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Research Article

Synthesis of [^{14}C]-labelled vardenafil hydrochloride and metabolites

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Summary

For studies of pharmacokinetics and drug metabolism of the new orally active, selective phosphodiesterase type V (PDE V) inhibitor vardenafil (Levitra®), the ^{14}C-labelled version was synthesised. Starting from the cyanation of 2-iodophenol with K^{14}CN, an 8-step synthesis led to two batches with 0.727 g (2.857 GBq) and 2.199 g (5.497 GBq) of [triazinone-^{14}C]vardenafil hydrochloride with different specific radioactivities. The label was located in position 2 of the imidazotriazinone moiety. Several carbon-14 labelled metabolites were synthesised as reference compounds for metabolism studies. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: Levitra®; PDE V inhibitor; carbon-14; cyanation; nucleophilic substitution; regioselective chlorosulfonation; semi-preparative chromatography

Introduction

Vardenafil (Levitra®) is a new orally active, selective phosphodiesterase type V (PDE V) inhibitor marketed for the treatment of male erectile dysfunction. It demonstrates excellent in vivo activity at the subnanomolar level.[1]

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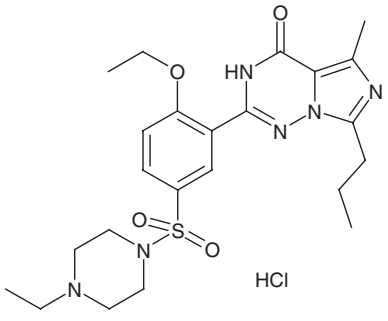


