Supporting Information

Cinnamic Anilides as New Mitochondrial Permeability Transition Pore Inhibitors Endowed with Ischemia-Reperfusion Injury Protective Effect in Vivo

Daniele Fancelli[†#], Agnese Abate[‡○], Raffaella Amici[†#], Paolo Bernardi[§], Marco Ballarini[†‖], Anna Cappa[‡#], Giacomo Carenzi[‡□], Andrea Colombo[⊥◊], Cristina Contursi[†=], Fabio Di Lisa[|], Giulio Dondio[⊥~], Stefania Gagliardi[⊥∫], Eva Milanesi[†], Saverio Minucci[‖⊤], Gilles Pain[†┣], Pier Giuseppe Pelicci[‖], Alessandra Saccani[†┤], Mariangela Storto[†‖], Florian Thaler[†#], Mario Varasi[‡#], Manuela Villa[†#] and Simon Plyte[†Δ].*

[†]Genextra Group, Congenia s.r.l., Via Adamello 16, 20139 Milan (Italy);
[‡]Genextra Group, DAC s.r.l., Via Adamello 16, 20139 Milan (Italy);
[‖]Department of Experimental Oncology, European Institute of Oncology IEO, Via Adamello 16, 20139 Milan, Italy;
[⊤]Department of Biosciences, University of Milan, 20100 Milan, Italy;
[§]Department of Biomedical Sciences, University of Padua, Via Ugo Bassi 58/B, 35121 Padua (PD), Italy;
[|] Department of Biomedical Sciences, University of Padua, Viale G. Colombo 3, 35131 Padua, Italy.
[⊥]NiKem Research s.r.l., Via Zambeletti 25, 20021 Baranzate (MI), Italy;

HPLC purity of target compounds

Method 1: Waters Acquity UPLC, Waters SQD single quadrupole. Column Acquity UPLC-BEH C18 50 x 2.1 mm x 1.7 μm. Flow rate: 0.6 ml/min. Mobile phase: A phase= water/CH_3CN 95/5 + 0.07% formic acid; B phase= CH_3CN + 0.05% formic acid. Gradient: 0 min (A: 98%, B: 2%), 3 min (A: 0%, B: 100%), 3.5 min (A: 0%, B: 100%). UV detection wavelength: 254 nm. Injection volume: 0.5μl

Method 2: Waters Acquity UPLC, Waters SQD single quadrupole. Column Acquity UPLC-BEH C18 50 x 2.1 mm x 1.7 μm. Flow rate: 0.6 ml/min. Mobile phase: A phase= water/CH_3CN 95/5 + 0.07% formic acid; B phase= CH3CN + 0.05% formic acid. Gradient: 0 min (A: 80%, B: 20%), 5 min (A: 30%, B: 70%). UV detection wavelength: 254 nm. Injection volume: 0.5μl.

