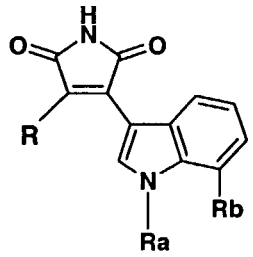
Indolylmaleimide Derivatives

The present invention relates to indolylmaleimide derivatives, process for their production and pharmaceutical compositions containing them.

More particularly the present invention provides a compound of formula I



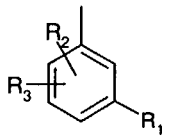
I

wherein

R_a is H; CH_3; CH_2-CH_3; or isopropyl,

R_b is H; halogen; C_{1-6}alkoxy; or C_{1-6}alkyl, and either

I. R is a radical of formula (a)



(a)

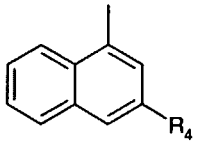
wherein

R_1 is piperazin-1-yl optionally substituted by CH_3 in position 3 or 4; or 4,7-diaza-spiro [2.5] oct-7-yl;

R_2 is Cl; Br; CF_3; or CH_3; and

R_3 is H; CH_3; or CF_3; R_2 being other than CH_3 or Cl when R_3 is H, R_a is H or CH_3, R_b is H and R_1 is 4-methyl-1-piperazinyl; or

II. R is a radical of formula (b)

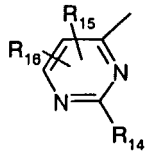


(b)

wherein

R_4 is 1-piperazinyl substituted in positions 3 and/or 4 by CH_3; or 4,7-diaza-spiro [2.5] oct-7-yl; R_a being other than H or CH_3 when R_4 is 4-methyl-1-piperazinyl; or

III. R is a residue of formula (c)



(c)

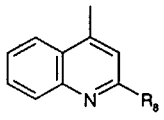
wherein

R_{14} is 1-piperazinyl optionally substituted by CH_3 in position 3 and/or 4 or in position 3 by ethyl, phenyl-C_{1-4}alkyl, C_{1-4}alkoxy-C_{1-4}alkyl or halogeno–C_{1-4}alkyl; or 4,7-diaza-spiro [2.5] oct-7-yl;

R_{15} is halogen; CF_3; or CH_3; R_{15} being other than CH_3 when R_{16} is CH_3, R_a is H or CH_3, R_b is H and R_{14} is 4-methyl-1-piperazinyl; and

R_{16} is H; CH_3; CH_2-CH_3; or CF_3; R_{16} being other than H when R_{15} is Cl, R_a is H or CH_3, R_b is H and R_{14} is 4-methyl-1-piperazinyl; or

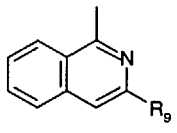
IV. R is a radical of formula (d)



(d)

wherein R_8 is 1-piperazinyl, 3-methyl-piperazin-1-yl or 4-benzyl-piperazin-1-yl; or

V. R is a radical of formula (e)



(e)

wherein R_9 is 4,7-diaza-spiro [2.5] oct-7-yl; or piperazin-1-yl substituted in position 3 by methyl or ethyl and optionally in position 4 by methyl.

The compounds of formula I may exist in free form or in salt form, e.g. addition salts with e.g. organic or inorganic acids, for example, hydrochloric acid, acetic acid, trifluoroacetic acid.

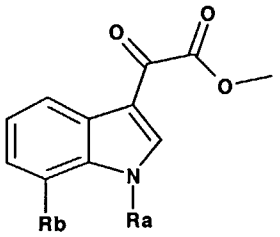
It will be appreciated that the compounds of formula I may exist in the form of optical isomers, racemates or diastereoisomers. For example, a ring carbon atom bearing a substituent in the position 3 of the piperazinyl residue is asymmetric and may have the R- or S- configuration. It is to be understood that the present invention embraces all enantiomers and their mixtures. Enantiomers are preferred over racemates. Similar considerations apply in relation to starting materials exhibiting asymmetric carbon atoms as mentioned.

Alkyl or alkoxy may be straight or branched. Phenyl-C_{1-4}alkyl is preferably benzyl or phenethyl. In C_{1-4}alkoxy-C_{1-4}alkyl the alkoxy moiety is preferably methoxy or ethoxy and the alkyl moiety preferably methyl or ethyl; a suitable example is e.g. 2-methoxyethyl. Halogen may be F, Cl, Br or I, preferably F, Cl or Br. Halogeno–C_{1-4}alkyl is alkyl wherein one or more H are replaced by halogen, e.g. Cl or F, e.g. CH_2Cl, CH_2F or CF_3

R is preferably a radical of formula (a), (c) or (e), preferably (e).

In the radical of formula (a) or (c), R_2 or R_{15} is preferably in para to R_1 or R_{14}, respectively. R_3 is preferably in meta to R_1. In the radical or formula (e), R_9 is preferably 4,7-diaza-spiro [2.5] oct-7-yl; when R_9 is piperazinyl substituted in position 3, it has the R or S configuration.

The present invention also includes a process for the preparation of a compound of formula I which process comprises reacting a compound of formula II



(II)

wherein R_a and R_b are as defined above,

with a compound of formula III

$$R - CH_2 - CO - NH_2 \qquad (III)$$

wherein R is as defined above,

and, where required, converting the resulting compound of formula I obtained in free form to a salt form or vice versa, as appropriate.

The process may conveniently be effected in the presence of a strong base, e.g. t-BuOK, e.g. as disclosed in WO02/38561, the contents of which being incorporated herein by reference, and as illustrated in the Examples.

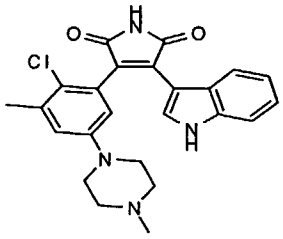
Compounds of formula II and III may be prepared in accordance with known methods, e.g. as disclosed in WO02/38561, the contents of which being incorporated herein by reference, and as illustrated in the Examples.

Insofar as the production of the starting materials is not particularly described, the compounds are known or may be prepared analogously to methods known in the art or as described hereafter.

The following examples are illustrative of the invention without any limitation.

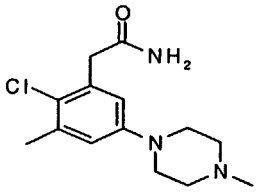
RT	=	room temperature
THF	=	tetrahydrofuran
FCC	=	flash column chromatography
TBAF	=	tetrabutyl ammonium fluoride
BINAP	=	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
$Pd_2(dba)_3$	=	Pd(0)-bis(dibenzylidenacetone)

Example 1: 3-[2-Chloro-3-methyl-5-(4-methyl-piperazin-1-yl)-phenyl]-4-(1H-indol-3-yl)-pyrrole-2,5-dione



2-[2-Chloro-3-methyl-5-(4-methyl-piperazin-1-yl)-phenyl]-acetamide (211 mg, 0.75 mmol) and 3-indoleglyoxylate (270 mg, 1.35 mmol) are dissolved in THF (5 mL). A solution of 1.0 M tert-BuOK in THF (2.98 mL, 4 eq) is added and the mixture is stirred at 35°C overnight. The reaction is diluted with AcOEt (20 mL) and washed with H_2O (20 mL) and brine (10 mL). The organic phase is dried over Na_2SO_4 and the solvent is evaporated. The residue is purified by FCC (AcOEt/AcOH/H_2O 7:1:1) to afford the title compound in the form of its acetate salt. ^{1}H NMR (DMSO, 400 MHz) δ 2.16 (s, 3H), 2.31 (s, 3H), 2.32-2.38 (m, 4H), 2.97-3.10 (m, 4H), 6.61 (d, J = 8.0 Hz, 1H), 6.71-6.77 (m, 2H), 7.04 (d, J = 2.8 Hz, 1H), 7.08 (dd, J = 7.4, 7.2 Hz, 1H), 7.41 (d, J = 8.2 Hz, 1H), 7.98 (d, J = 1.5 Hz, 1H), 11.05 (br s, 1H), 11.90 (br s, 1H); ES-MS: 435 $[M+H]^+$.

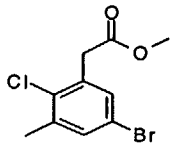
2-[2-Chloro-3-methyl-5-(4-methyl-piperazin-1-yl)-phenyl]-acetamide



used as starting material may be prepared as follows:

(5-Bromo-2-chloro-3-methyl-phenyl)-acetic acid methyl ester (2.0 g, 7.2 mmol), *N*-methylpiperazine (960 µL, 8.6 mmol) and Cs_2CO_3 (3.3 g, 10.1 mmol) are suspended in toluene (80 mL). $Pd(OAc)_2$ (81 mg, 0.36 mmol) and BINAP (224 mg, 0.36 mmol) are added

and the reaction is stirred at 100°C overnight. The mixture is filtered through Celite and the solvent is evaporated. The residue is purified by FCC (AcOEt/AcOH/H_2O 60:15:15) to afford 2-[2-chloro-3-methyl-5-(4-methyl-piperazin-1-yl)-phenyl]-acetic acid methyl ester. The ester is suspended in NH_4OH 25% (60 mL). The mixture is stirred overnight at RT and the precipitate is filtered off to yield the amide. ^1H NMR (DMSO, 400 MHz) δ 2.20 (s, 3H), 2.27 (s, 3H), 2.40-2.45 (m, 4H), 3.07-3.13 (m, 4H), 3.48 (s, 2H), 6.78 (s, 1H), 6.82 (s, 1H), 6.91 (br s, 1H), 7.34 (br s, 1H).