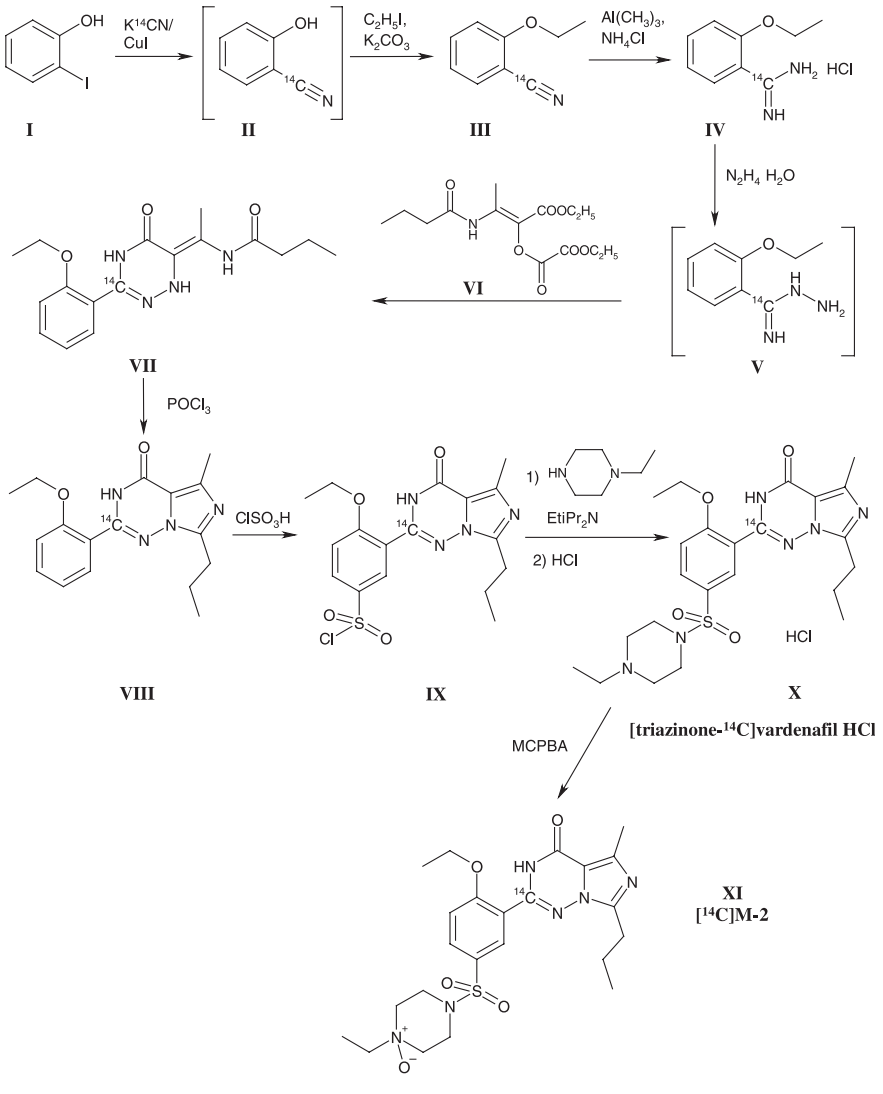
Vardenafil hydrochloride

For studies of pharmacokinetics and drug metabolism of vardenafil, the version with a metabolically stable carbon-14 label was required. In contrast to other piperazine containing molecules effective and quickly performed labelling in the piperazine moiety did not give a suitable label, because the piperazine moiety turned out to be the major site of metabolic degradation.[2] An 8-step radiosynthesis was necessary to insert the carbon-14 label in a stable position in the imidazotriazinone system of the molecule.[3]

For evaluation and interpretation of the metabolic studies several carbon-14 labelled metabolites were synthesized. The syntheses initiated from an appropriate intermediate of the main radiosynthesis.

Results and discussion

2-Hydroxybenzonitrile is the starting compound for the common vardenafil synthesis.[4] So we investigated the formation of the labelled version for introducing the carbon-14 label. The corresponding synthesis of the non-labelled compound has been described.[5] The authors used cuprous cyanide in the high boiling solvent DMF. Starting from readily available potassium [^{14}C]cyanide and 2-iodophenol we performed the reaction successfully in *N*-methylpyrrolidinone (NMP) at a temperature of 180°C with the less reactive reagent system K^{14}CN/CuI (reaction scheme 1). The subsequent phenol ether formation was carried out by conventional reaction with ethyl iodide and potassium carbonate. The 2-hydroxybenzo[^{14}C]nitrile was introduced into the etherification reaction without isolation and purification. The



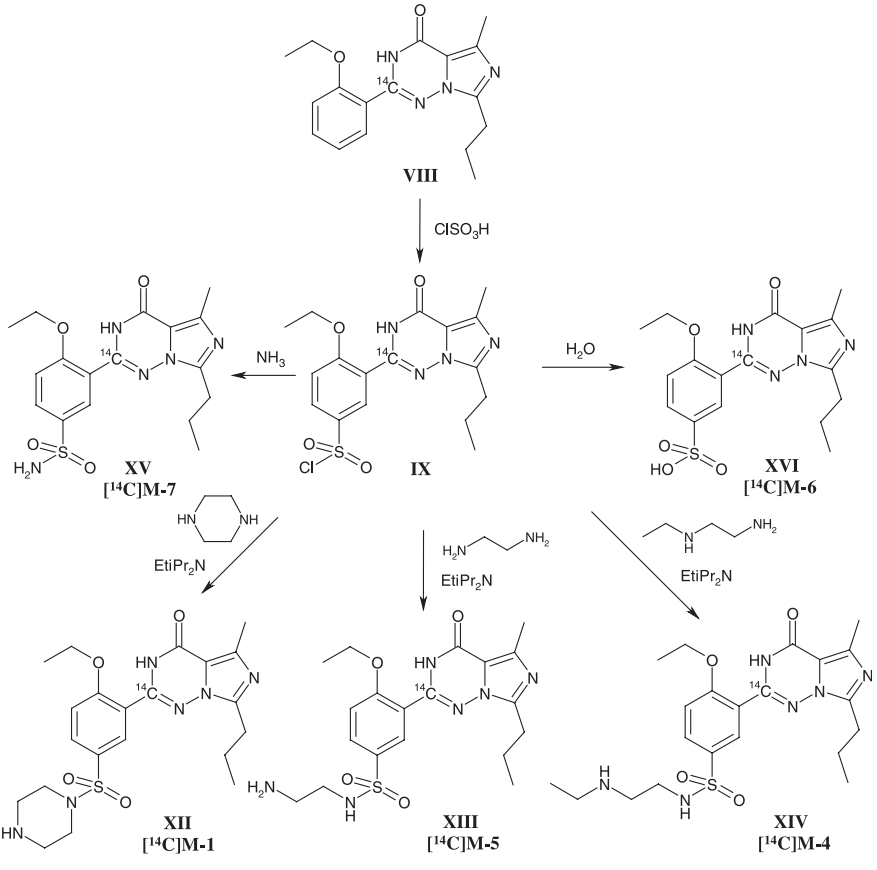
Reaction Scheme 1.

resulting 2-ethoxybenzo[^{14}C]nitrile (III) was then purified chromatographically on silica gel. The desired amidine derivative IV was obtained by reaction with trimethylaluminium and ammonium chloride. It was isolated in good yield as the hydrochloride as described in the literature.[4] The above steps were carried out in three separate batches due to the high level of radioactivity employed (starting activity 67 GBq).

