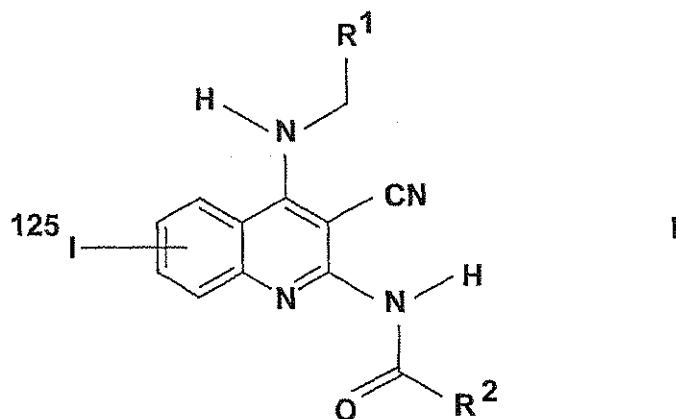
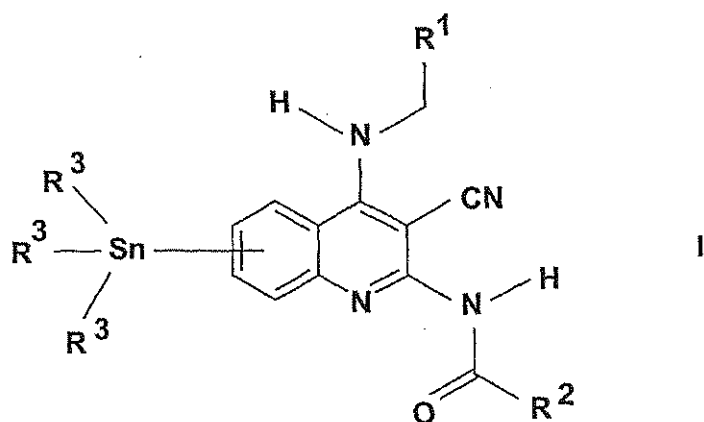


ISOTOPICALLY MARKED QUINOLINE DERIVATIVES AS ADENOSIN A₃ RECEPTOR LIGANDS

The present invention relates to adenosin A₃ receptor ligands labeled with iodine isotops of mass number 125, within those favourably to antagonists and their isomers, to the experimental materials containing them, to a process for the preparation of the compounds of the general formula (I)



and their isomers, to the new intermediates of the general formula (II)



and to the preparation thereof.

Adenosine is a well-known component of several endogenous molecules (ATP, NAD⁺, nucleic acids). Besides, it plays an important regulatory role in many physiological processes. The effect of adenosine on heart function was discovered already in 1929. (Drury and Szentgyörgyi, J Physiol 68:213, 1929). The identification of an increasing number of physiological functions mediated by adenosine and the discovery of new

adenosine receptor subtypes give possibilities for therapeutic application of specific ligands (Poulse, S. A. and Quinn, R. J. *Bioorganic and Medicinal Chemistry* 6:619, 1998).

5 To date, the receptors for adenosine have been classified into three main classes: A₁, A₂ and A₃. The A₁ subtype is partly responsible for inhibiting the adenylate cyclase by coupling to G_i membrane protein, partly influences other second messenger systems. The A₂ receptor subtype can be subdivided into two further subtypes – A_{2a} and A_{2b} -, which receptors
10 stimulate the adenylate cyclase activity. The sequence of adenosine A₃ receptors have been recently identified from rat testis cDNA library. Later it was proved that it corresponds to a novel, functional adenosine receptor. The activation of the A₃ receptors is connected also with several second-messenger systems: inhibiting of adenylate cyclase, stimulating
15 phospholipase C and D.

The adenosine receptors are found in several organs and regulate their functions. Both A₁ and A_{2a} receptors play important roles in the central nervous system and cardiovascular system. In the CNS, the adenosine inhibits the release of synaptic transmitters which effect is mediated by A₁ receptors.
20 In the heart, also the A₁ receptors mediate the negative inotropic, chronotropic and dromotropic effects of adenosine. The adenosine A_{2a} receptors, which located relatively in a higher amount in the striatum, display a functional interaction with dopamine receptors in regulating the synaptic transmission. The A_{2a} adenosine receptors on endothelial and smooth muscle cells are
25 responsible for adenosine-induced vasodilation.

On the basis of mRNA identification, the A_{2b} adenosine receptors are widely distributed in different tissues. They have been identified almost in every cell type, but its expression is the highest in the intestine and the

bladder. This subtype probably also has important regulatory function in the regulation of the vascular tone and plays a role in the function of mast cells.

5 Contrary to A_1 and A_{2a} receptors, where the tissue distribution was detected on the protein level, the presence of A_{2b} and A_3 receptors was detected on the basis of their mRNA level. Expression levels for A_3 adenosine receptors are rather low comparing to other subtypes and highly species dependent. A_3 adenosine receptors are expressed primarily in the central
10 nervous system, testis, immun system and appear to be involved in the modulation of mediator release from mast cells in immediate hypersensitivity reaction.

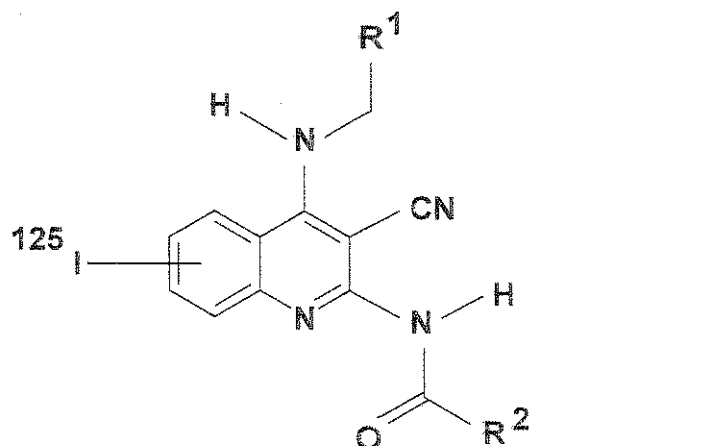
For therapeutic use it is essential to ensure that the molecule does not bind, or bind only in the case of very high concentration to the A_1 , A_{2a} and
15 A_{2b} sub-types of the adenosine receptor. Our present invention relates to the compounds of the general formula (I) labeled with iodo isotops of mass number 125, and to their salts, solvates and isomers, which have great selectivity for the A_3 sub-type of the adenosine receptor.

The [3H]-MRE 3008-F20 adenosine A_3 receptor antagonist radioligand
20 is known from the literature. (P.G. Baraldi, Bioorganic and Medicinal Chemistry Letters, 10, 209-211, 2000). Also known is the [3H]PSB-11 A_3 receptor antagonist radioligand (C.H. Müller, Bioorganic and Medicinal Chemistry Letters, 12, 501-503, 2002).

Our aim was to prepare A_3 radioligands of antagonistic effect labelled
25 with iodine isotope of mass number 125, since these have higher specific activity compared to those labelled with tritium. The goal was to prepare radioligands having strong affinity to the adenosin A_3 receptor, but at the same time showing high selectivity within the subtypes, i.e. binding in much higher concentration to the A_1 , A_{2a} and A_{2b} receptors. A further aim was to

have radioligands suitable for the characterisation of the A₃ receptor in the different tissues and for the study of the mechanism of action of A₃ antagonists.