$CuCl_2$ (8.0 g, 0.06 mol) and tert-butylnitrile (8.9 mL, 0.074 mol) are suspended in acetonitrile (60 mL). 1,1-dichloroethylene is added dropwise at 20°C. 5-Bromo-2-chloro-3-methyl-phenylamine (11.0 g, 0.05 mol), dissolved in acetonitrile (60 mL), is added and the reaction is stirred at RT for 3 h. The mixture is poured into aqueous 20% HCl (150 mL) and the aqueous phase is extracted with CH_2Cl_2 (2 x 150 mL). The organic phase is dried over Na_2SO_4 and the solvent is evaporated. The residue is purified by FCC (hexane/CH_2Cl_2 9:1) to afford 5-bromo-2-chloro-1-methyl-3-(2,2,2-trichloro-ethyl)-benzene. The intermediate is redissolved in MeOH (80 mL) and heated to 70°C. A solution of 5.4 M NaOMe in MeOH (28.4 mL) is added dropwise and the reaction is stirred at 70°C for 3 h. The reaction is cooled to RT and concentrated H_2SO_4 (10 mL) is added. The reaction is stirred at reflux for 1 h. The mixture is diluted with H_2O (200 mL) and extracted with CH_2Cl_2 (2 x 200 mL). The combined organic phases are dried over Na_2SO_4 and the solvent is evaporated. The residue is purified by FCC (hexane/CH_2Cl_2 9:1 to 1:1) to afford (5-bromo-2-chloro-3-methyl-phenyl)-acetic acid methyl ester. ^1H NMR ($CDCl_3$, 400 MHz) δ 2.26 (s, 3H), 3.61 (s, 3H), 3.63 (s, 2H), 7.15 (s, 1H), 7.21 (s, 1H).

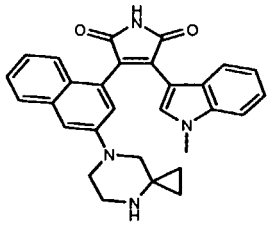
By following the procedure of Example 1 but using the appropriate starting materials, the compounds of formula I wherein R is a residue of formula (a), as indicated in Table 1 below may be obtained.

TABLE 1

Example	R_1	R_2	R_3	R_a	R_b	M.S. Data
2	-(4-methyl-piperazin-1-yl)	2-CH_3	3-CH_3	CH_3	H	MH^+ 429
3	-(4-methyl-piperazin-1-yl)	2-CH_3	3-CH_3	H	H	MH^+ 415
4	-(4-methyl-piperazin-1-yl)	2-Cl	3-CH_3	CH_3	H	MH^+ 449

5	1-piperazinyl	2-Cl	3-CH_3	H	H	MH^+ 421
6	1-piperazinyl	2-Cl	3-CH_3	CH_3	H	MH^+ 435
7	3-*R*-methyl-piperazin-1-yl	2-Cl	3-CH_3	CH_3	H	MH^+ 449
8	3-*R*-methyl-piperazin-1-yl	2-Cl	3-CH_3	H	H	MH^+ 435
9	1-piperazinyl	2-Cl	3-CF_3	CH_3	H	MH^+ 503
10	1-piperazinyl	2-Cl	3-CF_3	H	H	MH^+ 489
11	-(4-methyl-piperazin-1-yl)	2-Cl	3-CH_3	H	$CH(CH_3)_2$	MH^+ 477
12	-(4-methyl-piperazin-1-yl)	2-Cl	3-CH_3	H	CH_3	MH^+ 449
13	-(4-methyl-piperazin-1-yl)	2-Cl	3-CH_3	H	CH_2-CH_3	MH^+ 463
14	-(4-methyl-piperazin-1-yl)	2-Cl	3-CH_3	H	Cl	MH^+ 469
15	-(4-methyl-piperazin-1-yl)	2-Cl	3-CH_3	H	F	MH^+ 453
16	-(4,7-diaza-spiro[2.5]oct-7-yl)	2-Cl	H	H	CH_2-CH_3	MH^+ 462
17	-(4,7-diaza-spiro[2.5]oct-7-yl)	2-Cl	H	H	Cl	MH^+ 468
18	-(4,7-diaza-spiro[2.5]oct-7-yl)	2-Cl	H	H	CH_3	MH^+ 447
19	-(4,7-diaza-spiro[2.5]oct-7-yl)	2-Cl	H	H	H	MH^+ 434

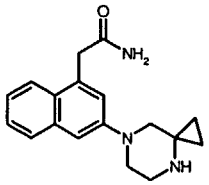
Example 20: 3-[3-(4,7-Diaza-spiro[2.5]oct-7-yl)-naphthalen-1-yl]-4-(1-methyl-1H-indol-3-yl)-pyrrole-2,5-dione



2-[3-(4,7-Diaza-spiro[2.5]oct-7-yl)-naphthalen-1-yl]-acetamide (100 mg, 0.30 mmol) and (1-methyl-1H-indol-3-yl)-oxo-acetic acid methyl ester (97 mg, 0.44 mmol) are azeotroped three times with dry THF and then dissolved in dry THF (3 ml). A solution of 1.0 M KOtBu in THF (1.2 ml) is added dropwise over 20 minutes at RT. After 5 minutes, TLC analysis indicates complete conversion of starting materials. The reaction is quenched by the additon of water (5 ml). The mixture is diluted with EtOAc and washed twice with saturated aq. NH_4Cl. The aqueous layers are back extracted twice with EtOAc. The combined organic layers are dried over Na_2SO_4 and the solvent is evaporated. The residue is purified by FCC (EtOAc/AcOH/H_2O 800:55:45) to afford the title compound as its acetate salt. The compound is dissolved in MeOH/TFA and the solvent is removed to yield the title compound as its trifluoroacetate salt. ^1H NMR (DMSO, 400 MHz) δ 0.95 (M, 4H), 3.39 (br, 4H), 3.49 (br, 2H),

3.86 (s, 3H), 6.16 (d, J = 7.9 Hz, 1H), 6.46 (dd, J = 6.6 / 7.9 Hz, 1H), 7.00 (dd, J = 6.6 / 7.9 Hz, 1H), 7.13 (dd, J = 7.6 / 7.6 Hz, 1H), 7.40 (m, 4H), 7.55 (d, J = 8.5 Hz, 1H), 7.78 (d, J = 7.6 Hz, 1H), 8.14 (s, 1H), 9.10 (br, 2H), 11.17 (s, 1H); ES-MS: 463 $[M+H]^+$.

2-[3-(4,7-Diaza-spiro[2.5]oct-7-yl)-naphthalen-1-yl]-acetamide, used as starting material may be prepared as follows:



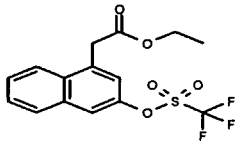
a) 2-[3-(4-Benzyl-4,7-diaza-spiro[2.5]oct-7-yl)-naphthalen-1-yl]-acetamide (280 mg, 0.73 mmol) is azeotroped twice with a 1.25 M solution of HCl in MeOH. The residue is dissolved in EtOH (10 ml). Palladium on charcoal (10%, 77 mg) is added, and the mixture is stirred under an atmosphere of hydrogen (1 atm) at RT for 14 h and at 50 °C for 2 h. The mixture is filtered, and the filtrate is concentrated. The residue is purified by FCC (EtOAc/AcOH/H_2O 750:83.68 to 600:150:150) to yield the title compound containing 0.7 eq of AcOH. ^1H NMR (DMSO, 400 MHz) δ 0.53 (m, 4H), 1.78 (s, AcOH), 2.93 (ddd, 2H), 3.04 (s, 2H), 3.16 (ddd, 2H), 3.76 (s, 2H), 6.94 (br, 1H), 6.99 (s, 1H), 7.25 (dd, 1H), 7.26 (s, 1H), 7.35 (dd, J = 6.7 / 7.8, 1H), 7.49 (br, 1H), 7.69 (d, J = 7.8, 1H), 7.88 (d, J = 10.0, 1H); ES-MS: 296 $[M+H]^+$.