Hydrazinolysis to the intermediate V was performed with hydrazine hydrate at a maximum temperature of 0°C within some minutes. The

2-ethoxybenzene[^{14}C]carboximidohydrazide (V) was subsequently reacted with the enolester VI to form the triazinone derivative VII. VI was synthesised via the Dakin-West reaction according to Hartley *et al.*[6] who described also some similar condensations. Optimisation experiments showed that the highest yields were achieved when both V and VI were used without isolation and purification. The obtained VII was purified by low pressure chromatography on silica gel due to the high amounts of by-products formed and to obtain a better yield in the subsequent cyclisation step.

Cyclisation of VII with phosphorus oxychloride gave the imidazotriazinone system VIII in good quality and yield. Due to its stability an aliquot of the compound was separated as starting compound for the synthesis of the carbon-14 labelled metabolites. Most of it was



Reaction Scheme 2.

converted to the sulfochloride derivative IX with chlorosulfonic acid. The reaction proceeded under very mild conditions with complete regioselectivity at the 5-position which seemed to be typical for this substitution pattern.[7] Final coupling with *N*-ethylpiperazine afforded the free base of X which was converted to [^{14}C]vardenafil hydrochloride by addition of a defined amount of hydrochloric acid. Purification was performed by recrystallization. Most of the product was diluted with non-labelled vardenafil in order to reduce the specific radioactivity for use in the ADME studies and to ensure better stability against radiolysis.

To aid in the interpretation of metabolic studies with [triazinone-^{14}C]vardenafil hydrochloride some important metabolites were required as carbon-14 labelled reference compounds. The syntheses of the derivatives with different functionalisation of the sulfonyl group started from the labelled intermediate VIII (reaction scheme 2). Sulfochloride IX was obtained from freshly purified VIII by a known procedure and used as a key intermediate for coupling with the different amines and water, respectively (reaction scheme 2).

The *N*-oxide metabolite [^{14}C]M-2 was synthesized directly by oxidation of the free base of X with 3-chloroperbenzoic acid (reaction scheme 1).

Most of the labelled metabolites had to be purified chromatographically.

Experimental

General methods

The ^{1}H NMR spectra were recorded on a Bruker DRX 400 nuclear magnetic resonance spectrometer. Radiochemical purities were determined with the GC system HP 5890 equipped with a radioactivity detector FHT 7000. HPLC analyses of the final products were performed on a HP 1050 Series II instrument. Nucleosil® 120 C-18 was used as stationary phase and acetonitrile/phosphate buffer (pH 6.5) as mobile phase. Radioactivity and UV signals (radioactivity detector Ramona® 92) were recorded. Radiochemical counting was performed on a liquid scintillation analyzer TRI-CARB® 2500 TR using Ultima Gold™ cocktail. GC/MS detection was performed on a HP 5890 equipped with MS HP 5970. LC mass spectrometric analysis was performed on a PE/Sciex API III mass spectrometer with MacIntosh

Quadra® 900. Semi-preparative chromatography was performed with WellChrom Maxi-Star® K 1000, Knauer variable wavelength monitor and Merck/Hitachi D-2500A Chromato-Integrator.

Labelling procedure

2-Hydroxy-benzo[^{14}C]nitrile, (II). Potassium [^{14}C]cyanide was obtained from Amersham International plc with a specific radioactivity of 30 MBq/mg. The chemical purity was 99.3%.

An aliquot of 28.9 GBq (13.7 mmol) of K^{14}CN was suspended in 17 ml of 1-methyl-2-pyrrolidinone (NMP) and 2.64 g (12 mmol) of 2-iodophenol together with 2.53 g (13.3 mmol) of cuprous iodide were added. The reaction mixture was stirred for 5 h at 180°C, then the conversion was checked as complete by GC. Two additional syntheses were performed in the same manner starting with 18.42 and 19.6 GBq of K^{14}CN, respectively. The product containing mixtures were used without isolation or purification in the subsequent etherification.