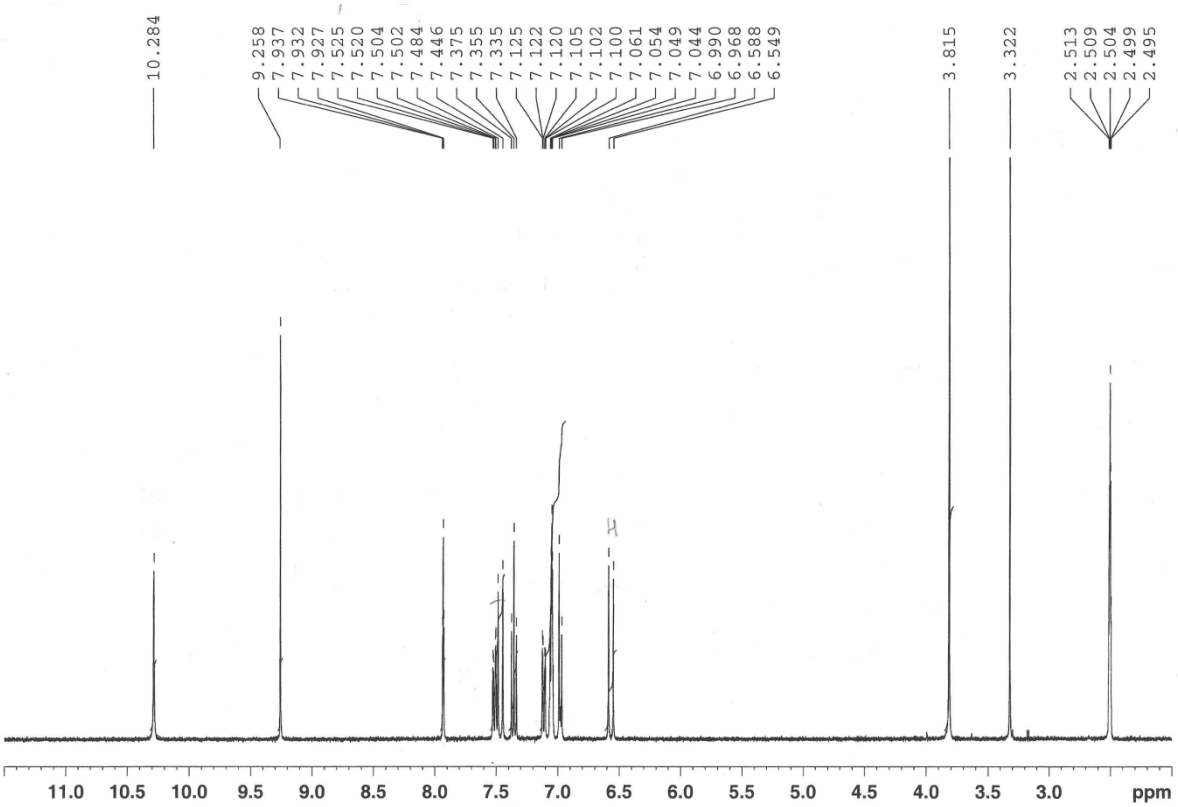
Method 3: Agilent 1100 HPLC, ion-trap Esquire 3000^+ with ESI (Bruker). Column Supelco Discovery 150 x 4.6 mm, 5 μm. Flow rate: 1.0 ml/min. Mobile phase: A phase= water + 0.05% TFA; B phase= CH_3CN + 0.05% TFA. Gradient: 0 min (A: 95%, B: 5%), 15 min (A: 50%, B: 50%). UV detection wavelength: 254 nm.

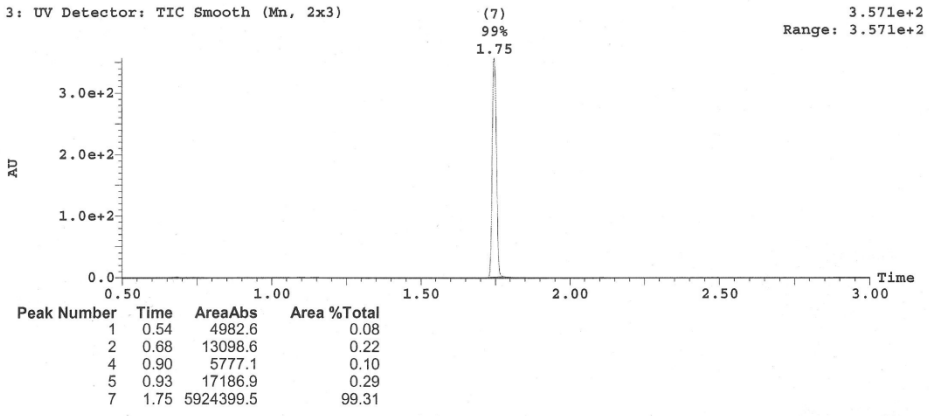
Method 4: Waters Acquity UPLC, Micromass ZQ 2000 Single quadrupole (Waters). Column Acquity UPLC-BEH C18 50 x 2.1 mm x 1.7 μm. Flow rate: 0.6 ml/min. Mobile phase: A phase= water/CH_3CN 95/5 + 0.1% TFA; B phase= water/CH_3CN 5/95 + 0.1% TFA. Gradient: 0-0.25min (A: 95%, B: 5%), 0.25-3.30 min (A: 0%, B: 100%), 3.30-4.00 min (A: 0%, B: 100%). UV detection wavelength: 254 nm. Injection volume: 2 μl

Method 5: Agilent 1100 HPLC, ion-trap Esquire 3000^+ with ESI (Bruker). Column Supelco Discovery 150 x 4.6 mm, 5 μm. Flow rate: 1.0 ml/min. Mobile phase: A phase= water + 0.05% TFA; B phase= CH_3CN + 0.05% TFA. Gradient: 0 min (A: 80%, B: 20%), 15 min (A: 0%, B: 90%). UV detection wavelength: 254 nm.