In a first aspect, there is provided compounds of the general formula (I)

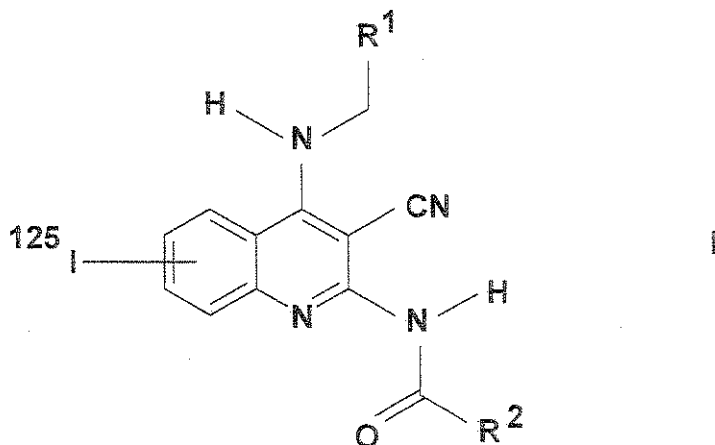


5 and their isomers – where in the formula

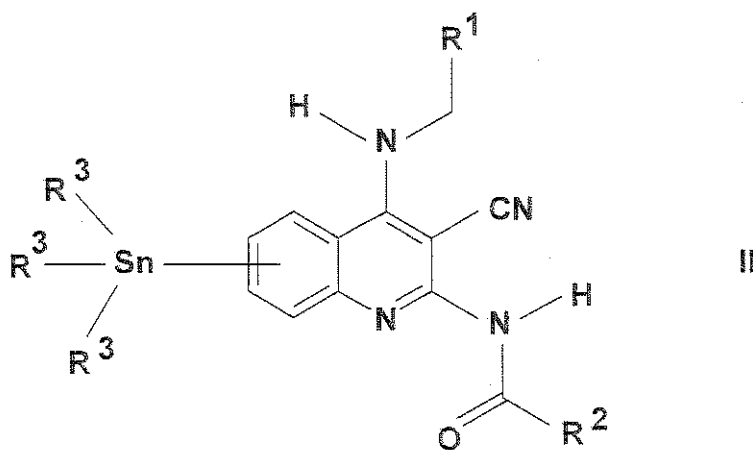
- 10 R¹ stands for hydrogen atom or a straight or branched C₁₋₄ alkyl group, or a C₃₋₆ cycloalkyl group, or a phenyl group, thienyl group, or furyl group, optionally substituted with one or more straight or branched C₁₋₄ alkyl group, straight or branched C₁₋₄ alkoxy group, or halogen atom, or for a 5- or 6-membered heteroaromatic ring -containing one, two or three nitrogen atoms-, or for a 5-membered heteroaromatic ring -containing one nitrogen atom and one oxygen atom or one nitrogen atom and one sulphur atom- optionally substituted with one or more straight or branched C₁₋₄ alkyl group, straight or branched C₁₋₄ alkoxy group, or halogen atom;
- 15 R² stands for hydrogen atom or for a straight or branched C₁₋₄ alkyl group, or for a phenyl-, benzyl-, thienyl- or furyl-group -optionally substituted with a methylenedioxy group, or one or more straight or branched C₁₋₄ alkyl group, or straight or branched C₁₋₄ alkoxy-, hydroxyl-, trifluoromethyl- or cyano-group, or halogen atom-, or for a 5- or 6-membered heteroaromatic ring -
- 20 containing one, two or three nitrogen atoms, or one nitrogen atom and one oxygen atom, or one nitrogen atom

and one sulphur atom -optionally substituted with one or more straight or branched C₁₋₄ alkyl group, straight or branched C₁₋₄ alkoxy group, or halogen atom.

In a second aspect, there is provided a process for the preparation of the compounds of the general formula (I)

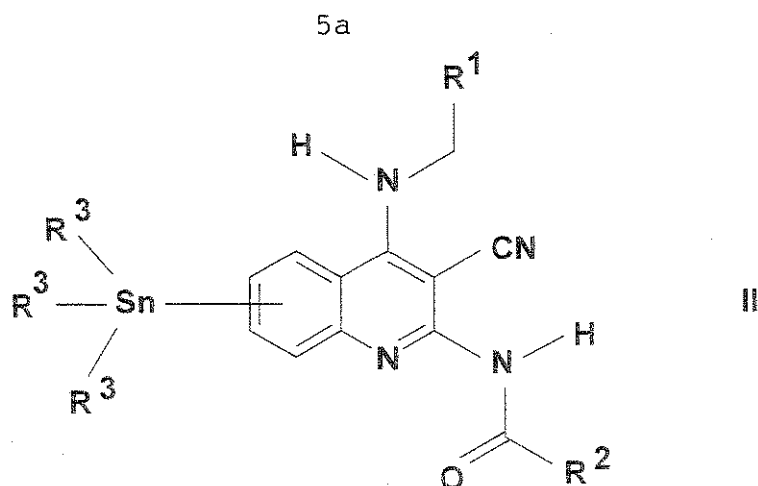


- where R¹ and R² have the meanings as defined in Claim 1.- characterized in that a trialkyl-stannyl compound of the general formula (II)



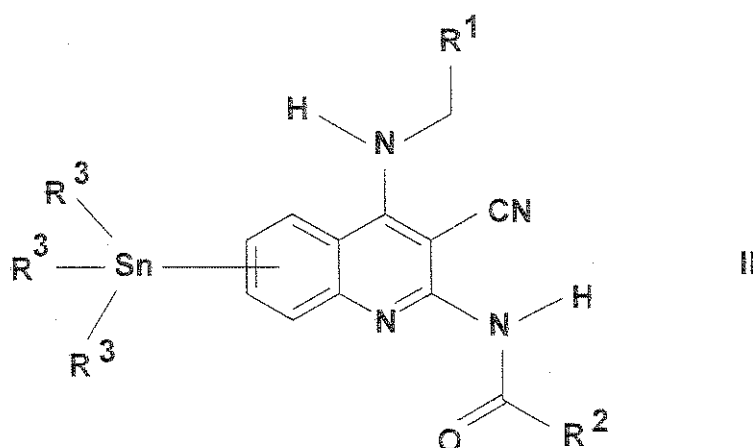
- where R¹ and R² have the meanings as defined in Claim 1. and R³ stands for straight or branched C₁₋₄ alkyl group- is reacted with [¹²⁵I]NaI, in the presence of an oxidant.