2-Ethoxy-benzo[^{14}C]nitrile, (III). Potassium carbonate (7.81 g, 56.5 mmol) and ethyl iodide (1.37 ml, 17.1 mmol) were added to the mixture of crude II of the first batch. Then 35 ml of acetone was added and the reaction mixture was heated at 80°C for 70 min. The acetone was evaporated off and 150 ml of water and 250 ml of dichloromethane were added to the residue. A precipitate was removed by filtration and the filter cake was washed four times with 20 ml of dichloromethane. The organic layer of the mother liquor was separated and evaporated to dryness. The oily residue contained III and NMP. Two additional syntheses were performed in the same manner with the second and third batch of II. The residues of the three 2-ethoxy-benzo[^{14}C]nitrile batches were combined (total of 38.4 g), dissolved in 35 ml of dichloromethane and purified chromatographically in 17 runs on a column Lobar® Si 60 size C using CH_2Cl_2/CH_3OH 9:1 as eluent, a flow of 15 ml/min. and UV detection at 230 nm.The product containing fractions were combined and evaporated.

Yield: 6.14 g of compound III, radiochemical purity (radio-GC): 95%, chemical purity (GC): 65.5%. Residual NMP was detected as 30%. With this value the yield of III was calculated as 27 mmol which corresponds to 85% of theoretical related to K^{14}CN which was used in slight excess. The structure was confirmed by GC/MS with $m/z = 149$ $[^{14}C\text{-}M]^+$.

2-Ethoxy-benz[^{14}C]amidine hydrochloride, (IV). Ammonium chloride (2.5 g, 46.7 mmol) was suspended in 32 ml of toluene under an argon atmosphere. The mixture was cooled in an ice bath and 24 ml (48 mmol) of trimethylaluminium (2 M in toluene) was added dropwise. The mixture was stirred without further cooling until gas formation had ceased. Then an aliquot of III (2 g, 8.8 mmol) was added and the reaction mixture was stirred for 9 hs under reflux. After cooling to room temperature 1.6 g of silica gel was added and excess trimethylaluminium quenched by careful addition of 16 ml of methanol with ice bath cooling. The resulting suspension was diluted with 16 ml of dichloromethane and stirred without further cooling until hydrogen formation had ceased. The solid was removed by filtration and washed four times with 8 ml of methanol. After the addition of 5 drops of 37% hydrochloric acid the mother liquor was evaporated, the residue was dissolved in 24 ml of CH_2Cl_2/CH_3OH (9:1) and filtered off from a small amount of insoluble solid. The solution was evaporated to dryness and the semi-solid residue was dissolved in 3 ml of CH_2Cl_2/CH_3OH 9:1. Then 1 ml of 37% hydrochloric acid was added and the mixture was evaporated again. The residue was suspended in 25 ml of diethyl ether and stirred for 30 min at room temperature. The clear supernatant was decanted and the residue washed twice with 3 ml of diethyl ether and dried under vacuum.

Yield: 2.43 g of compound IV.

The rest of compound III was converted in two separate syntheses in the same manner.

N-{1-[3-(2-Ethoxy-phenyl)-5-oxo-4,5-dihydro-1H-[3-^{14}C][1,2,4]triazin-6-ylidene]-ethyl}-butyramide, (VII). An aliquot of compound IV (3.15 g) was dissolved in 16 ml of anhydrous ethanol. The mixture was cooled in an ice bath and 0.79 ml (15.3 mmol) of hydrazine hydrate was added over the course of 15 min. Ammonium chloride was precipitated. Then 19 g of oxalic acid 2–butyrylamino-1-ethoxycarbonyl-propenyl ester ethyl ester (VI), diluted with 10 ml of ethanol, was added. The reaction mixture was stirred for 30 min at room temperature and subsequently for 5 hs under reflux. The solvent was removed by evaporation and the oily residue was partitioned between 50 ml of dichloromethane and 20 ml of water. The organic layer was evaporated to dryness and the residue dried azeotropically twice by addition and evaporation of 25 ml of ethanol. The crude VII (16.55 g) was dissolved in 33 ml of dichloromethane and purified chromatographically in 21 runs on a

column Lobar® Si 60 size C using CH_2Cl_2/CH_3OH (95:5) as eluent, a flow of 25 ml/min. and UV detection at 230 nm.The product containing fractions were combined and evaporated.

Yield: 2.35 g (6.48 mmol) of compound VII as a thicky yellow oil, chemical purity (GC): 91%.