b) 2-[3-(4-Benzyl-4,7-diaza-spiro[2.5]oct-7-yl)-naphthalen-1-yl]-acetamide [3-(4-Benzyl-4,7-diaza-spiro[2.5]oct-7-yl)-naphthalen-1-yl]-acetic acid ethyl ester (347 mg, 0.84 mmol) and formamide (126 mg, 2.80 mmol) are dissolved under an atmosphere of argon in DMF (1 ml). The solution is heated to 105 °C, and NaOMe (155 µL of a 5.4 M solution in MeOH, 45 mg, 0.84 mmol) is added dropwise during 15 minutes. After 30 minutes at 105 °C, TLC analysis indicates complete consumption of starting material. The reaction mixture is cooled to RT, diluted with water, and extracted with EtOAc. The EtOAc layers are washed twice with water. Removal of solvent and purification by FCC (EtOAc/MeOH 98:2 to 96:4 to 90:10) yielded the title compound. ^1H NMR ($CDCl_3$, 400 MHz) δ 0.71 (ddd, 2H), 0.89 (ddd, 2H), 3.10 (ddd, 2H), 3.16 (s, 2H), 3.31 (ddd, 2H), 3.93 (s, 2H), 3.99 (s, 2H), 5.33 (br, 1H), 5.42 (br, 1H), 7.11 (s, 1H), 7.23 (d, J = 2.0, 1H), 7.32 (m, 5H), 7.37 (dd, J = 6.7 Hz, 1H), 7.45 (dd, J = 6.7 Hz, 1H), 7.73 (d, J = 8.9 Hz, 1H), 7.85 (d, J = 8.9 Hz, 1H); ES-MS: 386 $[M+H]^+$.

c) [3-(4-Benzyl-4,7-diaza-spiro[2.5]oct-7-yl)-naphthalen-1-yl]-acetic acid ethyl ester

(3-Trifluoromethanesulfonyloxy-naphthalen-1-yl)-acetic acid ethyl ester (500 mg, 1.38 mmol) is dissolved under an atmosphere of argon in dry THF (10 ml). 4-Benzyl-4,7-diaza-spiro[2.5]octane (325 mg, 1.61 mmol) is added, followed by the addition of K_3PO_4 (410 mg, 1.93 mmol), $Pd_2(dba)_3$ (62 mg, 0.069 mmol) and biphenyl-2-yl-di-tert-butyl-phosphane (21 mg, 0.069 mmol). The reaction mixture is heated to 80 °C. After 4 h, TLC analysis indicates complete consumption of starting materials. The reaction mixture is cooled to RT, filtered and concentrated. The residue is purified by FCC (hexanes/EtOAc 100:0 to 90:10 to 80:20 to 70:30 to 0:100) to afford the title compound. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.69 (ddd, 2H), 0.86 (ddd, 2H), 1.24 (t, J = 7.0 Hz, 3H), 3.09 (ddd, 2H), 3.19 (s, 2H), 3.31 (ddd, 2H), 3.93 (s, 2H), 4.02 (s, 2H), 4.17 (q, J = 7.0 Hz, 2H), 7.08 (s, 1H), 7.21 (s, 1H), 7.33 (m, 6H), 7.42 (dd, J = 8.8 Hz, 1H), 7.71 (d, J = 8.8 Hz, 1H), 7.88 (d, J = 8.8 Hz, 1H); ES-MS: 415 $[M+H]^+$.

d) (3-Trifluoromethanesulfonyloxy-naphthalen-1-yl)-acetic acid ethyl ester



(3-Hydroxy-naphthalen-1-yl)-acetic acid ethyl ester (1.67 g, 7.25 mmol) is dissolved in CH_2Cl_2 (20 ml) under an atmosphere of argon. Pyridine (1.17 ml, 1.15 g, 14.50 mmol) is added, and the reaction mixture is cooled to 0 °C, whereupon trifluoromethanesulfonic anhydride (1.79 ml, 3.07 g, 10.88 mmol) is added dropwise. The reaction mixture is warmed to RT, and after 1 h a RT, TLC analysis indicates complete consumption of starting material. The reaction mixture is diluted with EtOAc and washed twice with H_2O. The combined organic layers are dried over Na_2SO_4, the solvent is removed, and the residue is purified by FCC (hexanes/EtOAc 100:0 to 97:3 to 95:5 to 93:7 to 90:10) to yield the title compound. ^{1}H NMR (DMSO, 400 MHz) δ 1.19 (t, J = 7.2 Hz, 3H), 4.11 (q, J = 7.2 Hz, 2H), 4.38 (s, 2H), 7.60 (d, J = 3.0 Hz, 1H), 7.70 (m, 2H), 8.03 (m, 1H), 8.12 (m, 2H); ES-MS: 362 $[M+H]^+$.

e) (3-Hydroxy-naphthalen-1-yl)-acetic acid ethyl ester

(3-Benzyloxy-naphthalen-1-yl)-acetic acid ethyl ester (2.43 g, 7.58 mmol) is dissolved in MeOH (50 ml). Palladium on charcoal (807 mg) is added, and the reaction mixture is stirred at RT under an atmosphere of hydrogen (1 atm) for 14 h. The reaction mixture is filtered. Concentration yields the pure title compound . ^{1}H NMR (DMSO, 400 MHz) δ 1.18 (t, J = 7.2 Hz, 3H), 4.05 (s, 2H), 4.10 (q, J = 7.2 Hz, 2H), 7.04 (d, J = 1.8 Hz, 1H), 7.06 (d, J = 1.8, 1H), 7.30 (t, J = 7.8, 1H), 7.40 (t, J = 7.8, 1H), 7.70 (d, J = 7.8, 1H), 7.80 (d, J = 7.8 Hz, 1H); ES-MS: 230 $[M+H]^+$.

f) (3-Benzyloxy-naphthalen-1-yl)-acetic acid ethyl ester

3-Benzyloxy-1-bromo-naphthalene (5.64 g, 18.01 mmol) is dissolved under an atmosphere of argon in dry DMF (100 ml). Tributylstannanyl-acetic acid ethyl ester (7.47 g, 19.81 mmol) is added, as well as [bis(tri-ortho-tolyl-phosphine)]palladium (II) dichloride (2.83 g, 3.60 mmol) and zinc(II) bromide (5.27 g, 23.41 mmol). The reaction mixture is heated to 80 °C for 3 h. The reaction mixture is diluted with EtOAc and washed twice with diluted brine (back extracted). The combined organic layers are dried over Na_2SO_4, the solvent is removed, and the residue is purified by FCC (hexane/EtOAc 100:0 to 97.5:2.5 to 95:5 to 90:10). The resulting oil, still containing tin residues, is stirred in a 1:1 mixture of EtOAc/1N NaOH (200 ml) for 1 h. The mixture is extracted twice with EtOAc. The combined organic layers are washed twice with H_2O (back extracted), dried over Na_2SO_4 and concentrated. The residue is purified by FCC (hexane/EtOAc 100:0 to 97:3 to 95:5 to 93:7 to 92:8 to 90:10) to yield the title compound. ^1H NMR (DMSO, 400 MHz) δ 1.18 (t, J = 7.2 Hz, 3H), 4.10 (q, J = 7.2 Hz, 2H), 4.11 (s, 2H), 5.25 (s, 2H), 7.21 (d, J = 3.0 Hz, 1H), 7.41 (m, 8H), 7.53 (d, J = 6.6 Hz, 1H), 7.84 (dd, J = 7.2 Hz, 1H); ES-MS: 320 $[M+H]^+$.

g) 3-Benzyloxy-1-bromo-naphthalene

4-Bromo-naphthalen-2-ol (5.0 g, 22.41 mmol) is dissolved in dry DMF (50 ml) under an atmosphere of argon. Sodium hydride (986 mg of a 60% suspension in mineral oil, 592 mg, 24.65 mmol) is added and the mixture is stirred at 50 °C for 1 h. After re-cooling to RT, benzyl bromide (3.46 ml, 4.98 g, 29.14 mmol) and tetrabutyl ammoniumiodide (828 mg, 2.24 mmol) are added. After 16 h at RT, the reaction mixture is diluted with EtOAc. The solution is washed twice with semi-concentrated brine (back extracted). The combined organic layers are dried over Na_2SO_4, the solvent is removed, and the residue is purified by FCC (hexane/EtOAc 100:0 to 95:5 to 90:10) to yield the title compound. ^1H NMR ($CDCl_3$, 400 MHz) δ 5.22 (s, 2H), 7.25 (d, J = 1.5 Hz, 1H), 7.45 (m, 7H), 7.64 (d, J = 2.4, 1H), 7.75 (d, J = 7.8, 1H), 8.17 (d, J = 7.8, 1H); ES-MS: 312 $[M+H]^+$.

By following procedure of example 20, the compounds of formula I wherein R is a residue of formula (b), as indicated in Table 2 may be obtained.

TABLE 2

Example	R_4	R_a	M.S. Data
21	3-*R*-methyl-piperazin-1-yl	CH_3	MH^+ 452
22	-(4,7-diaza-spiro[2.5]oct-7-yl)	CH_3	MH^+ 464
23	-(4,7-diaza-spiro[2.5]oct-7-yl)	H	MH^+ 450

24	3-*R*-methyl-piperazin-1-yl	H	MH^+ 438
25	3-*S*-methyl-piperazin-1-yl	CH_3	MH^+ 452
26	3-*S*-methyl-piperazin-1-yl	H	MH^+ 438
27	4-methyl-3-*S*-methyl-piperazin-1-yl	CH_3	MH^+ 466
28	4-methyl-3-*S*-methyl-piperazin-1-yl	H	MH^+ 452

By following the procedure as disclosed above or in Example 56 in WO02/38561, but using the appropriate starting materials the compounds of formula I wherein R is a residue of formula (c), as indicated in Table 3 below may be obtained.