In a third aspect, there is provided compounds of the general formula (II)

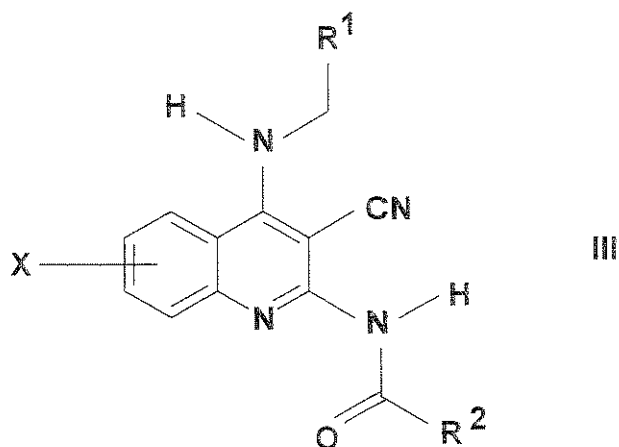


and their isomers – where R^1 and R^2 have the meanings as defined in Claim 1. and R^3 stands for straight or branched C_{1-4} alkyl group.

In a fourth aspect, there is provided a process for the preparation of the compounds of the general formula (II)



- where in the formula R^1 and R^2 have the meanings as defined in Claim 1. and R^3 stands for straight or branched C_{1-4} alkyl group– characterized in that a compound of the general formula (III)



- where in the formula R^1 and R^2 have the meanings as defined in Claim 1. and X stands for iodo- or bromo atom – is reacted with hexaalkyl distannane, in an organic solvent, in the presence of a base and palladium catalyst.

Detailed meanings of the above substituents are as follows:

5 By straight or branched C_{1-4} alkyl group we mean methyl-, ethyl-, propyl-, isopropyl-, butyl-, isobutyl-, secondary-butyl-, tert.-butyl-, preferably ethyl- or methyl group.

By straight or branched C_{1-4} alkoxy group we mean methoxy-, ethoxy-, propoxy-, isopropoxy-, butoxy-, isobutoxy-, sec.-butoxy-, tert.-butoxy-, preferably
10 ethoxy- or methoxy group.

By C_{3-6} cycloalkyl group we mean cyclopropyl-, cyclobutyl-, cyclopentyl- or cyclohexyl group.

The heteroaromatic ring containing one or two or three nitrogen atoms may mean pyrrole, imidazole, pyrazole, 1,2,3-triazole, 1,2,4-triazole, pyridine,
15 pyrimidine, pyridazine, pyrazine and 1,3,4-triazine ring. The ring is optionally substituted with a C_{1-4} alkyl, or alkoxy group or by a halogen atom.

The heteroaromatic ring containing one nitrogen atom and one oxygen or one sulphur atom means oxazole, isoxazole, thiazole, isothiazole ring. The ring is optionally substituted with a C_{1-4} alkyl, or alkoxy group or by a halogen atom.

20 A favourable group of compounds of the general formula (I) is formed by the compounds -wherein

R^1 stands for phenyl-, thienyl- or furyl group

R^2 stands for 4-methoxyphenyl-, 3-methylphenyl-, 3-methoxyphenyl-, 2-thienyl-, 3-thienyl-, 2-furyl- or 3-furyl group.

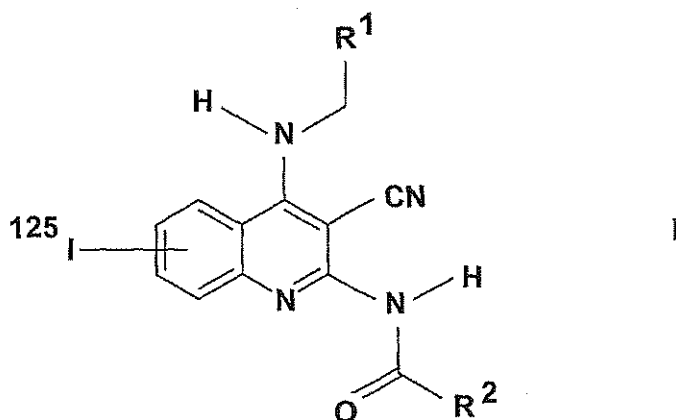
25

Especially favourable are the following compounds complying with the above criteria:

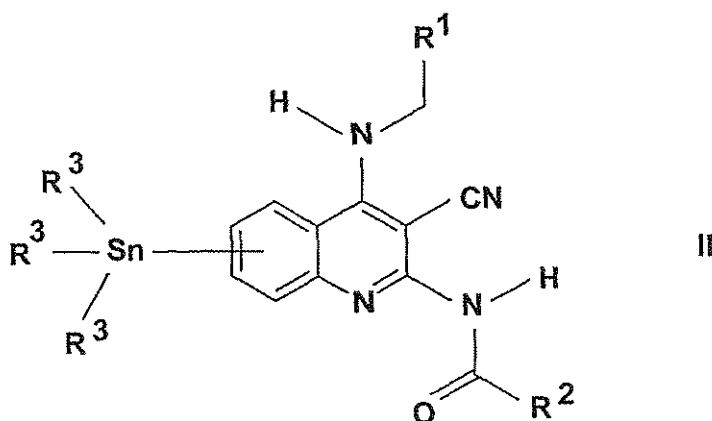
5 4-Methoxy-*N*-(6- ^{125}I)iodo-4-benzylamino-3-cyanoquinolin-2-yl)benzamide

4-Methoxy-*N*-(6- ^{125}I)iodo-4-[2-thienylmethylamino]-3-cyanoquinolin-2-yl)benzamide.

10 Further subject of the invention is the preparation of the compounds of the general formula (I)



and of the intermediates of the general formula (II).



The intermediates of general formula (II) which are used in the preparation process according to the invention, are novel. In the general formula (II) substituents R^1 and R^2 have the meanings as defined above, R^3 stands for straight or branched C_{1-4} alkyl group, preferably methyl- or butyl group.

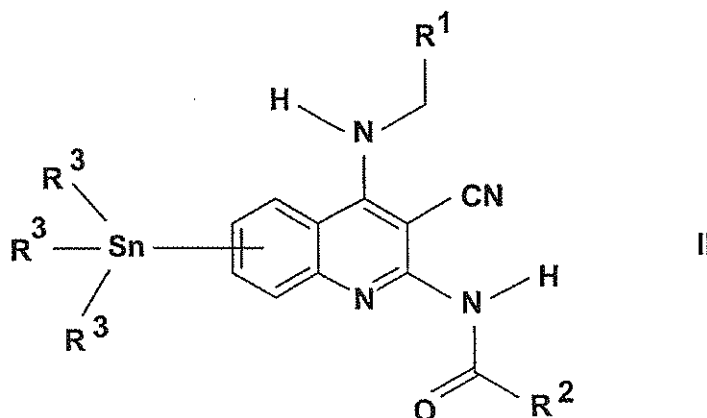
In the process according to our invention the compounds of the general formula (I) are prepared by reacting the appropriate compounds of the general formula (II) with unsupported $[^{125}\text{I}]\text{NaI}$, in the presence of an oxidant.