A second batch started from 3.15 g of IV and gave 1.99 g (5.55 mmol) of compound VII, chemical purity (GC): 92%.

2-(2-Ethoxy-phenyl)-5-methyl-7-propyl-3H-[2-^{14}C]imidazo[5,1-f]triazin-4-one, (VIII). The first batch of compound VII (2.35 g) was dissolved in 27 ml of dichloroethane. Phosphoryl chloride (1.35 ml, 14.37 mmol) was added and the reaction mixture was stirred for 2 hours under reflux. Then it was evaporated, the residue was dissolved in 25 ml of dichloromethane, washed twice with 10 ml of saturated sodium bicarbonate solution and subsequently with water, dried over sodium sulphate and evaporated carefully due to vigorous foaming of the product.

Yield: 1.717 g (5.23 mmol) of compound VIII, chemical purity (GC): 92%, radiochemical purity (radio-GC): 86%.

A second batch starting from 1.99 g of VII, gave 1.69 g (4.93 mmol) of compound VIII, chemical purity (GC): 91%.

Both batches were combined, dissolved in dichloromethane and diluted to exactly 25 ml.The total radioactivity was determined by LS counting to be 17.953 GBq. This amount corresponds to 26.8% of the starting radioactivity. With a specific radioactivity of 2.035 GBq/mmol a product content of 8.82 mmol (2.75 g) was calculated.

4-Ethoxy-3-(5-methyl-4-oxo-7-propyl-3,4-dihydro-[2-^{14}C]imidazo[5,1-f][1,2,4]-triazin-2-yl)-benzene-sulfonyl chloride, (IX). An aliquot of compound VIII (14.31 GBq, 2.19 g, dissolved in 20 ml of dichloromethane) was cooled in an ice bath and 3.5 ml (52.5 mmol) of chlorosulfonic acid was added dropwise whilst cooling. The mixture was stirred for one hour in an ice bath and subsequently for 16 hs at room temperature. Then 12 ml of ice cold water was added carefully whilst cooling. The organic layer was washed four times with 8 ml of cold water, dried over sodium sulphate and evaporated to dryness under reduced pressure.

Yield: 2.95 g of compound IX.

The crude product was used without further purification in the next step.

[^{14}C]Vardenafil hydrochloride, 2-[2-ethoxy-5-(4-ethyl-piperazine-1-sulfonyl)-phenyl]-5-methyl-7-propyl-3H-[2–^{14}C]imidazo[5,1-f][1,2,4]triazin-4-one hydrochloride, (X). The labelled IX (2.95 g) was dissolved in 14 ml of dichloromethane and 1.06 g (8.25 mmol) of *N*-ethyl-diisopropylamine and subsequently a solution of 0.82 g (7.18 mmol) of *N*-ethyl-piperazine in 4 ml of dichloromethane was added. The reaction mixture was stirred for 16 hs at room temperature. The solution was washed three times with 12 ml of water, twice with 12 ml of 5% sodium bicarbonate solution and finally with 12 ml of water. The organic layer was dried over sodium sulphate and evaporated to dryness giving 2.99 g of crude X.

An aliquot of 2.8 mg was dissolved in acetonitrile and diluted exactly to 10 ml. The content of pure X was determined as 2.25 mg by UV quantification using defined non-labelled reference compound. From this result the total amount of pure X was calculated as 2.4 g (4.91 mmol). The total radioactivity was 10.782 GBq which corresponds to a total yield of 20.1%, based on the starting K^{14}CN.

For formation of the desired hydrochloride the crude product was dissolved in 17 ml of boiling acetone and 0.64 ml (4.93 mmol) of 25% hydrochloric acid was added. The product crystallised during cooling to room temperature. An additional 2 ml of acetone was added and the suspension stored for 65 hs in a refrigerator. The product was filtered off, washed three times with 6 ml of acetone (a remarkable solubility was observed) and dried in an evacuated desiccator over Blaugel.

Yield: 0.73 g of [triazinone-^{14}C]vardenafil hydrochloride (1.38 mmol), radiochemical purity: $\geq$ 97% by radio-HPLC, chemical purity (HPLC): $\geq$ 99%.

