $NH_{ind}$), 13.72 (s, 1H, $NH_{pyr}$); $^{13}$C NMR (DMSO-$d_6$) δ: 10.89 (4-$CH_3$), 11.05 ($CH_2\underline{CH_3}$), 13.75 (2-$CH_3$), 35.67 ($CONH\underline{CH_2}CH_2$), 42.13 ($\underline{CH_2}CH_3$), 46.25 ($CONHCH_2\underline{CH_2}$), 106.10 (d, 1C, $^2J$ (C,F) = 25.5 Hz, C-4′), 110.20 (d, 1C, $^3J$ (C,F) = 8.7 Hz, C-7′), 112.58 (d, 1C, $^2J$ (C,F) = 24.0 Hz, C-6′), 115.00 (dd, 1C, $^1J$ (C,C) = 71.3 Hz, $^4J$ (C,F) = 2.7 Hz, C-3′), 119.92 (d, 1C, $^3J$ (C,C) = 3.0 Hz, C-3), 124.96 (3′-CH-5), 126.02 (d, 1C, $^1J$ (C,C) = 68.9 Hz, C-5), 127.23 (d, 1C, $^3J$ (C,F) = 9.4 Hz, C-3a′), 130.63 (d, 1C, $^2J$ (C,C) = 4.7 Hz, C-4), 134.72 (d, 1C, $^4J$ (C,F) = 4.5 Hz, C-7a′), 137.22 (C-2), 158.37 (d, 1C, $^1J$ (C,F) = 233 Hz, C-5′), 165.43 ($\underline{C}ONHCH_2CH_2$), 169.70 (d, 1C, $^2J$ (C,C) = 2.7 Hz, C-2′). LC-MS: purity 99.3%. $MS^2$: $ESI^+$ 372.1 ($[C^{13}_{19}CH_{23}FN_4O_2+H]^+$, 16%), 327.1 ($[C^{13}_{19}CH_{23}FN_4O_2-C_2H_6N]^+$, 13%), 283.9 ($[C^{13}_{19}CH_{23}FN_4O_2-C_4H_{11}N_2]^+$, 100%); $ESI^-$ 369.9 ($[C^{13}_{19}CH_{23}FN_4O_2-H]^-$, 100%), 255.9 ($[C^{13}_{19}CH_{23}FN_4O_2-C_5H_{11}N_2O]^-$, 43%).

### 2-(2-(Ethylamino)ethyl)-1*H*-isoindole-1,3(2*H*)-dione hydrochloride (7)

A mixture of phthalimide (50.0 mmol, 7.36 g) and *N*-ethylethylenediamine (50.0 mmol, 4.41 g) was heated to 100°C for 3 h, followed by an additional 1 h at 130°C. Ethanol (100 mL) was added to the hot mixture and, upon cooling to room temperature, gaseous hydrogen chloride was introduced to obtain a white precipitate. The suspension was extended with ethanol (150 mL) and heated for recrystallization to obtain **7** as white needles (8.21 g, 64%), mp 236°C, lit.[20] 232–234°C. $^1$H NMR (DMSO-$d_6$) δ: 1.18 (t, 3H, $^3J$ (H,H) = 7.3 Hz, $CH_2\underline{CH_3}$), 2.95 (app d, 2H, $^3J$ (H,H) = 6.3 Hz, $\underline{CH_2}CH_3$), 3.16 (app s, 2H, $(CO)_2NCH_2\underline{CH_2}$), 3.91 (t, 2H, $^3J$ (H,H) = 6.0 Hz, $(CO)_2N\underline{CH_2}CH_2$), 7.78–7.90 (m, 4H, 4-H, 5-H, 6-H, 7-H), 9.21 (s, 2H, $N^+H_2$); $^{13}$C NMR (DMSO-$d_6$) δ: 10.92 ($CH_2\underline{CH_3}$), 34.03 ($(CO)_2NCH_2\underline{CH_2}$), 41.83 ($\underline{CH_2}CH_3$), 44.27 ($(CO)_2N\underline{CH_2}CH_2$), 123.17 (C-4, C-7), 132.12 (C-3a, C-7a), 134.46 (C-5, C-6), 168.05 (C-1, C-3). HPLC purity: 95.8%.

### *N* - (2 - ([$^2H_5$]Diethylamino)ethyl)- 5-((5-fluoro-2-oxo-1,2-dihydro - 3*H* - indol-3-ylidene)methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxamide (8, $^2H_5$-SU11248)