10 The reaction is carried out in aqueous methanolic medium (pH 3), at room temperature.

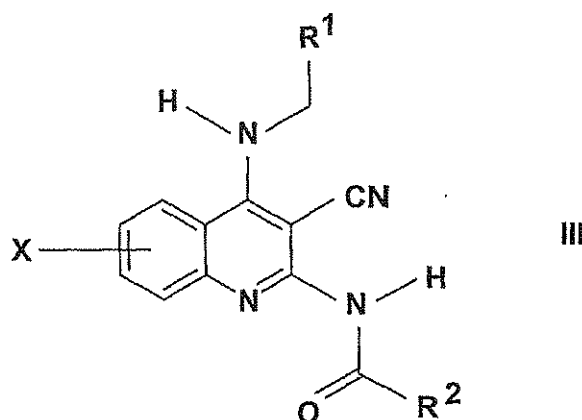
As oxidizing agents, peroxides for example hydrogen peroxide, *N*-halogeno-succinamides e.g. *N*-chloro-succinamide, or chlorosulphonamides, preferably chloramine-T can be used.

15 The product is purified by reversed-phase high-performance liquid chromatographic (RP-HPLC) method using silica gel based C_{18} modified packing as stationary phase and methanol-water binary mixture containing 0.1% (v/v) trifluoroacetic acid as eluent system, with flow rate of 0.9 ml/min. Detection is carried out by UV at 272 nm; detection of radioactivity is
20 carried out by flow liquid scintillation method.

According to our invention the compound of general formula (II)

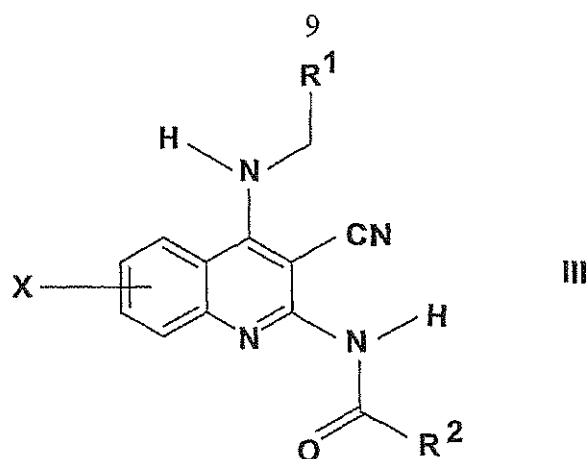


can be prepared from the appropriate quinoline of the general formula (III)



wherein R¹ and R² have the meanings as defined above and X stands for
 5 ✓ iodo- or bromo atom— by reacting it in the presence of palladium catalyst,
 organic or inorganic base and organic solvent, with a hexaalkyl-distannane
 compound. The product of general formula (II) is isolated. The exchange of
 the halogen atom for trialkyl-stannyl group is carried out in an organic
 solvent, for example in dioxane or dimethylformamide, or favourably in *N*-
 10 methyl-2-pyrrolidone. The reaction can be performed in a wide temperature
 range, preferably between 20°C - 100°C. As organic base trialkylamines,
 preferably triethylamine can be applied. For inorganic base alkali hydroxides,
 carbonates and acetates, preferably potassium acetate can be used. In the
 reaction palladium acetate, palladium chloride or
 15 tetrakis(triphenylphosphine)palladium(0) catalysts can be used (Z.P. Zhuang,
 M.P. Kung, C. Hou, D.M. Skovronsky, T.L. Gur, K. Plössl, J.Q. Trojanowski,
 V.M.Y. Lee, H.F. Kung, *J. Med. Chem.* **44**, 1905, (2001)), but we have found
 that with tetrakis(tri(*o*-tolyl)phosphine)palladium(0) catalyst faster reaction
 and better yield is achieved.

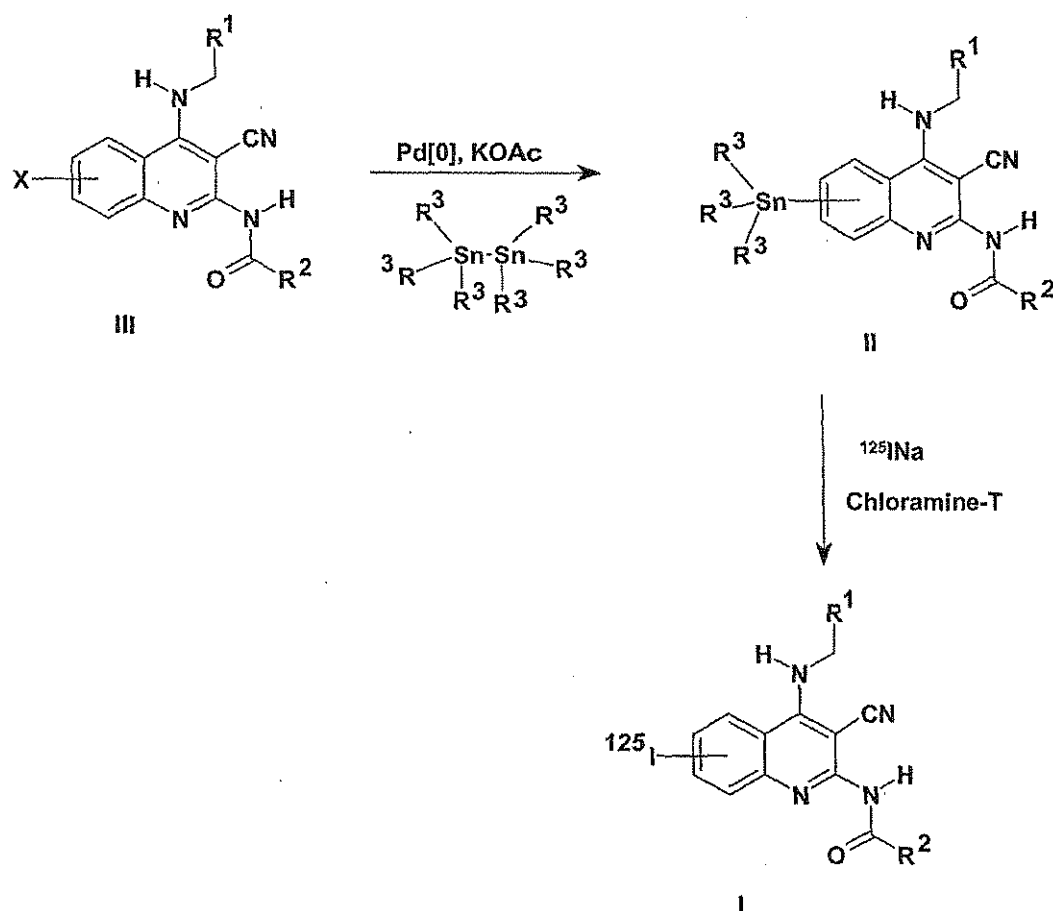
20 The compounds of the general formula (III)



can be synthesized by the method described in patent application WO 02/096879.

The process according to our invention is shown in Reaction Scheme 1.

5



Further details of the invention are demonstrated in the Examples, without limiting the claims to the Examples.

Examples

Example 1.