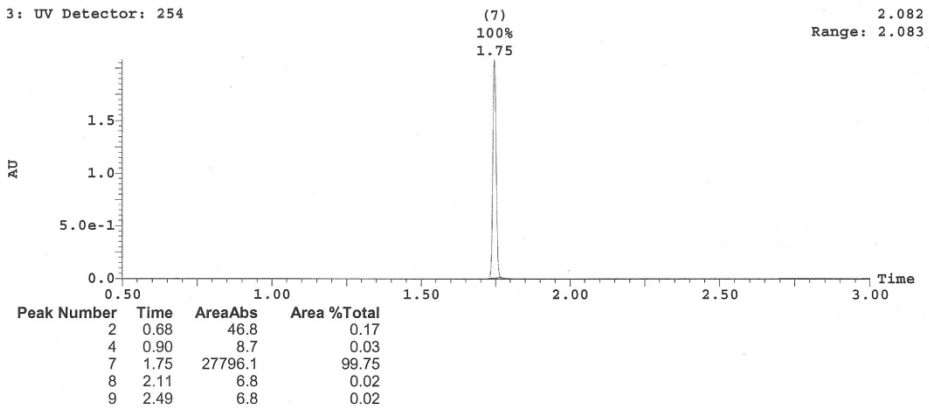
Compound	Purity (raw area % at 254 nm)	Method	Retention time (min)
3	100	2	1.78
4	99	1	1.81
5	100	2	2.18
6	100	1	1.75
7	95	1	1.61
8	98	5	11.4
9	99	1	2.05
10	90	5	7.30
11	90	3	10.6
12	99	5	11.7
13	100	1	1.88
14	100	1	1.47
15	100	5	10.79
16	99	5	8.77
17	100	5	10.67
18	99	5	3.93
19	100	5	4.73
20	100	5	9.80
21	100	5	10.3
22	99	1	1.68
23	100	1	1.78
24	97	5	5.38
25	99	5	11.87
26	100	5	9.55
27	100	5	10.95
28	98	5	9.16
29	97	1	1.8
30	100	5	9.71
31	95	1	1.47
32	100	1	1.38
33	98	1	1.2
34	100	5	9.96
37	99	5	11.02
41	95	1	1.53
44	98	5	11.7
46	100	5	9.95