From the above mother liquor and washing liquors three batches of free base [triazinone-^{14}C]vardenafil were isolated by concentration, dissolution in water and addition of saturated sodium bicarbonate solution. The combined amounts (767 mg + 392 mg + 418 mg) were purified by recrystallisation from 17.5 ml of acetone/water (1:1) giving 1.28 g (2.61 mmol) of [triazinone-^{14}C]vardenafil (free base), radiochemical purity: $\geq$ 98% by radio-HPLC, chemical purity (HPLC): $\geq$ 96%.

For formation of the desired hydrochloride and reduction of the specific radioactivity the total amount was dissolved in 80 ml of methanol and 0.815 g (1.55 mmol) of non-labelled vardenafil hydrochloride and 2.55 ml of 1 M hydrochloric acid (2.55 mmol)

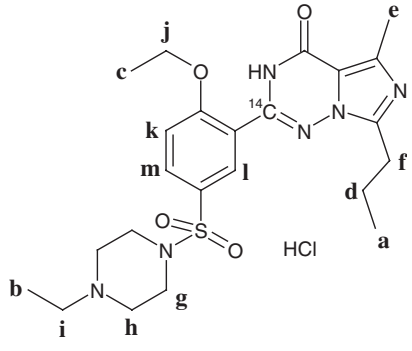
were added. Very small amounts of insoluble components were removed by filtration and the solution was used for characterisation and storage.

The total radioactivity was 5.5 GBq. The weight was calculated as 2.199 g (4.17 mmol) of [triazinone-^{14}C]vardenafil hydrochloride with a specific radioactivity of 2.50 MBq/mg. The radiochemical purity was determined as $\geq 97\%$; the chemical purity (HPLC) was $\geq 97\%$.

Determination of the specific radioactivity

The specific radioactivity of both product batches was determined by two different methods. A weighed amount of the labelled compound was dissolved in water and the total radioactivity was determined by LS counting. Secondly the concentration of [triazinone-^{14}C]vardenafil hydrochloride in solution was determined by HPLC/UV and quantified using non-labelled reference compound. The specific radioactivity of batch 1 (non-diluted) was 2.063 GBq/mmol (3.93 MBq/mg) and of batch 2 (diluted) as 1.313 GBq/mmol (2.50 MBq/mg).

The total radioactivity of 8.354 GBq (2.87 GBq of batch 1 and 5.5 GBq of batch 2) corresponds to a yield of 16% of the theory, based on the starting K^{14}CN.



NMR (DMSO-d_6) δ (ppm); 0.93 (m, 3H, **a**), 1.20 (t, 3H, **b**), 1.33 (t, 3H, **c**), 1.74 (m, 2H, **d**), 2.50 (s, 3H, **e**), 2.80 (multiplets, 4H, **f**+**g**), 3.09+3.42 (multiplets, 6H, **i**+**h**), 3.81 (d, 2H, **g'**), 4.22 (q, 2H, **j**), 7.41 (d, 1H, **k**), 7.93 (s+d, 2H, **l**+**m**), 10.68 (broad, 1H, **HCl**), 11.84 (s, 1H, **NH**).

Metabolite syntheses

2-[2-Ethoxy-5-(4-ethyl-4-oxo-piperazine-1-sulfonyl)-phenyl]-5-methyl-7-propyl-3H-[2–^{14}C]imidazo-[5,1–f][1,2,4]triazin-4-one,[^{14}C]M-2,(XI). The free base [triazinone-^{14}C]vardenafil was liberated from 25.7 mg (0.049 mmol) of X with sodium bicarbonate solution, extracted with dichloromethane and isolated by evaporation. The solid was dissolved in 1.5 ml of dichloromethane and a solution of 20 mg of 3-chloroperbenzoic acid (50–60%) in 0.5 ml of dichloromethane was added. The reaction mixture was stirred for 5 hs at room temperature, then the solvent was removed by passing argon over the surface of a warm solution. The remaining solid was dissolved in 2 ml of acetonitrile and purified in 4 runs by semipreparative HPLC on Nucleosil® 100 C 18 (125 × 16 mm; 7 µm) with the eluent acetonitrile/0.02% NEt_3-solution 50:50 (v/v), a flow of 5 ml/min. and UV detection at 230 nm. The combined product containing fractions were evaporated to dryness.