4-Methoxy-*N*-(6-[¹²⁵I]iodo-4-benzylamino-3-cyanoquinolin-2-yl)benzamide

In the general formula (I) R¹ stands for phenyl group, R² stands for 4-methoxyphenyl group.

a.) To 8 µl of 0.1 mg/ml methanolic solution of 4-methoxy-*N*-(6-tributylstannyl-4-benzylamino-3-cyanoquinolin-2-yl)benzamide are added 25 µl (1.1 nmol) of 1% (v/v) methanolic solution of trifluoroacetic acid and 70 MBq [¹²⁵I]NaI solution (21 µl). To the reaction mixture 5 µl (2 nmol) of 0.1 mg/ml aqueous solution of chloramine-T is added and the mixture is stirred at room temperature for 15 minutes. After the incubation period the reaction is stopped by the addition of 7 µl (3.5 nmol) of 0.1 mg/ml sodium pyrosulfite solution and the product is immediately purified by using RP-HPLC method, applying UV and radioactivity detection. By this manner 31 MBq of the title compound is obtained (molar activity 81.4 GBq/mmol), radiochemical purity >95%. The purified product is stored in methanol-water (0.1% TFA) 3:1 mixture (activity concentration: 29 MBq/ml).

b.) 4-Methoxy-*N*-(6-tributylstannyl-4-benzylamino-3-cyanoquinolin-2-yl)benzamide.

0.33 g of 4-methoxy-*N*-(6-iodo-4-benzylamino-3-cyanoquinolin-2-yl)benzamide is dissolved in 5 ml of *N*-methyl-2-pyrrolidone and to the solution 240 mg of potassium acetate, 50 mg of tetrakis(tri(*o*-tolyl)phosphine)palladium(0) and 0.6 ml of hexabutyl distannane are added. The reaction mixture is stirred at room temperature under argon atmosphere for 16 hours, then it is poured onto 20 ml of water and extracted with 2 x 20 ml of toluene. The united toluene phase is dried over sodium sulfate, filtered and evaporated under reduced pressure. The residue is chromatographed on a

silicagel coloumn using chloroform – ethyl acetate (10 : 0.5) mixture as eluent. After evaporation of the pure fractions 250 mg of the title compound is obtained.

¹H-NMR (CDCl₃) δ 0.9 (m, 9H), 1.1 (M, 6H), 1.3 (m, 6H), 1.55 (m, 6H), 3.8
5 (s, 3H), 5.08 (d, 2H), 7.06 (d, 2H), 7.2-7.4 (m, 5H), 7.55 (d, 2H), 7.9-8.1 (m, 3H), 8.66 (m, 1H), 10.7 (s, 1H).

Example 2.

4-Methoxy-*N*-(6-[¹²⁵I]iodo-4-[2-thienylmethylamino]-3-cyanoquinolin-
10 2-yl)benzamide

In the general formula (I) R¹ stands for thienyl group, R² stands for 4-methoxyphenyl group.

a.) To 8 µl (1.1 nmol) of 0.1 mg/ml methanolic solution of 4-methoxy-*N*-(6-tributylstannyl-4-[2-thienylmethylamino]-3-cyanoquinolin-2-
15 yl)benzamide are added 25 µl (1.1 nmol) of 1% (v/v) methanolic solution of trifluoroacetic acid and 70 MBq [¹²⁵I]NaI solution (21 µl). To the reaction mixture 5 µl (2 nmol) of 0.1 mg/ml aqueous solution of chloramine-T is added and the mixture is stirred at room temperature for 15 minutes. After the incubation period the reaction is stopped by the addition of 7µl (3.5 nmol) of
20 0.1 mg/ml sodium pyrosulfite solution and the product is immediately purified by RP-HPLC method, while applying UV and radioactivity detection. By this manner 28 MBq of the title compound is obtained (molar activity 81.4 GBq/mmol), radiochemical purity >95%. The purified product is stored in methanol-water (0.1% TFA) 3:1 solvent mixture (activity concentration: 28
25 MBq/ml).

b.) 4-Methoxy-*N*-(6-tributylstannyl-4-[2-thienylmethylamino]-3-cyanoquinolin-2-yl)benzamide.

0.3 g 4-Methoxy-*N*-(6-iodo-4-[2-thienylmethylamino]-3-cyanoquinolin-2-yl)benzamide is dissolved in 5 ml of *N*-methyl-2-pyrrolidone

and to the solution 240 mg of potassium acetate, 50 mg of tetrakis(tri(o-
tolyl)phosphine)palladium(0) and 0.6 ml of hexabutyl distannane are added.
The reaction mixture is stirred at room temperature under argon atmosphere
for 16 hours, then it is poured onto 20 ml of water and extracted with 2 x 20
5 ml of toluene. The united toluene phase is dried over sodium sulfate, filtered
and evaporated under reduced pressure. The residue is chromatographed on a
silicagel column using chloroform – ethyl acetate (10 : 0.5) mixture as
eluent. By evaporating the pure fractions 235 mg of the title compound is
obtained.

10 ¹H-NMR (CDCl₃) δ 0.9 (m, 9H), 1.1 (M, 6H), 1.3 (m, 6H), 1.55 (m, 6H), 3.85
(s, 3H), 5.1 (d, 2H), 6.9-7.17 (m, 4H), 7.43-7.54 (m, 2H), 8.03 (m, 3H), 8.72-
8.82 (m, 2H), 10.86 (s, 1H).

Example 3.

15

A./ Biological methods

Human Adenozin A₁ receptor binding

Preparing membrane suspension: collect ovarium cells of cloned
Chinese hamster expressing human A₁ receptors (further: CHO-hA₁), wash
20 them three times with PBS, centrifuge (1000 x g 10 min.) and homogenize
(B.Braun Potter S) at 1500/min rotation speed. Buffer: 50 mM Tris HCl, pH
7,4. Centrifuge this homogenized mixture (43.000 g, 10 min), suspense the
pellet in the above buffer with adjustment of the protein concentration to 5
mg/mL (Bradford method) and complete with 2 U/mL ADA.

25

Binding protocol: incubate CHO-hA₁ membrane preparation (50 µg
protein content), in the presence of the test compound and 10 nM [³H]CCPA
(2-chloro-N⁶-cyclopenthyll-adenosine) (80.000 dpm) in incubation buffer (50
mM Tris HCl, pH 7.4, 2 U/mL adenosine deaminase). The non-specific

binding is defined in the presence of 10 μ M R-PIA (N^6 -[L-2-phenylisopropyl]adenosine) in a total volume of 100 μ L for 3 hr at room temperature. Filter over Whatman GF/B glass fibre filters (presoaked in 0.5% polyethylimine for 3 hours), wash 4x with 1 mL ice-cold 50 mM Tris HCl (pH 7.4) on 96-well Brandel Cell Harvester. Detection of activity: in 96-well plate in the presence of HiSafe-3 cocktail in beta-counter (1450 Microbeta, Wallac). Inhibition [%] = $100 - ((\text{activity in the presence of test compound} - \text{non-specific activity}) / (\text{total activity} - \text{non-specific activity})) * 100$

10 *Human adenosine A_{2a} receptor binding*

Incubate 7 μ g of membranes (human A_{2a} adenosine receptors transfected into HEK-293 cells, source: Receptor Biology, Inc.), in the presence of the test compound and 20 nM [3 H]CGS-21680 (2-[p-(2-carbonylethyl)phenylethylamino]-5'-N-ethylcarboxamido-adenosine) (200,000 dpm) in incubation buffer (50 mM Tris HCl, 10 mM $MgCl_2$, 1 mM EDTA, 2 U/mL adenosine deaminase, pH 7.4). The non-specific binding is defined in the presence of 100 μ g NECA (5'-N-ethylcarboxamido-adenosine) in a total volume of 100 μ L for 90 min at room temperature. Filter in vacuum over Whatman GF/B glass fibre filters (presoaked for 3 hours in 0.5% polyethylimine), wash 4x with 1 mL ice-cold buffer (50 mM Tris HCl, 10 mM $MgCl_2$, 1 mM EDTA, 0.9 % NaCl, pH 7.4) on 96-well Brandel Cell Harvester. Detection of activity: in beta-counter (1450 Microbeta, Wallac) in the presence of 200 μ L HiSafe-3 cocktail. Inhibition [%] = $100 - ((\text{activity in the presence of test compound} - \text{non-specific activity}) / (\text{total activity} - \text{non-specific activity})) * 100$.