TABLE 3

Ex.	R_{14}	R_{15} in 4	R_{16} in 5	R_a	R_b	M.S. Data
29	-(4-methyl-piperazin-1-yl)	Cl	CH_3	H	H	MH^+ 437
30	-(4-methyl-piperazin-1-yl)	Br	H	H	H	MH^+ 469
31	-(4-methyl-piperazin-1-yl)	Br	CH_3	H	H	MH^+ 483
32	-(4-methyl-piperazin-1-yl)	Br	H	CH_3	H	MH^+ 483
33	-(4-methyl-piperazin-1-yl)	CF_3	H	H	H	MH^+ 457
34	-(4-methyl-piperazin-1-yl)	CF_3	H	CH_3	H	MH^+ 471
35	3-*R*-methyl-piperazin-1-yl	Cl	CH_3	H	H	MH^+ 437
36	-(4,7-diaza-spiro[2.5]oct-7-yl)	Cl	CH_3	H	H	MH^+ 449
37	1-piperazinyl	Cl	CH_3	H	H	MH^+ 423
38	4-methyl-3-*R*-methyl-piperazin-yl	Cl	CH_3	H	H	MH^+ 451
39	3-R-methoxyethyl-piperazin-1-yl	Cl	CH_3	H	H	MH^+ 481
40	3-R-ethyl-piperazin-1-yl	Cl	CH_3	H	H	MH^+ 451
41	3-R-benzyl-piperazin-1-yl	Cl	CH_3	H	H	MH^+ 514
42	3-S-methyl-piperazin-1-yl	Cl	CH_3	H	H	MH^+ 437
43	4-methyl-piperazin-1-yl	Cl	CH_3	H	CH_2-CH_2-CH_3	MH^+ 479
44	3-CH_2F-piperazin-1-yl	Cl	CH_3	H	H	MH^+ 453
45	4-methyl-piperazin-1-yl	Cl	CH_3	H	F	MH^+ 455
46	4-methyl-piperazin-1-yl	Cl	CH_3	H	$CH(CH_3)_2$	MH^+ 479
47	4-methyl-piperazin-1-yl	Cl	CH_3	H	Cl	MH^+ 471
48	4-methyl-piperazin-1-yl	Cl	CH_3	H	OCH_3	MH^+ 467
49	4-methyl-piperazin-1-yl	Cl	CH_3	H	CH_3	MH^+ 451
50	4-methyl-piperazin-1-yl	Cl	CH_3	H	CH_2-CH_3	MH^+ 465

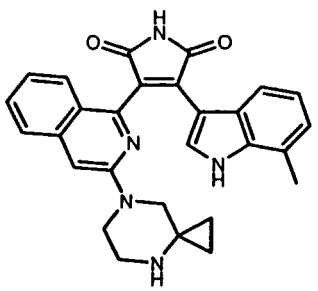
51	4-methyl-piperazin-1-yl	CF_3	H	H	CH_2-CH_3	MH^+ 485
52	4-methyl-piperazin-1-yl	CF_3	H	H	CH_3	MH^+ 471
53	4-methyl-piperazin-1-yl	F	H	H	H	MH^+ 407
54	4-methyl-piperazin-1-yl	F	H	H	CH_3	MH^+ 421
55	4-methyl-piperazin-1-yl	F	H	H	CH_2-CH_3	MH^+ 435
56	4-methyl-piperazin-1-yl	F	CH_2-CH_3	H	CH_3	MH^+ 449
57	4-methyl-piperazin-1-yl	F	CH_2-CH_3	H	H	MH^+ 435
58	4-methyl-piperazin-1-yl	F	CH_2-CH_3	H	CH_2-CH_3	MH^+ 463
59	4-methyl-piperazin-1-yl	F	CH_3	H	H	MH^+ 421
60	4-methyl-piperazin-1-yl	F	CH_3	H	CH_3	MH^+ 435
61	4-methyl-piperazin-1-yl	F	CH_3	H	CH_2-CH_3	MH^+ 449

By following the procedure as disclosed above or in Example 163 in WO 02/38561, but using the appropriate starting material the compounds of formula I wherein R is a residue of formula (d), as indicated in Table 4 below, may be obtained.

TABLE 4

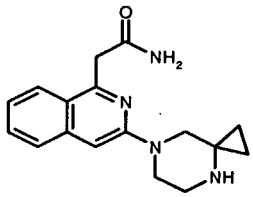
Example	R_8	R_a	M.S. Data
62	3-*S*-methyl-piperazin-1-yl	CH_3	MH^+ 452
63	3-*R*-methyl-piperazin-1-yl	H	MH^+ 438
64	3-*R*-methyl-piperazin-1-yl	CH_3	MH^+ 452
65	4-benzyl-1-piperazinyl	H	MH^+ 514
66	4-benzyl-1-piperazinyl	CH_3	MH^+ 528
67	1-piperazinyl	CH_3	MH^+ 438
68	1-piperazinyl	H	MH^+ 424

Example 69: 3-[3-(4,7-Diaza-spiro[2.5]oct-7-yl)-isoquinolin-1-yl]-4-(7-methyl-1H-indol-3-yl)-pyrrole-2,5-dione



2-[3-(4,7-Diaza-spiro[2.5]oct-7-yl)-isoquinolin-1-yl]-acetamide (4.95 g, 16.70 mmol) and (7-Methyl-1H-indol-3-yl)-oxo-acetic acid methyl ester (5.44 g, 25.05 mmol) are azeotroped twice with dry THF. Dry THF (100 ml) is then added, and under an atmosphere of argon KOtBu (1.0 M in THF, 50 ml, 50 mmol) is added dropwise during 20 min. After an additional 90 min, TLC analysis indicates complete conversion of starting materials. The reaction mixture is diluted with H_2O and extracted twice with EtOAc. The combined organic layers are washed twice with saturated aq NH_4Cl solution (back extracted), dried over Na_2SO_4 and concentrated. Purification by FCC (CH_2Cl_2/MeOH 100:0 to 98:2 to 96:4 to 94:6 to 92:8 to 90:10) yields the title compound, which is converted to its acetate salt by concentration of a EtOH/AcOH solution. ^{1}H NMR (DMSO, 400 MHz) δ 0.26 - 053 (br, 4 H), 1.89 (s, 3H, CH_3COOH), 2.36 (s, 3H), 2.80 (br m, 2 H), 3.15 – 3.48 (br m, 2H), 6.14 (d, J = 8.2 HZ, 1H), 6.44 (dd, J = 8.2 / 7.4 Hz, 1H), 6.75 (d, J = 7.4 Hz, 1H), 7.00 (s, 1H), 7.02 (dd, J = 8.2 / 8.2 Hz, 1H), 7.40 (dd, J = 8.2 / 8.2 Hz, 1H), 7.59 (d, J = 8.2 Hz, 1H), 7.63 (d, J = 8.2 Hz, 1H), 7.63 (d, J = 8.2 Hz, 1H), 7.97 (d, J = 2.9 Hz, 1H), 11.04 – 11.21 (br, 1H), 11.86 (d, J = 2.9 Hz, 1H); ES-MS: 464 $[M+H]^+$.

2-[3-(4,7-Diaza-spiro[2.5]oct-7-yl)-isoquinolin-1-yl]-acetamide used as starting material may be prepared as follows:



a) 2-[3-(4-Benzyl-4,7-diaza-spiro[2.5]oct-7-yl)-isoquinolin-1-yl]-acetamide (490 mg, 1.27 mmol) is dissolved in absolute MeOH (5 ml). Pd on charcoal (140 mg) is added, as well as ammonium formate (200 mg, 3.17 mmol). After refluxing for 1 h (T = 75 °C), an additional charge of ammonium formate (200 mg, 3.17 mmol) is added. 1 h later, TLC analysis indicates complete conversion of the starting material. After filtration and concentration, the residue is taken up in CH_2Cl_2 and washed with water (pH 10 by addition of 2 N NaOH). The organic layer is dried over Na_2SO_4 and the solvent is removed. Purification by FCC (EtOAc/AcOH/H_2O 750:83:68 to 700:110:90 to 650:130:120 to 600:150:150) affords the title compound as its bis-acetate salt. ^{1}H NMR (DMSO, 400 MHz) δ 0.46 – 0.52 (m, 4H), 2.88 (t, J = 5.5, 2H), 3.35 (s, 2H), 3.49 (t, J = 5.5, 2H), 3.94 (s, 2H), 6.77 (s, 1H), 7.00 (br s, 1H), 7.18 – 7.25 (m, 1H), 7.45 – 7.56 (m, 2H), 7.60 – 7.65 (m, 1H), 7.95 (d, J = 9.9, 1H). ES-MS: 297 $[M+H]^+$.