A suspension of 2-(2-(ethylamino)ethyl)-1*H*-isoindole-1,3(2*H*)-dione hydrochloride (**7**; 10.0 mmol, 2.55 g), [$^2H_5$]ethyl iodide (12.0 mmol, 1.93 g) and potassium carbonate (20.0 mmol, 2.75 g) in acetonitrile (25 mL) was stirred 16 h at room temperature. The solvent was removed and the residue subjected to column chromatography on silica using ethyl acetate containing 5% triethylamine. Fractions containing 2-(2-([$^2H_5$]diethylamino)-ethyl)-1*H*-isoindole-1,3(2*H*)-dione were collected to obtain a viscious oil (5.5 mmol, 55%). Hydrazine (5.5 mmol, 0.28 g hydrazine hydrate, 100%) and ethanol (20 mL) were added and the reaction mixture refluxed for 1 h. The solvent was removed and the residue dissolved in *N,N*-dimethylformamide (10 mL). In a separate flask, 5-((5-fluoro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene) methyl) - 2,4 - dimethyl - 1*H* - pyrrole - 3 -carboxylic acid (**4a**; 5.0 mmol, 1.51 g), 1-hydroxybenzotriazole (7.5 mmol, 1.00 g), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide were stirred in *N,N*-dimethylformamide (50 mL) for 2 h at room temperature. The solution prepared above was added and the mixture stirred for additional 16 h. Upon solvent removal, the residue was taken up in a mixture of ethyl acetate (100 mL) and methanol (10 mL), heated to reflux and filtered hot. The remaining solid was subjected to preparative HPLC on $C_{18}$-silica using a gradient of methanol–water to afford **8** as a yellow powder (1.28 g, 63%), mp 217°C. $^1$H NMR (DMSO-$d_6$) δ: 0.97 (t, 3H, $^3J$ (H,H) = 7.1 Hz, $CH_2\underline{CH_3}$), 2.41 (s, 3H, 2-$CH_3$), 2.43 (s, 3H, 4-$CH_3$), 2.52 (app q, 2H, $^3J$ (H,H) = 7.6 Hz, $\underline{CH_2}CH_3$), 2.55 (app t, 2H, $^3J$ (H,H) = 6.7 Hz, $CONHCH_2\underline{CH_2}$), 3.28 (app q, 2H, $^3J$ (H,H) = 6.7 Hz, $CONH\underline{CH_2}CH_2$), 6.83 (dd, 1H, $^3J$ (H,H) = 8.5 Hz, $^4J$ (H,F) = 4.4 Hz, 7′-H), 6.91 (ddd, 1H, $^3J$ (H,F) = 9.0 Hz, $^3J$ (H,H) = 9.0 Hz, $^4J$ (H,H) = 2.6 Hz, 6′-H), 7.40 (t, 1H, $^3J$ (H,H) = 5.1 Hz, $CONHCH_2CH_2$), 7.70 (s, 1H, 3′-CH-5), 7.73 (dd, 1H, $^3J$ (H,F) = 9.5 Hz, $^4J$ (H,H) = 2.5 Hz, 4′-H), 10.85 (s, 1H, $NH_{ind}$), 13.66 (s, 1H, $NH_{pyr}$); $^{13}$C NMR (DMSO-$d_6$) δ: 10.71 (4-$CH_3$), 11.96 ($CH_2\underline{CH_3}$, $C^2H_2\underline{C^2H_3}$), 13.46 (2-$CH_3$), 37.07 ($CONH\underline{CH_2}CH_2$), 46.63 ($\underline{CH_2}CH_3$, $\underline{C^2H_2}C^2H_3$), 51.75 ($CONHCH_2\underline{CH_2}$), 106.03 (d, 1C, $^2J$ (C,F) = 25.5 Hz, C-4′), 110.13 (d, 1C, $^3J$ (C,F) = 8.4 Hz, C-7′), 112.48 (d, 1C, $^2J$ (C,F) = 24.0 Hz, C-6′), 114.73 (d, 1C, $^4J$ (C,F) = 3.0 Hz, C-3′), 120.85 (C-3), 124.98 (3′-CH-5), 125.92 (C-5), 127.30 (d, 1C, $^3J$ (C,F′) = 9.4 Hz, C-3a′), 130.28 (C-4), 134.66 (C-7a′), 136.69 (C-2), 158.36 (d, 1C, $^1J$ (C,F) = 233 Hz, C-5′), 164.65 ($\underline{C}ONHCH_2CH_2$), 169.70 (C-2′). LC-MS: purity 99.8%. $MS^2$: $ESI^+$ 404.1 ($[C_{22}H^2_{22}H_5FN_4O_2+H]^+$, 36%), 326.1 ($[C_{22}H^2_{22}H_5FN_4O_2-C_4H^2_5H_5N]^+$, 49%), 282.9 ($[C_{22}H^2_{22}H_5FN_4O_2-C_6H^2_{10}H_5N_2]^+$, 100%); $ESI^-$ 401.9 ($[C_{22}H^2_{22}H_5FN_4O_2-H]^-$, 100%), 254.9 ($[C_{22}H^2_{22}H_5FN_4O_2-C_7H^2_{10}H_5N_2O]^-$, 36%).

## Conclusion

A concise five-step synthesis was developed to obtain either $^{13}$C- or $^2$H-labelled sunitinib (SU11248) or its primary metabolite (SU12662). All compounds were characterized in detail by NMR and/or LC-MS techniques; $^{13}$C NMR assignments of the sunitinib scaffold are reported for the first time. The reference compounds and internal standards were used to establish a LC-MS procedure that allows for the quantification of both compounds in human blood samples as a basis for PK/PD modelling studies of sunitinib, such as CESAR P-I-007. Details of which will be disclosed in a future communication.

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*Supporting Information*: HPLC chromatograms for compounds **1, 2a-b, 3a-b, 4a-b**, and **7**, as well as LC-MS traces of compounds **5, 6a-b**, and **8** are available at http://www.interscience.wiley.com.

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