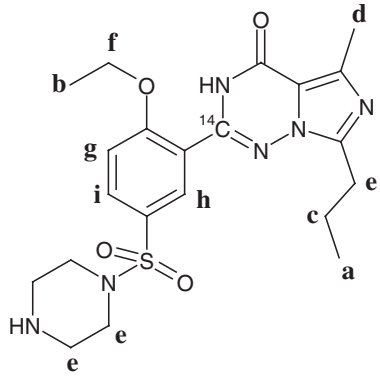
Yield: 18 mg (0.035 mmol) of XI, radiochemical purity = 98% by radio-HPLC, chemical purity (HPLC) = 99%.

The specific radioactivity was calculated from the ratio of the molecular ion peaks $[^{14}C–M+H]^+$ = 394 and $[^{12}C\text{-}M+H]^+$ = 392 as 2.034 GBq/mmol (4.03 MBq/mg).

2-[2-Ethoxy-5-(piperazine-1-sulfonyl)-phenyl]-5-methyl-7-propyl-3H-[2–^{14}C]imidazo-[5,1–f][1,2,4]triazin-4-one, [^{14}C]M-1,(XII). The labelled intermediate IX was freshly prepared from the above aliquot of VIII. Piperazine (69 mg, 0.8 mmol) and *N*-ethyl-diisopropylamine (70 µl, 0.4 mmol) were dissolved in 1.4 ml of dichloromethane. A solution of 129 mg of the sulfochloride IX in 1.5 ml of dichloromethane was added over a course of 5 min. at room temperature. The mixture was stirred for 16 hours at room temperature. Then 2 ml of water was added, the organic layer separated, washed with 2 ml of water, dried over sodium sulphate and evaporated. The crude XII (148 mg) was dissolved in 7 ml of acetonitrile and purified in 14 runs by semipreparative HPLC on Nucleosil® 100 C 18 (125 × 16 mm; 7 µm) with the eluent acetonitrile/0.1% ammonia 30:70 (v/v), a flow of 6 ml/min. and UV detection at 230 nm. The combined product containing fractions were concentrated to approximately 15 ml. The product was extracted three times with 10 ml of dichloromethane. The combined extracts were evaporated to dryness.

Yield: 91 mg (0.198 mmol) of XII, radiochemical purity = 98% by radio-HPLC, chemical purity (HPLC) = 97%.

The specific radioactivity was determined by the above mentioned gravimetric method as 2.023 GBq/mmol (4.39 MBq/mg). The structure was confirmed by electrospray-MS with m/z = 467 $[^{14}C\text{-}M + H]^+$ and by ^1H NMR.



NMR (DMSO-d_6) δ (ppm); 0.92 (m, 3H, **a**), 1.31 (t, 3H, **b**), 1.72 (m, 2H, **c**), 2.49 (s, 3H, **d**), 2.71 + 2.80 (m + m, 10H, **e**), 4.20 (q, 2H, **f**), 7.38 (d, 1H, **g**), 7.80 (s, 1H, **h**), 7.83 (d, 1H, **i**).

N-(2-Aminoethyl)-4-ethoxy-3-(5-methyl-4-oxo-7-propyl-3,4-dihydro[2–^{14}C] imidazo-[5,1–f][1,2,4]triazin-2-yl)benzenesulfonamide, [^{14}C]M-5, (XIII). XIII was prepared according to the synthesis of XII using 16.7 µl (0.25 mmol) of 1,2-diaminoethane and 22.6 µl (0.13 mmol) of *N*-ethyl-diisopropylamine in 0.5 ml of dichloromethane and a solution of 41 mg of freshly prepared sulfochloride IX in 1 ml of dichloromethane. The eluent of the chromatography was changed to acetonitrile/0.1% ammonia 22:78. The isolated product (40 mg) was digested in 7.5 ml of methanol and some insoluble components were removed by filtration. The filtrate was evaporated.

Yield: 27.6 mg (0.064 mmol) of XIII, radiochemical purity = 97% by radio-HPLC, chemical purity (HPLC) = 97%.

The specific radioactivity was determined by the above mentioned gravimetric method as 2.017 GBq/mmol (4.64 MBq/mg). The structure was confirmed by electrospray-MS with *m/z* = 437 $[^{14}C\text{-}M + H]^+$.

4-Ethoxy-N-(2-ethylamino-ethyl)-3-(5-methyl-4-oxo-7-propyl-3,4-dihydro[2–^{14}C]imidazo-[5,1–f][1,2,4]triazin-2-yl)benzenesulfonamide, [^{14}C]M-4, (XIV). XIV was prepared according to the synthesis of XII using 26.3 µl (0.25 mmol) of 2-ethylamino-ethylamine, 22.6 µl (0.13 mmol) of *N*-ethyl-diisopropylamine and 41 mg of freshly prepared sulfochloride IX. The eluent of the chromatography was varied to acetonitrile/0.1% ammonia 20:80. The isolated product was digested in 7.5 ml of methanol and some insoluble components were removed by filtration. The filtrate was evaporated.