b) 2-[3-(4-Benzyl-4,7-diaza-spiro[2.5]oct-7-yl)-isoquinolin-1-yl]-acetamide

[3-(4-Benzyl-4,7-diaza-spiro[2.5]oct-7-yl)-isoquinolin-1-yl]-acetic acid ethyl ester (700 mg, 1.68 mmol) is dissolved in dry DMF under an atmosphere of argon. Formamide (224 µl, 254 mg, 5.64 mmol) is added, and the reaction mixture is heated to 105 °C. At this temperature, NaOMe (312 µl of a 5.4 M solution in MeOH, 91 mg, 1.68 mmol) is added dropwise during 20 minutes. After 30 minutes at 105 °C, TLC analysis indicates complete conversion of starting material. Cooling the reaction mixture to RT is followed by addition of water and extraction with EtOAc. The organic layers are washed with H_2O (twice), dried over Na_2SO_4 and concentrated. The residue is purified by FCC (hexanes/EtOAc 1:1 to 1:3 to 0:100 to EtOAc/MeOH 98:2) to afford the title compound. ^{1}H NMR (DMSO, 400 MHz) δ 0.68 – 0.70 (m, 2H), 0.82 – 0.88 (m, 2H), 3.08 (t, J = 4.4, 2H), 3.45 (s, 2H), 3.58 (t, J = 4.4, 2H), 3.96 (s, 2H), 4.27 (s, 2H), 5.3 – 5.5 (br, 1H), 6.55 – 6.7 (br, 1H), 6.72 (s, 1H), 7.26 – 7.36 (m, 6H), 7.51 (t, J = 8.8, 1H), 7.60 d, J = 9.9, 1H), 8.02 (d, J = 9.9, 1H); ES-MS: 387 $[M+H]^+$.

c) [3-(4-Benzyl-4,7-diaza-spiro[2.5]oct-7-yl)-isoquinolin-1-yl]-acetic acid ethyl ester

(3-Chloro-isoquinolin-1-yl)-acetic acid ethyl ester (2.50 g, 10.01 mmol), 4-benzyl-4,7-diaza-spiro[2.5]octane (2.23 g, 11.01 mmol), NaOtBu (1.06 g, 11.01 mmol), BINAP (249 mg, 0.40 mmol) and Palladium(II)-acetate (180 mg, 0.80 mmol) are mixed under argon. After addition of degassed, dry dioxane (36 ml), the suspension is heated to 85 °C. After 25 minutes at 85 °C, HPLC analysis indicates a conversion of 71%. The mixture is cooled to RT, diluted with EtOAc, and washed with H_2O and sat. aq. NH_4Cl (back extracted). The combined organic layers are dried over Na_2SO_4, the solvent is removed and the residue purified by FCC (hexane/EtOAc 100:0 to 96:4 to 93:7 to 90:10 to 85:15) to afford the title compound. ^{1}H NMR (DMSO, 400 MHz) δ 0.58 – 0.61 (m, 2H), 0.70 – 0.73 (m, 2H), 1.18 (t, J = 8.8, 3H), 2.98 (t, J = 5.5, 2H), 3.39 (s, 2H), 3.49 (t, J = 5.5, 2H), 3.86 (s, 2H), 4.12 (s, 2H), 4.12 (q, J = 8.8, 2H), 5.59 (s, 1H), 7.14 – 7.19 (m, 6H), 7.39 (t, J = 8.8, 1H), 7.51 (d, J = 9.9, 1H), 7.78 (d, J = 9.9, 1H); ES-MS: 417 $[M+H]^+$.

d) (3-Chloro-isoquinolin-1-yl)-acetic acid ethyl ester

1,1,1,3,3,3-Hexamethyl-disilazane (27.4 ml, 20.37 g, 126.2 mmol) are dissolved in dry toluene (150 ml). After cooling to –78 °C, n-BuLi (79 ml of a 1.6 M solution in hexanes, 126.2 mmol) is slowly added during 20 minutes. The white suspension is stirred at –78 °C for 15 minutes and at RT for 15 minutes, after which time a clear bright yellow solution is obtained. This solution is canulated into a second two-necked flask, containing $Pd_2(dba)_3$ (1.39 g, 1.51 mmol) and (2'-Dicyclohexylphosphanyl-biphenyl-2-yl)-dimethyl-amine (1.25 g, 3.18 mmol).

After stirring at RT for 10 minutes, the clear dark red solution is cooled to –10 °C. Acetic acid tert-butyl ester (15.7 ml, 13.5 g, 116.1 mmol) is added during 5 min. After 10 minutes at –10 °C, 1,3-Dichloro-isoquinoline (10.0 g, 50.49 mmol) is added in one portion. The dark red solution is allowed to warm to RT. After 30 minutes at RT, TLC analysis indicates complete conversion of the starting material. The reaction mixture is filtered through a 2-cm pad of silica, which is rinsed with EtOAc/MeOH 98:2. After concentration, the residue is purified by FCC (toluene/CH_2Cl_2 2:1 to toluene/EtOAc 100:0 to 99:1 to 98:2 to 97:3 to 96:4 to 94:6 to 90:10) to afford (3-Chloro-isoquinolin-1-yl)-acetic acid tert.-butyl ester. This compound is dissolved in a saturated ethanolic solution of HCl (200 ml) and refluxed for 15 minutes. Concentration affords the title compound in quantitative yield. ^{1}H NMR (DMSO, 400 MHz) δ 1.17 (t, J = 8.8, 3H), 4.11 (q, J = 8.8, 2H), 4.28 (s, 2H), 7.51 – 7.57 (m, 1H), 7.61 (s, 1H), 7.61 – 7.66 (m, 1H), 7.72 (d, J = 8.8, 1H), 7.98 (d, J = 8.8, 1H); ES-MS: 250 $[M+H]^+$.

By following the procedure of Example 69 but using the appropriate starting materials, the compounds of formula I wherein R is residue of formula (e), as indicated in Table 5 below, may be obtained.

TABLE 5

Example	R_9	R_a	R_b	M.S. Data
70	-(4,7-diaza-spiro[2.5]oct-7-yl)	CH_3	H	MH^+ 465
71	3-ethyl-piperazin-1-yl	CH_3	H	MH^+ 467
72	-(4,7-diaza-spiro[2.5]oct-7-yl)	H	H	MH^+ 451
73	3-ethyl-1-piperazinyl	H	H	MH^+ 453
74	3-*R*-methyl-piperazin-1-yl	CH_3	H	MH^+ 453
75	3-*R*-methyl-piperazin-1-yl	H	H	MH^+ 439
76	3-*S*-methyl-piperazin-1-yl	CH_3	H	MH^+ 453
77	3-*S*-methyl-piperazin-1-yl	H	H	MH^+ 439
78	4-methyl-3-*S*-methyl-piperazin-1-yl	CH_3	H	MH^+ 467
79	4-methyl-3-*S*-methyl-piperazin-1-yl	H	H	MH^+ 453
80	4,7-diaza-spiro [2.5] 7ct-7-yl	CH_3	H	MH^+ 464
81	4,7-diaza-spiro [2.5] 7ct-7-yl	H	F	MH^+ 479
82	4,7-diaza-spiro [2.5] 7ct-7-yl	CH_2-CH_3	H	MH^+ 479
83	4,7-diaza-spiro [2.5] 7ct-7-yl	H	$CH(CH_3)_2$	MH^+ 495
84	4,7-diaza-spiro [2.5] 7ct-7-yl	H	OCH_3	MH^+ 471

85	4,7-diaza-spiro [2.5] 7ct-7-yl	CH_3	CH_2-CH_3	MH^+ 451
86	4,7-diaza-spiro [2.5] 7ct-7-yl	H	CH_2-CH_3	MH^+ 465
87	4,7-diaza-spiro [2.5] 7ct-7-yl	$CH(CH_3)_2$	H	MH^+ 451
88	4,7-diaza-spiro [2.5] 7ct-7-yl	CH_3	CH_3	MH^+ 479
89	4,7-diaza-spiro [2.5] 7ct-7-yl	CH_3	Cl	MH^+ 499
90	4,7-diaza-spiro [2.5] 7ct-7-yl	H	Cl	MH^+ 485
91	4,7-diaza-spiro [2.5] 7ct-7-yl	CH_2-CH_3	CH_3	MH^+ 492
92	3-ethyl-piperazin-1-yl	CH_2-CH_3	CH_2-CH_3	MH^+ 494

* The compound of Example 80 is converted into its bis-trifluoroacetate or acetate salt.

The compounds of formula I in free form or in pharmaceutically acceptable salt form exhibit valuable pharmacological properties, e.g. inhibiting Protein Kinase C (PKC), e.g. PKC isoforms like α, β, δ, ε, η or θ activity, inhibiting T-cell activation and proliferation, e.g. by inhibiting production by T-cells or cytokines, e,g, IL-2, by inhibiting the proliferative response of T-cells to cytokines, e.g. IL-2, e.g. as indicated in in vitro and in vivo tests and are therefore indicated for therapy.