Human adenosine A_{2b} receptor binding

Binding protocol: incubate 20.8 μ g of membranes (human A_{2b} adenosine receptors transfected into HEK-293 cells, source: Receptor

Biology, Inc.), in the presence of the test compound and 32.4 nM [^3H]DPCPX (8-cyclopentyl-1,3-dipropylxanthine) (800.000 dpm) in incubation buffer (50 mM Tris-HCl, 10 mM MgCl_2 , 1 mM EDTA, 0.1 mM benzamidine, 2 U/mL adenosine deaminase, pH 6.5). The non-specific binding is defined in the presence of 100 μM NECA (5'-N-ethylcarboxamido-adenosine) in a total volume of 100 μL for 30 min at room temperature. Filter under 25 Hgmm vacuum over Whatman GF/C glass fibre filters (presoaked in 0.5% polyethylimine for 3 hours), wash 4x with 1 mL ice-cold 50 mM Tris HCl (pH 6.5) on 96-well Brandel Cell Harvester. Detection of activity: in beta-counter (1450 Microbeta, Wallac) in the presence of 200 μL of HiSafe-3 cocktail. Inhibition [%] = $100 - ((\text{activity in the presence of test compound} - \text{non-specific activity}) / (\text{total activity} - \text{non-specific activity})) * 100$

15 *Human adenosine A_3 receptor binding*

Preparing membrane suspension: collect ovarium cells of cloned Chinese hamster expressing human A_3 receptors (further: CHO-h A_3), wash them three times with PBS, centrifuge (1000 x g 10 min.) and homogenize (B.Braun Potter S) at 1500/min rotation speed. Buffer: 50 mM Tris, 10mM MgCl₂, 1 mM EDTA, pH 8.0. Centrifuge this homogenized mixture (43.000 g, 10 min), suspense the pellet in the above buffer with adjustment of the protein concentration to 0.1 mg/mL (Bradford method) and complete with 2 U/mL ADA.

25 *Receptor binding in the presence of [^{125}I]AB-MECA :*

Incubate the CHO-h A_3 membrane preparation (protein content 2 μg) in the presence of the test compound and 0.5 nM [^{125}I]AB-MECA (4-amino-3-iodo-benzyl-5'-N-methylcarboxamide-adenosine) (100.000 cpm) in incubation buffer (50 mM Tris, 10 mM MgCl_2 , 1 mM EDTA, 2 U/mL

adenosine deaminase, pH 8.0). The non-specific radioligand binding is defined in the presence of 100 μ M R-PIA (N^6 -[L-2-phenylisopropyl]adenosine) in a total volume of 50 μ L for 60 min at room temperature. Filter under 25 Hgmm vacuum over Whatman GF/C glass fibre
5 filters (presoaked in 0.5% polyethylimine for 3 hours), wash 4x with 1 mL ice-cold buffer (50 mM Tris, 10 mM $MgCl_2$, 1 mM EDTA, pH 8) on 96-well Brandel Cell Harvester. Detection of radioactivity: in gamma-counter (1470 Wizard, Wallac). Inhibition [%] = $100 - ((\text{activity in the presence of test compound} - \text{non-specific activity}) / (\text{total activity} - \text{non-specific activity})) * 100$.

10

Receptor binding in the presence of the radioactive iodine-containing compound of Example 2/a:

Incubate the CHO-hA₃ membrane preparation (protein content 4 μ g) in the presence of the test compound and 0.5 nM of the compound containing
15 radioactive iodine (100.000 cpm), described in Example 2/a, in incubation buffer (50 mM Tris, pH 8.0, 10 mM $MgCl_2$, 1 mM EDTA, 0.08% CHAPS, 0.5% BSA, 2 U/mL adenosine deaminase). The non-specific radioligand binding is defined in the presence of 100 μ M R-PIA (N^6 -[L-2-phenylisopropyl]adenosine) in a total volume of 50 μ L for 60 min at room
20 temperature. Keep the isotop preparation and the reaction mixture in polyethylene tube to decrease adsorption. Filter under 25 Hgmm vacuum over Whatman GF/C glass fibre filters (presoaked in 0.5% polyethylimine for 3 hours), wash 4x with 1 mL ice-cold buffer (50 mM Tris (pH 8), 10 mM $MgCl_2$, 1 mM EDTA, 0.08% CHAPS, 0.25% BSA) on 96-well Brandel Cell
25 Harvester. Detection of radioactivity: in gamma-counter (1470 Wizard, Wallac). Inhibition [%] = $100 - ((\text{activity in the presence of test compound} - \text{non-specific activity}) / (\text{total activity} - \text{non-specific activity})) * 100$.

B./ Biological results

I Affinity of the un-labeled iodine-containing compound given in Example 2/b as starting material (i.e. the un-labeled analogue of the new radioligand given in Example 2/a) to the adenosine receptor sub-types in the presence of known radioligands

The affinity of the starting compound of Example 2/b to the adenosine A₃ receptor ($K_i = 1.5$ nM) exhibits at least thousand-fold selectivity compared to the other adenosine receptor sub-types (Table 1).

Table 1 Characterisation of the starting compound of Example 2/b as regards its affinity to the adenosine receptors

	hA ₃	Inhibition in 1 μ M		
	[¹²⁵ I]AB-MECA	hA ₁ [³ H]CCPA	hA _{2A} [³ H]CGS21680	hA _{2B} [³ H]DPCPX
The starting compound of Example 2/b	$K_i = 1.5$ nM	4 %	29 %	-16 %

15

II/A Investigation of the new radioactive iodine-containing compound of Example 2/a on the human adenosine A₃ receptor by Scatchard analysis used for the characterisation of radioligands

On the basis of radioisotop saturation curves, by Scatchard analysis (G. Scatchard, Ann. N. Y. Acad. Sci. 51:660, 1949) the dissociation constant (K_D) of the new radioligand of Example 2/a on CHO-hA₃ membrane preparation is determined. In the investigated concentration range (0.156 nM – 10 nM) the radioligand binds to only one binding place in the presence of a membrane

20

preparate containing 4 μ g of protein. The value of K_D was found to be 4 nM, the maximal binding capacity 985 femtomol/1 mg protein (see Figure 1).