Yield: 14.7 mg (0.032 mmol) of XIV, radiochemical purity = 97% by radio-HPLC, chemical purity (HPLC) = 97%.

The specific radioactivity was determined by the above mentioned gravimetric method as 1.964 GBq/mmol (4.24 MBq/mg). The structure was confirmed by electrospray-MS with m/z = 465 $[^{14}C\text{-}M+H]^+$.

4-Ethoxy-3-(5-methyl-4-oxo-7-propyl-3,4-dihydro-[2-^{14}C]imidazo[5,1-f][1,2,4]triazin-2-yl)-benzenesulfonamide, [^{14}C]M-7, (XV). Due to the fact that the very pure intermediate VIII provides pure sulfochloride IX, which gives pure compounds XV and XVI, the crude starting intermediate VIII (378 mg) was purified chromatographically on Lobar® Si 60 (310 × 25 mm) with the eluent dichloromethane/methanol 95:5 (v/v), a flow of 6 ml/min. and UV detection at 254 nm (14 runs) giving 303 mg of pure VIII, radiochemical purity ≥97% by radio-HPLC, chemical purity (HPLC) ≥97%.

For formation of the desired amide 0.5 ml of 25% ammonia solution was added to 33 mg (0.08 mmol) of fresh sulfochloride IX, prepared from purified VIII. The mixture was stirred for 2 h at room temperature, then water and ammonia were removed by passing argon over the surface of a warm solution. The solid residue was suspended in 1 ml of ice-cold water, filtered off, washed twice with 1 ml of ice-cold water and dried for 18 hours in an evacuated desiccator over Blaugel.

Yield: 26.6 mg (0.068 mmol) of XV, radiochemical purity = 96% by radio-HPLC, chemical purity (HPLC) = 95%.

The specific radioactivity was calculated from the ratio of the molecular ion peaks $[^{14}C\text{-}M+H]^+$ = 394 and $[^{12}C\text{-}M+H]^+$ = 392 as 2.100 GBq/mmol (5.37 MBq/mg).

4-Ethoxy-3-(5-methyl-4-oxo-7-propyl-3,4-dihydro-[2-^{14}C]imidazo[5,1-f][1,2,4]triazin-2-yl)-benzenesulfonic acid, [^{14}C]M-6, (XVI). Sulfochloride IX from the above batch (33 mg, 0.08 mmol) was dissolved in 0.5 ml

of acetonitrile and 1 ml of water was added. The mixture was stirred for 18 hs at room temperature, then the solvents were removed by passing argon over the surface of a warm solution.

Yield: 28 mg (0.071 mmol) of XVI, radiochemical purity = 97% by radio-HPLC, chemical purity (HPLC) = 97%.

The specific radioactivity was calculated from the ratio of the molecular ion peaks $[^{14}C–M+H]^+$ = 395 and $[^{12}C\text{-}M+H]^+$ = 393 as 2.100 GBq/mmol (5.35 MBq/mg).

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References

1. Bischoff E, Niewöhner U, Es Sayed M, Schenke T, Schlemmer K-H *J Urol* 2000; **165**: 1316.
2. Radtke M, Karl W, Seidel D, Schnorbus H, Schreiber A, Boberg M. *Abstract 231*, 6th *International ISSX Meeting*, Oct. 7–11, 2001, Munich, Germany (*Drug Metab Rev* 2001; **33**: Suppl. 1).
3. Seidel D. *J Label Compd Radiopharm* 2000; **44**: 961.
4. Haning H, Niewöhner U, Schenke T, Es Sayed M, Schmidt G, Lampe T, Bischoff E. *Bioorg Med Chem Lett* 2002; **12**: 865.
5. Sakai K, Isida I. *Patent application* JP 60-184059, 1985.
6. Charles I, Latham DWS, Hartley D, Oxford AW, Scopes D.I.C. *J Chem Soc Perkin Trans I* 1980: 1139.
7. Terrett NK, Bell AS, Brown D, Ellis P. *Bioorg Med Chem Lett* 1996; **6**: 1819.