A. In vitro

1. Protein Kinase C assay

The compounds of formula I are tested for their activity on different PKC isoforms according to a published method (D. Geiges et al. Biochem. Pharmacol. 1997;53:865-875) The assay is performed in a 96-well polypropylene microtiterplate (Costar 3794) that has been previously siliconized with Sigmacote (Sigma SL-2). The reaction mixture (50 µl) contains 10 µl of the relevant PKC isozyme together with 25 µl of the test compound and 15 µl of a mix solution that contains 200 µg/ml protamine sulfate, 10 mM $Mg(NO_3)_2$, 10 µM ATP (Boehringer 519987) and 3750 Bq of ^{33}P-ATP (Hartmann Analytic SFC301, 110TBq/mmol) in 20 mM Tris-buffer pH 7.4 + 0.1% BSA. Incubation is performed for 15 min at 32°C in a microtiterplate shaking incubator (Biolabo Scientific Instruments). Reaction is stopped by adding 10 µl of 0.5 M Na_2EDTA, pH 7.4. 50 µl of mixture are pipetted onto a pre-wetted phosphocellulose paper (Whatmann 3698-915) under gentle pressure. Non-incorporated ATP is washed away with 100 µl bi-dist H_2O. The paper is washed twice in 0.5% H_3PO_4 for 15 min followed by 5 min in EtOH. Thereafter the paper is dryed and placed in an omnifilter (Packard 6005219), and overlayed with 10 µl/well of Microscint-O (Packard 6013611) before

counting in a Topcount radioactivity counter (Packard). IC_{50} measurement is performed on a routine basis by incubating a serial dilution of inhibitor at concentrations ranging between 1-1000 μM according to the method described above. IC_{50} values are calculated from the graph by sigmoidal curve fitting.

2. Protein Kinase C θ Assay

Human recombinant PKCθ is used under the assay conditions as described above. In this assay, compounds of formula I inhibit PKC θ with an $IC_{50} \leq 1$ μM. Compound of Examples 33 and 69 inhibit PKCθ in this assay with an IC_{50} 6.8 and 12.1 nM, respectively.

3. Protein Kinase Cα Assay

Human recombinant PKCα was obtained from Oxford Biomedical Research and is used under the assay conditions as described under Section A.1 above. In this assay, compounds of formula I inhibit PKC θ with an $IC_{50} \leq 1$ μM. Compound of Example 29 inhibits PKCα in this assay with an IC_{50} of 4.3 nM.

4. Protein Kinase Cβ1 Assay

Human recombinant PKCβ1 was obtained from Oxford Biomedical Research and is used under the assay conditions as described under Section A.1 above. In this assay, compounds of formula I inhibit PKC θ with an $IC_{50} \leq 1$ μM. Compound of Example 33 inhibits PKCβ1 in this assay with an IC_{50} of 19.6 nM.

5. Protein Kinase Cδ Assay

Human recombinant PKCδ was obtained from Oxford Biomedical Research and is used under the assay conditions as described under Section A.1 above. In this assay, compounds of formula I inhibit PKC θ with an $IC_{50} \leq 1$ μM. Compound of Example 29 inhibits PKCδ in this assay with an IC_{50} of 20 nM.

6. Protein Kinase Cε Assay

Human recombinant PKCε was obtained from Oxford Biomedical Research and is used under the assay conditions as described under Section A.1 above. In this assay, compounds of formula I inhibit PKC θ with an $IC_{50} \leq 1$ μM. Compound of Example 69 inhibits PKCε in this assay with an IC_{50} of 18 nM.

7. Protein Kinase Cη Assay

Human recombinant PKCη was obtained from PanVera and is used under the assay conditions as described under Section A.1 above. In this assay, compounds of formula I

inhibit PKC θ with an $IC_{50} \leq 1$ µM. Compound of Example 29 inhibits PKCη in this assay with an IC_{50} of 27.4 nM.

8. CD28 costimulation assay

The assay is performed with Jurkat cells transfected with a human interleukin-2 promoter/reporter gene construct as described by Baumann G et al. in Transplant.Proc. 1992;24:43-8, the ß-galactosidase reporter gene being replaced by the luciferase gene (de Wet J., et al., Mol. Cell Biol. 1987, 7(2), 725-737). Cells are stimulated by solid phase-coupled antibodies or phorbol myristate acetate (PMA) and the Ca^{++} ionophore ionomycin as follows. For antibody-mediated stimulation Microlite TM1 microtiter plates (Dynatech) are coated with 3 µg/ml goat anti-mouse IgG Fc antibodies (Jackson) in 55 µl phosphate-buffered saline (PBS) per well for three hours at RT. Plates are blocked after removing the antibodies by incubation with 2% bovine serum albumin (BSA) in PBS (300 µl per well) for 2 hours at RT. After washing three times with 300 µl PBS per well, 10 ng/ml anti-T cell receptor antibodies (WT31, Becton & Dickinson) and 300 ng/ml anti-CD28 antibodies (15E8) in 50 µl 2% BSA/PBS are added as stimulating antibodies and incubated overnight at 4°C. Finally the plates are washed three times with 300 µl PBS per well. Seven three-fold serial dilutions of test compounds in duplicates in assay medium (RPMI 1640/10% fetal calf serum (FCS) containing 50 µM 2-mercaptoethanol, 100 units/ml penicillin and 100 µg/ml streptomycin) are prepared in separate plates, mixed with transfected Jurkat cells (clone K22 290_H23) and incubated for 30 minutes at 37°C in 5% CO_2. 100 µl of this mixture containing 1×10^5 cells are then transferred to the antibody-coated assay plates. In parallel 100 µl are incubated with 40 ng/ml PMA and 2 µM ionomycin. After incubation for 5.5 hours at 37°C in 5% CO_2, the level of luciferase is determined by bioluminescence measurement. The plates are centrifuged for 10 min at 500 g and the supernatant is removed by flicking. Lysis buffer containing 25 mM Tris-phosphate, pH 7.8, 2 mM DTT, 2 mM 1.2-diaminocyclohexane-N,N,N',N-tetraacetic acid, 10 % (v/v) glycerol and 1 % (v/v) Triton X-100 is added (20 µl per well). The plates are incubated at RT for 10 minutes under constant shaking. Luciferase activity is assessed with a bioluminescence reader (Labsystem, Helsinki, Finland) after automatic addition of 50 µl per well luciferase reaction buffer containing 20 mM Tricine, 1.07 mM $(MgCO_3)_4Mg(OH)_2x5H_2O$, 2.67 mM $MgSO_4$, 0.1 mM EDTA, 33.3 mM DTT, 270 µM coenzyme A, 470 µM luciferin (Chemie Brunschwig AG), 530 µM ATP, pH 7.8. Lag time is 0.5 seconds, total measuring time is 1 or 2 seconds. Low control values are light units from anti-T cell receptor- or PMA-stimulated cells, high controls are from anti-T cell receptor/anti-

CD28- or PMA/ionomycin-stimulated cells without any test sample. Low controls are subtracted from all values. The inhibition obtained in the presence of a test compound is calculated as percent inhibition of the high control. The concentration of test compounds resulting in 50% inhibition (IC_{50}) is determined from the dose-response curves. In this assay, compounds of formula I inhibit anti-T cell receptor/anti-CD28 and PMA/ionomycin stimulated Jurkat cells with an $IC_{50} \leq 1$ μM. Compound of Example 29 has an IC_{50} of 20 nM in this assay.

9. Allogeneic Mixed Lymphocyte Reaction (MLR)

The two-way MLR is performed according to standard procedures (J. Immunol. Methods, 1973, 2, 279 and Meo T. et al., Immunological Methods, New York, Academic Press, 1979, 227-39). Briefly, spleen cells from CBA and BALB/c mice (1.6×10^5 cells from each strain per well in flat bottom tissue culture microtiter plates, 3.2×10^5 in total) are incubated in RPMI medium containing 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco BRL, Basel, Switzerland), 50 μM 2-mercaptoethanol (Fluka, Buchs, Switzerland) and serially diluted compounds. Seven three-fold dilution steps in duplicates per test compound are performed. After four days of incubation 1 μCi ^{3}H-thymidine is added. Cells are harvested after an additional five-hour incubation period, and incorporated ^{3}H-thymidine is determined according to standard procedures. Background values (low control) of the MLR are the proliferation of BALB/c cells alone. Low controls are subtracted from all values. High controls without any sample are taken as 100% proliferation. Percent inhibition by the samples is calculated, and the concentrations required for 50% inhibition (IC_{50} values) are determined. In this assay, Compound of Example 29 has an IC_{50} of 28 nM. Compounds of formula I also exhibit IC_{50} values in the nM range when tested in the human MLR.