5

Figure 1: Scatchard saturation curve of the new radioligand of Example 2/a in the presence of CHO-hA₃ membrane preparation

10 **II/B Comparison of a known adenosine A₃ radioligand with the new radioligand of Example 2/a on the basis of the affinity values of reference compounds, in the presence of 4 μ g CHO-hA₃ preparate**

Knowing the K_D values, by the Cheng-Prusoff equation (Y. J. Cheng
15 and W. H. Prusoff, Biochem. Pharmacol. 22:3099, 1973) the K_i constants of the investigated reference compounds and that of the starting compound of Example 2/b (i.e. of the un-labeled analogue of the new radioligand of Example 2/a) were calculated on the basis of the IC₅₀ values. The reference compounds exhibited similar affinity values in the presence of the known and
20 of the new radioligands, proving the suitability of the radioligand of Example 2/a. The un-labeled analogue of the new radioligand exhibited a K_i value nearly equal to the K_D value of the isotop-labeled form (4.0 nM and 1.3 nM), also proving the specific binding of the new radioligand (see Table 2).

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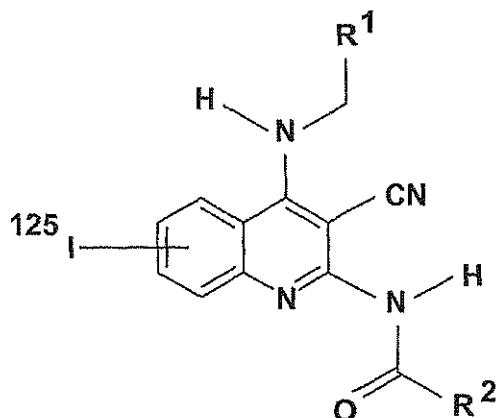
Table 2. Comparison of a known adenosine A₃ radioligand and the new radioligand of Example 2/a with the help of affinity values of reference compounds, in the presence of 4 µg CHO-hA₃ preparation.

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	^[125] I]AB-MECA	The radioligand given in Example 2/a
	K _i	
R-PIA	65 nM	150 nM
Cl-IB-MECA	3.3 nM	5.9 nM
The starting compound of Example 2/b	1.5 nM	1.3 nM

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Claims

1. Compounds of the general formula (I)



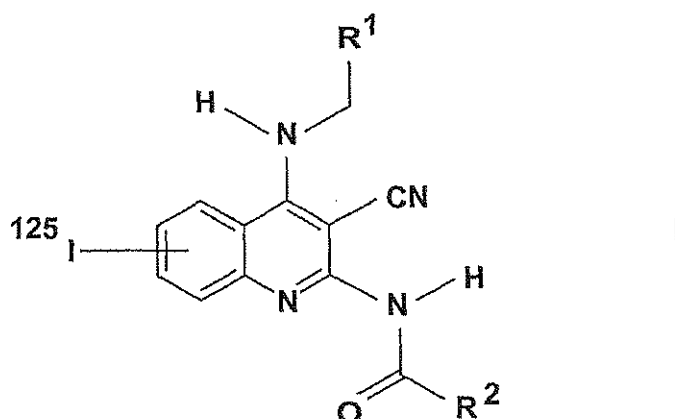
5 and their isomers – where

R^1 stands for hydrogen atom or a straight or branched C_{1-4} alkyl group, or a C_{3-6} cycloalkyl group, or a phenyl group, thienyl group, or furyl group, optionally substituted with one or more straight or branched C_{1-4} alkyl group, straight or branched C_{1-4} alkoxy group, or halogen atom, or for a 5- or 6
 10 membered heteroaromatic ring -containing one, two or three nitrogen atoms-, or 5-membered heteroaromatic ring -containing one nitrogen atom and one oxygen atom or one nitrogen atom and one sulphur atom- optionally substituted with one or more straight or branched C_{1-4} alkyl group, straight or branched C_{1-4} alkoxy group, or halogen atom;

15 R^2 stands for hydrogen atom or for a straight or branched C_{1-4} alkyl group, or for a phenyl-, benzyl-, thienyl- or furyl-group -optionally substituted with a methylenedioxy group, or one or more straight or branched C_{1-4} alkyl group, or straight or branched C_{1-4} alkoxy-, hydroxyl-, trifluoromethyl- or cyano-group or halogen atom-, or for a 5- or 6-membered heteroaromatic ring
 20 -containing one, two or three nitrogen atoms, or one nitrogen atom and one oxygen atom, or one nitrogen atom and one sulphur atom- optionally substituted with one or more straight or branched C_{1-4} alkyl group, straight or branched C_{1-4} alkoxy group, or halogen atom.

20

2. Compounds of the general formula (I)



and their isomers, according to Claim 1. – wherein

R^1 stands for phenyl- or thienyl- or furyl-group

5 R^2 stands for 4-methoxyphenyl-, 3-methylphenyl-, 3-methoxyphenyl-,
2-thienyl-, 3-thienyl-, 2-furyl- or 3-furyl group.

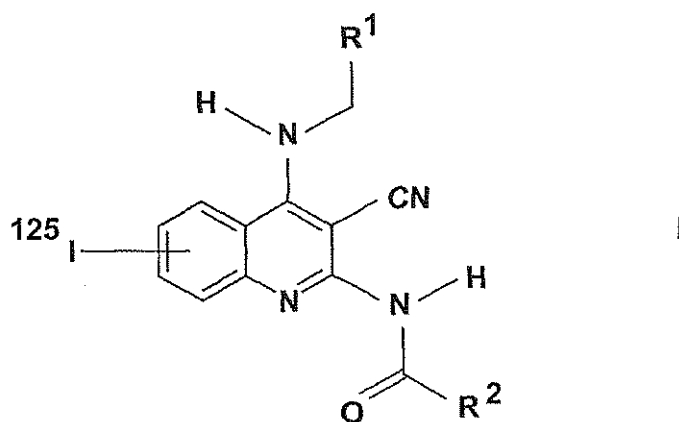
3. The following compounds according to Claim 1-2:

4-Methoxy-*N*-(6- 125 I]iodo-4-benzylamino-3-cyanoquinolin-2-yl)benzamide

10 4-Methoxy-*N*-(6- 125 I]iodo-4-[2-thienylmethylamino]-3-cyanoquinolin-2-yl)benzamide.

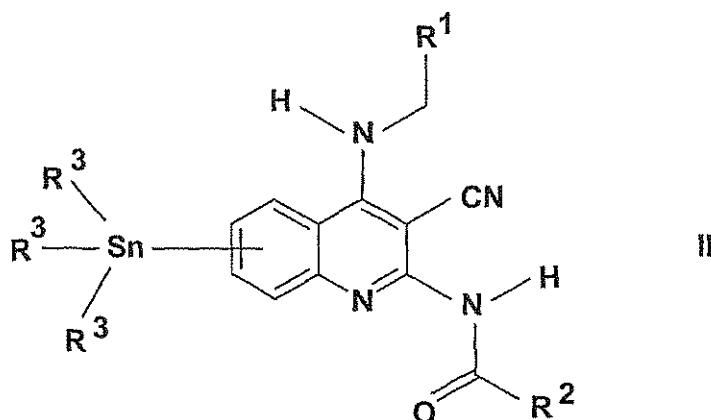
4. Process for the preparation of the compounds of the general formula

(I)



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- where R^1 and R^2 have the meanings as defined in Claim 1.- characterized in that a trialkyl-stannyl compound of the general formula (II)



5 - where R^1 and R^2 have the meanings as defined in Claim 1. and R^3 stands for straight or branched C_{1-4} alkyl group- is reacted with $[^{125}\text{I}]\text{NaI}$, in the presence of an oxidant.

5. Process as defined in Claim 4, characterized in that as oxidant a peroxide, like hydrogen peroxide, or an *N*-halogeno-succinamide, like *N*-chloro-succinamide, or a chlorosulfonamide, preferably chloramin-T is used.

6. Process as defined in Claim 4., characterized in that un-supported $[^{125}\text{I}]\text{NaI}$ is used.

15 7. Process as defined in Claim 4., characterized in that the reaction is carried out in aqueous-methanolic medium (pH 3), at room temperature.

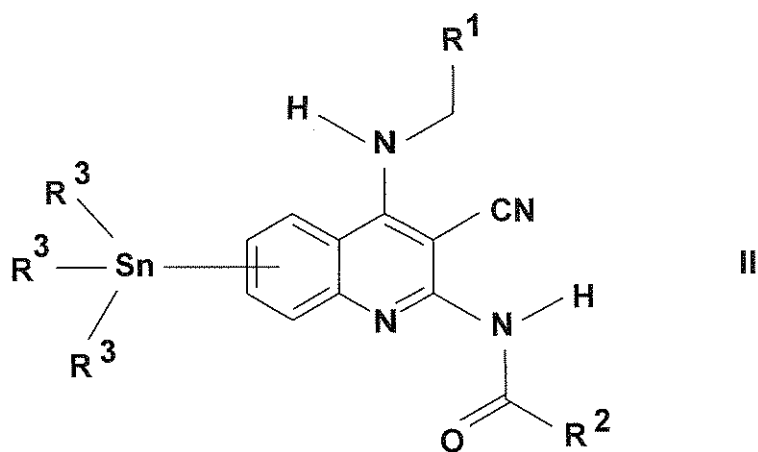
8. Process as defined in Claim 4., characterized in that the product is isolated from the reaction mixture by RP-HPLC method.

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9. Process as defined in Claim 8., characterized in that during the isolation by RP-HPLC method, silica gel based C_{18} modified packing is used as stationary phase and the separation is performed using methanol-water

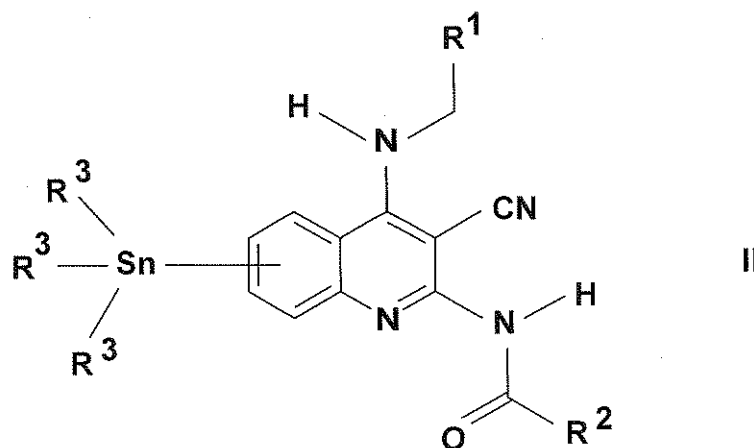
binary eluent system containing 0.1% (v/v) trifluoroacetic acid, with flow-rate of 0.9 ml/min, under UV detection and on-line radioactivity detection.

10. Compounds of the general formula (II)



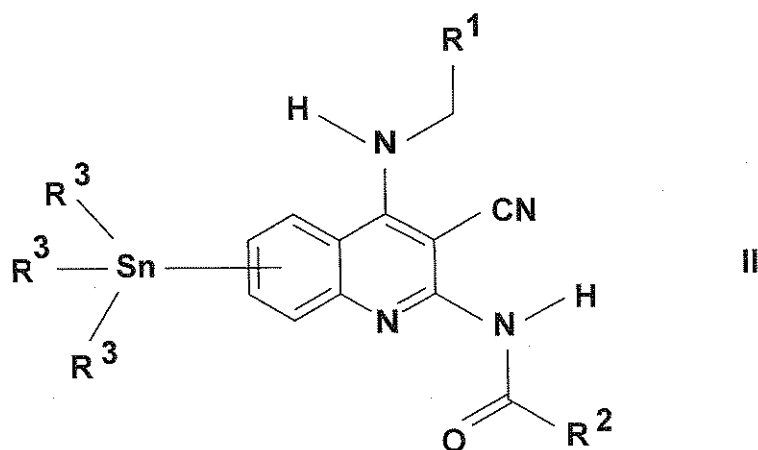
and their isomers – where R^1 and R^2 have the meanings as defined in Claim 1. and R^3 stands for straight or branched C_{1-4} alkyl group.

11. Compounds of the general formula (II)

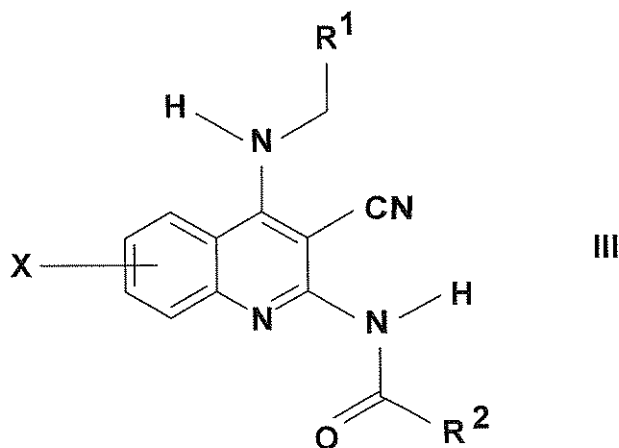


according to Claim 10. – where R^1 and R^2 stand for phenyl or thienyl group and R^3 for methyl or butyl group.

12. Process for the preparation of the compounds of the general formula (II)



- where in the formula R^1 and R^2 have the meanings as defined in Claim 1. and R^3 stands for straight or branched C_{1-4} alkyl group— characterized in that a compound of the general formula (III)



- where in the formula R^1 and R^2 have the meanings as defined in Claim 1. and X stands for iodo- or bromo atom — is reacted with hexaalkyl distannane, in an organic solvent, in the presence of a base and palladium catalyst.

13. Process according to Claim 12., characterized in that as organic solvent dioxane, dimethyl formamide, preferably *N*-methyl-2-pyrrolidone is used.

14. Process according to Claim 12., characterized in that as base, an organic base, like trialkylamine, preferably triethylamine, or an inorganic

base, like alkali hydroxide, -carbonate or -acetate, preferably potassium acetate is used.

15. Process according to Claim 12., characterized in that as catalyst,
5 palladium acetate, palladium chloride,
tetrakis(triphenylphosphine)palladium(0), preferably tetrakis(tri(o-
tolyl)phosphine)palladium(0) catalyst is used.

16. Process according to Claim 12., characterized in that as hexaalkyl
10 distannane, hexabutyl distannane is used.