10. Inhibition of GSK-3β

The GSK-3β binding assay is performed in 50μl reactions in 96 well polypropylene plate, each reaction containing 20mM magnesium chloride, 40 μM ATP, 2mM DTT, 88.5μM biotinylated and phosphorylated CREB-peptide substrate (biotin-KRREILSRRPS(PO_4)YR-OH ; Q. M. Wang et al., J. Biol. Chem. 269, 14566-14574, 1994), [γ-^{33}P]ATP (1μCi) and 2μl of the compound to be tested in DMSO(various concentrations). 15μl of GSK-3β (various concentrations) is added and the mixture is incubated at 30°C for 1 hour. The reaction is stopped by transferring 25 μl of the mixture to a phosphocellulose plate containing 130μl of 1.85% phosphoric acid. The free radionucleotides in the membrane are washed off under vacuum with 1.85% phosphoric acid (5 times). After the last wash, the plate is transferred to

an adaptor plate and 50µl of scintillation cocktail (Microscint-20, Packard, cat. # 20-133) is added to each well and the amount of radioactivity is counted in a top counter. Compounds of formula I are active in this assay. Compounds of formula I may also be tested in other standard GSK-3β binding assays using other substrates, e.g. as commercially available.

B. In vivo

Rat Heart transplantation

The strain combination used: Male Lewis (RT^1 haplotype) and BN (RT^1 haplotype). The animals are anaesthetised using inhalational isofluorane. Following heparinisation of the donor rat through the abdominal inferior vena cava with simultaneous exsanguination via the aorta, the chest is opened and the heart rapidly cooled. The aorta is ligated and divided distal to the first branch and the brachiocephalic trunk is divided at the first bifurcation. The left pulmonary artery is ligated and divided and the right side divided but left open. All other vessels are dissected free, ligated and divided and the donor heart is removed into iced saline.

The recipient is prepared by dissection and cross-clamping of the infra-renal abdominal aorta and vena cava. The graft is implanted with end-to-side anastomoses, using 10/0 monofilament suture, between the donor brachiocephalic trunk and the recipient aorta and the donor right pulmonary artery to the recipient vena cava. The clamps are removed, the graft tethered retroabdominally, the abdominal contents washed with warm saline and the animal is closed and allowed to recover under a heating lamp. Graft survival is monitored by daily palpation of the beating donor heart through the abdominal wall. Rejection is considered to be complete when heart beat stops. Increases of graft survival are obtained in animals treated with a compound of formula I administered orally at a daily dose of 1 to 30 mg/kg bid.

Graft v. Host Model

Spleen cells ($2x10^7$) from Wistar/F rats are injected subcutaneously into the right hind footpad of (Wistar/F x Fischer 344)F_1 hybrid rats. The left footpad is left untreated. The animals are treated with the test compounds on 4 consecutive days (0-3). The popliteal lymph nodes are removed on day 7, and the weight differences between two corresponding lymph nodes are determined. The results are expressed as the inhibition of lymph node enlargement (given in percent) comparing the lymph node weight differences in the experimental groups to the weight difference between the corresponding lymph nodes from

a group of animals left untreated with a test compound. In this assay, an inhibition of 100% is obtained with compound of Example 29 when administered at a dose of 30 mg/kg/day bid.

The compounds of formula I are, therefore, useful in the treatment and/or prevention of diseases or disorders mediated by T lymphocytes and/or PKC, e.g. acute or chronic rejection of organ or tissue allo- or xenografts, graft versus host diseases, atherosclerosis, vascular occlusion due to vascular injury such as angioplasty, restenosis, obesity, syndrome X, impaired glucose tolerance, polycystic ovary syndrome, hypertension, heart failure, chronic obstructive pulmonary disease, CNS diseases such as Alzheimer disease or amyotrophic lateral sclerosis, cancer, infectious diseases such as AIDS, septic shock or adult respiratory distress syndrome, ischemia/reperfusion injury e.g. myocardial infarction, stroke, gut ischemia, renal failure or hemorrhage shock, or traumatic shock, e.g. traumatic brain injury. The compounds of formula I are also useful in the treatment and/or prevention of T-cell mediated acute or chronic inflammatory diseases or disorders or autoimmune diseases e.g. rheumatoid arthritis, osteoarthritis, systemic lupus erythematosus, Hashimoto's thyroidis, multiple sclerosis, myasthenia gravis, diabetes type I or II and the disorders associated therewith, e.g. angiopathy, diabetic proliferative retinopathy, diabetic macular edema, nephropathy, neuropathy and dawn phenomenon, respiratory diseases such as asthma or inflammatory lung injury, inflammatory liver injury, inflammatory glomerular injury, cutaneous manifestations of immunologically-mediated disorders or illnesses, inflammatory and hyperproliferative skin diseases (such as psoriasis, atopic dermatitis, allergic contact dermatitis, irritant contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis), inflammatory eye diseases, e.g. Sjoegren's syndrome, keratoconjunctivitis or uveitis, inflammatory bowel disease, Crohn's disease or ulcerative colitis. For the above uses the required dosage will of course vary depending on the mode of administration, the particular condition to be treated and the effect desired. In general, satisfactory results are indicated to be obtained systemically at daily dosages of from about 0.1 to about 100 mg/kg body weight. An indicated daily dosage in the larger mammal, e.g. humans, is in the range from about 0.5 mg to about 2000 mg, conveniently administered, for example, in divided doses up to four times a day or in retard form.

The compounds of formula I may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets or capsules, or parenterally, e.g. in the form of injectable solutions or suspensions, topically, e.g. in the form of lotions, gels, ointments or creams, or in a nasal or a suppository form. Pharmaceutical compositions comprising a compound of formula I in free form or in pharmaceutically acceptable salt form in association

with at least one pharmaceutical acceptable carrier or diluent may be manufactured in conventional manner by mixing with a pharmaceutically acceptable carrier or diluent. Unit dosage forms for oral administration contain, for example, from about 0.1 mg to about 500 mg of active substance.

Topical administration is e.g. to the skin. A further form of topical administration is to the eye.

The compounds of formula I may be administered in free form or in pharmaceutically acceptable salt form e.g. as indicated above. Such salts may be prepared in conventional manner and exhibit the same order of activity as the free compounds.

In accordance with the foregoing the present invention further provides:

1.1 A method for preventing or treating disorders or diseases mediated by T lymphocytes and/or PKC or GSK-3β, e.g. such as indicated above, in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof;

1.2 A method for preventing or treating acute or chronic transplant rejection or T-cell mediated inflammatory or autoimmune diseases, e.g. as indicated above, in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof;

2. A compound of formula I, in free form or in a pharmaceutically acceptable salt form for use as a pharmaceutical, e.g. in any of the methods as indicated under 1.1 and 1.2 above.

3. A pharmaceutical composition, e.g. for use in any of the methods as in 1.1 and 1.2 above comprising a compound of formula I in free form or pharmaceutically acceptable salt form in association with a pharmaceutically acceptable diluent or carrier therefor.

4. A compound of formula I or a pharmaceutically acceptable salt thereof for use in the preparation of a pharmaceutical composition for use in any of the method as in 1.1 and 1.2 above.

Compounds of formula I may be administered as the sole active ingredient or together with other drugs in immunomodulating regimens or other anti-inflammatory agents e.g. for the treatment or prevention of allo- or xenograft acute or chronic rejection or inflammatory or

autoimmune disorders. For example, they may be used in combination with cyclosporines, or ascomycines or their immunosuppressive analogs or derivatives, e.g. cyclosporin A, ISA Tx247, FK-506, ABT-281, ASM 981; an mTOR inhibitor, e.g. rapamycin, 40-O-(2-hydroxy)ethyl-rapamycin, CCI779, ABT578 or a rapalog, e.g. AP23573 etc.; corticosteroids; cyclophosphamide; azathioprene; methotrexate; an EDG receptor agonist having accelerating lymphocyte homing properties, e.g. FTY 720 or an analogue thereof; leflunomide or analogs thereof; mizoribine; mycophenolic acid; mycophenolate mofetil; 15-deoxyspergualine or analogs thereof; immunosuppressive monoclonal antibodies, e.g., monoclonal antibodies to leukocyte receptors, e.g., MHC, CD2, CD3, CD4, CD 11a/CD18, CD7, CD25, CD 27, B7, CD40, CD45, CD58, CD 137, ICOS, CD150 (SLAM), OX40, 4-1BB or their ligands, e.g. CD154; or other immunomodulatory compounds, e.g. a recombinant binding molecule having at least a portion of the extracellular domain of CTLA4 or a mutant thereof, e.g. an at least extracellular portion of CTLA4 or a mutant thereof joined to a non-CTLA4 protein sequence, e.g. CTLA4Ig (for ex. designated ATCC 68629) or a mutant thereof, e.g. LEA29Y, or other adhesion molecule inhibitors, e.g. mAbs or low molecular weight inhibitors including LFA-1 antagonists, Selectin antagonists and VLA-4 antagonists. Compounds of formula I may also be administered together with an antiproliferative drug, e.g. a chemotherapeutic drug, e.g. as used in cancer treatment, including but not limited to aromatase inhibitors, antiestrogens, topoisomerase I inhibitors, topoisomerase II inhibitors, microtubule active agents, alkylating agents, histone deacetylase inhibitors, farnesyl transferase inhibitors, COX-2 inhibitors, MMP inhibitors, mTOR inhibitors, antineoplastic antimetabolites, platin compounds, compounds decreasing the protein kinase activity and further anti-angiogenic compounds, gonadorelin agonists, anti-androgens, bengamides, bisphosphonates, antiproliferative antibodies and temozolomide, or with an anti-diabetic drug, an insulin secretagogue or insulin secretion enhancer, e.g. a sulphonyl urea, e.g. tolbutamide, chlorpropamide, tolazamide, acetohexamide, 4-chloro-N-[(1-pyrolidinylamino)carbonyl]-benzensulfonamide (glycopyramide), glibenclamide (glyburide), gliclazide, 1-butyl-3-metanilylurea, carbutamide, glibonuride, glipizide, gliquidone, glisoxepid, glybuthiazole, glibuzole, glyhexamide, glymidine, glypinamide, phenbutamide or tolylcyclamide, an oral insulinotropic agent derivative, e.g. a short acting insulin enhancer, e.g. meglitinide, repaglinide, a phenyl acetic acid derivative, e.g.nateglinide, a DPP IV inhibitor, e.g. 1-{2-[(5-cyanopyridin-2-yl)amino]ethylamino}acetyl-(2S)-cyano-pyrrolidine dihydrochloride, LAF237, GLP-1 or a GLP-1 agonist analog, or an insulin sensitizer e.g. a peroxisome proliferator activated receptor γ agonist (PPARγ), e.g. a glitazone, a non-

glitazone type such as a N-(2-benzoylphenyl)-L-tyrosine analogue, e.g. GI-262570, or an oxolidinedione, e.g. JTT501, a dual PPARγ/PPARα agonist, e.g. DRF-554158, NC-2100 or NN-622, a retinoid X receptor agonist or a rexinoid, e.g. 2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-cyclopropyl]-pyridine-5-carboxylic acid, 4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-2-carbonyl]-benzoic acid, 9-cis retinoic acid or an analog, derivative or a pharmaceutically acceptable salt thereof, in diabetes therapy,

In accordance with the foregoing the present invention provides in a yet further aspect:

5. A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of an inhibitor of GSK-3β, PKC or of T-cell activation and proliferation, e.g. a compound of formula I in free form or in pharmaceutically acceptable salt form, and a second drug substance, said second drug substance being an immunosuppressant, immunomodulatory, anti-inflammatory, antiproliferative or anti-diabetic drug, e.g. as indicated above.

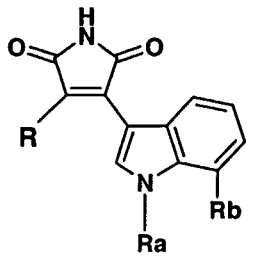
6. A therapeutic combination, e.g. a kit, comprising a) an inhibitor of GSK-3β, PKC or of T-cell activation and proliferation, e.g. a compound of formula I in free form or in pharmaceutically acceptable salt form, and b) at least one second agent selected from an immunosuppressant, immunomodulatory, anti-inflammatory, antiproliferative and anti-diabetic drug. Component a) and component b) may be used concomitantly or in sequence. The kit may comprise instructions for its administration.

Where an inhibitor of GSK-3β, PKC or of T-cell activation and proliferation, e.g. a compound of formula I, is administered in conjunction with other immunosuppressive/immunomodulatory, anti-inflammatory, antiproliferative or anti-diabetic therapy, e.g. for preventing or treating acute or chronic graft rejection or inflammatory or autoimmune disorders as hereinabove specified, dosages of the co-administered immunosuppressant, immunomodulatory, anti-inflammatory, antiproliferative or anti-diabetic compound will of course vary depending on the type of co-drug employed, e.g. whether it is a steroid or a cyclosporine, on the specific drug employed, on the condition being treated and so forth.

Compounds of formula I have an interesting pharmacokinetic profile and interesting in vitro and in vivo activities.

CLAIMS

1. A compound of formula I



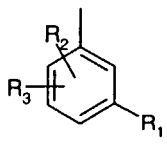
I

wherein

R_a is H; CH_3; CH_2-CH_3; or isopropyl,

R_b is H; halogen; C_{1-6}alkoxy; or C_{1-6}alkyl, and either

I. R is a radical of formula (a)



(a)

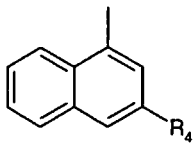
wherein

R_1 is piperazin-1-yl optionally substituted by CH_3 in position 3 or 4; or 4,7-diaza-spiro [2.5] oct-7-yl;

R_2 is Cl; Br; CF_3; or CH_3; and

R_3 is H; CH_3; or CF_3; R_2 being other than CH_3 or Cl when R_3 is H, R_a is H or CH_3, R_b is H and R_1 is 4-methyl-1-piperazinyl; or

II. R is a radical of formula (b)

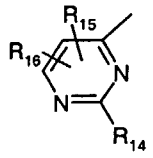


(b)

wherein

R_4 is piperazin-1-yl substituted in positions 3 and/or 4 by CH_3; or 4,7-diaza-spiro [2.5] oct-7-yl; R_a being other than H or CH_3 when R_4 is 4-methyl-1-piperazinyl; or

III. R is a residue of formula (c)



(c)

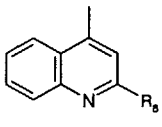
wherein

R_{14} is piperazin-1-yl optionally substituted by CH_3 in position 3 and/or 4 or in position 3 by ethyl, phenyl-C_{1-4}alkyl, C_{1-4}alkoxy-C_{1-4}alkyl or halogeno–C_{1-4}alkyl; or 4,7-diaza-spiro [2.5] oct-7-yl;

R_{15} is halogen; CF_3; or CH_3; R_{15} being other than CH_3 when R_{16} is CH_3, R_a is H or CH_3, R_b is H and R_{14} is 4-methyl-1-piperazinyl; and

R_{16} is H; CH_3; CH_2-CH_3; or CF_3; R_{16} being other than H when R_{15} is Cl, R_a is H or CH_3, R_b is H and R_{14} is 4-methyl-1-piperazinyl; or

IV. R is a radical of formula (d)

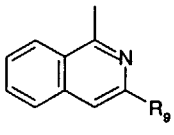


(d)

wherein

R_8 is 1-piperazinyl, 3-methyl-piperazin-1-yl or 4-benzyl-piperazin-1-yl; or

V. R is a radical of formula (e)



(e)

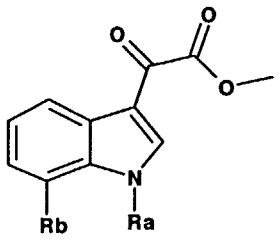
wherein

R_9 is 4,7-diaza-spiro [2.5] oct-7-yl; or 1-piperazinyl substituted in position 3 by methyl or ethyl and optionally in position 4 by methyl,

or a salt thereof.

2. A compound according to claim 1 which is selected from 3-[5-chloro-6-methyl-2-(4-methyl-piperazin-1-yl)-pyrimidin-4-yl]-4-(1H-indol-3-yl)-pyrrole-2,5-dione, 3-[3-(4,7-diaza-spiro[2.5]oct-7-yl)-isoquinolin-1-yl]-4-(7-methyl-1H-indol-3-yl)-pyrrole-2,5-dione and 3-(1H-indol-3-yl)-4-[2-(4-methyl-piperazin-1-yl)-5-trifluoromethyl-pyrimidin-4-yl]pyrrole-2,5-dione or a salt thereof.

3. A process for the preparation of a compound of formula I according to claim 1, which process comprises reacting a compound of formula II



(II)

wherein R_a and R_b are as defined in claim 1,

with a compound of formula III

$$R - CH_2 - CO - NH_2 \qquad (III)$$

wherein R is as defined in claim 1,

and, where required, converting the resulting compound of formula I obtained in free form to a salt form or vice versa, as appropriate.

4. A compound of formula I according to claim 1 or 2, in free form or in a pharmaceutically acceptable salt form for use as a pharmaceutical.

5. A pharmaceutical composition comprising a compound of formula I according to claim 1 or 2 or a pharmaceutically acceptable salt thereof in association with a pharmaceutically acceptable diluent or carrier therefor.

6. A compound of formula I according to claim 1 or 2 or a pharmaceutically acceptable salt thereof for use in the preparation of a pharmaceutical composition for use in the treatment or prevention of disorders or diseases mediated by T lymphocytes and/or PKC or GSK-3β.

7. A compound of formula I according to claim 1 or 2 or a pharmaceutically acceptable salt thereof for use in combination with an immunosuppressant, immunomodulatory, anti-inflammatory, antiproliferative or anti-diabetic drug.

8. A combination comprising a) a compound of formula I in free form or in pharmaceutically acceptable salt form, and b) at least one second agent selected from an immunosuppressant, immunomodulatory, anti-inflammatory, antiproliferative and anti-diabetic drug.

9. A method for preventing or treating disorders or diseases mediated by T lymphocytes and/or PKC or GSK-3β in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I according to claim 1 or 2 or a pharmaceutically acceptable salt thereof.

10. A method according to claim 9 comprising co-administration concomitantly or in sequence of a therapeutically effective amount of a compound of formula I in free form or in pharmaceutically acceptable salt form, and a second drug substance, said second drug substance being an immunosuppressant, immunomodulatory, anti-inflammatory, antiproliferative or anti-diabetic drug.