^{1}H NMR and UPLC traces of compound 6



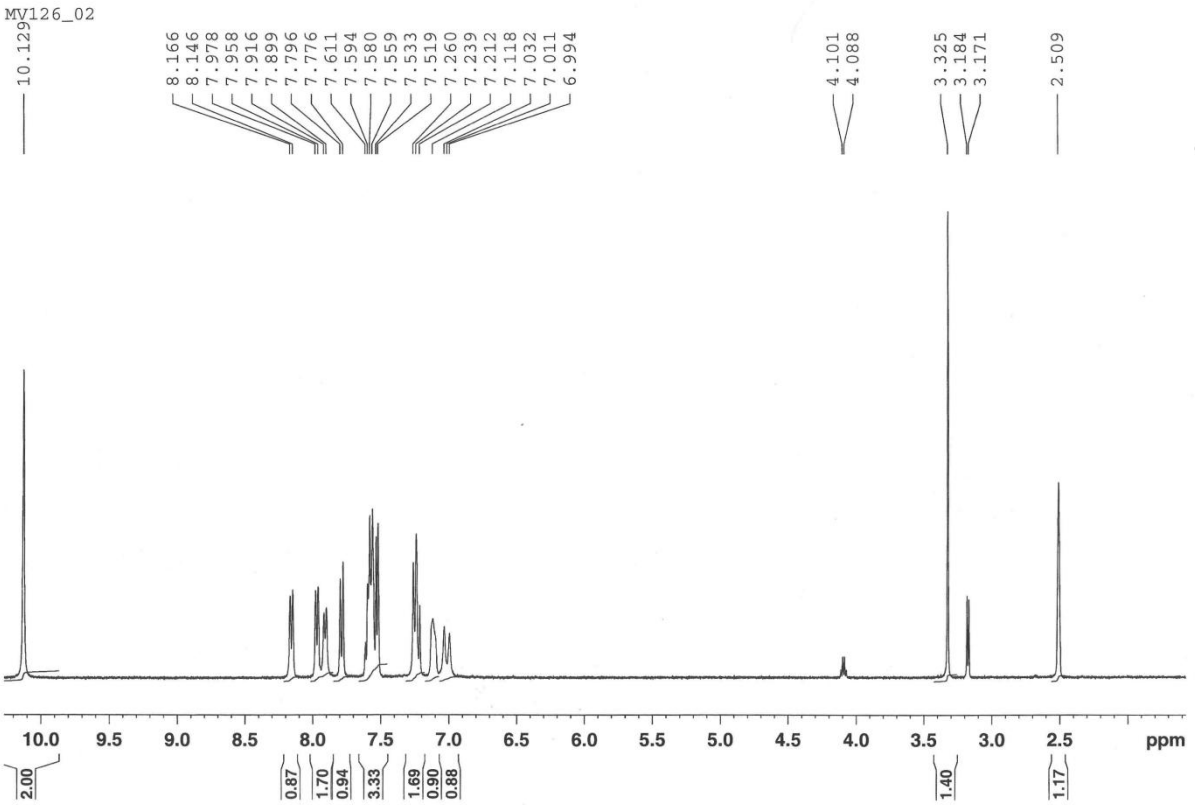


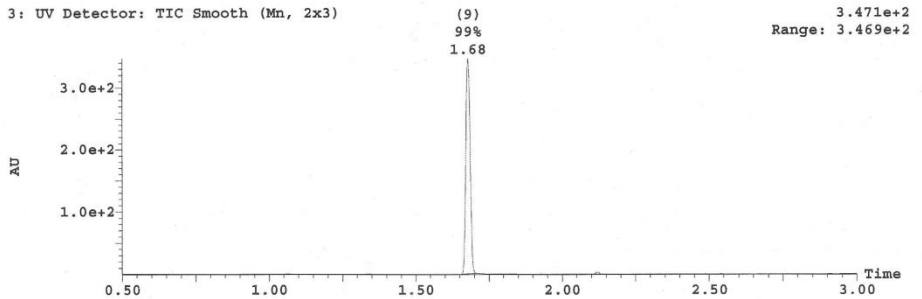
Peak Number	Time	AreaAbs	Area %Total
1	0.54	4982.6	0.08
2	0.68	13098.6	0.22
4	0.90	5777.1	0.10
5	0.93	17186.9	0.29
7	1.75	5924399.5	99.31



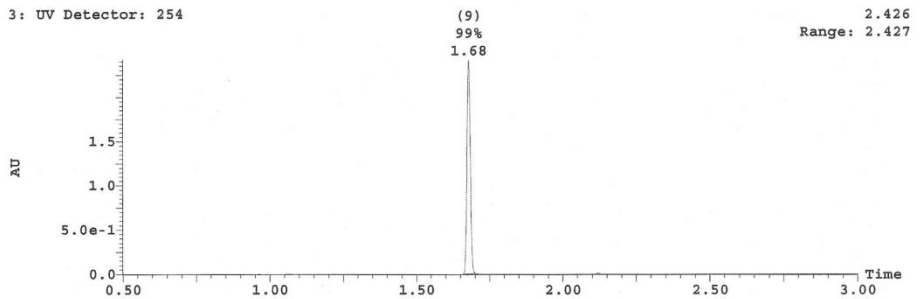
Peak Number	Time	AreaAbs	Area %Total
2	0.68	46.8	0.17
4	0.90	8.7	0.03
7	1.75	27796.1	99.75
8	2.11	6.8	0.02
9	2.49	6.8	0.02

[1]H NMR and UPLC traces of compound 22





Peak Number	Time	AreaAbs	Area %Total
4	0.94	10869.4	0.18
5	0.96	12093.1	0.20
6	1.06	14859.7	0.25
9	1.68	5903955.0	98.69
11	2.12	40724.3	0.68



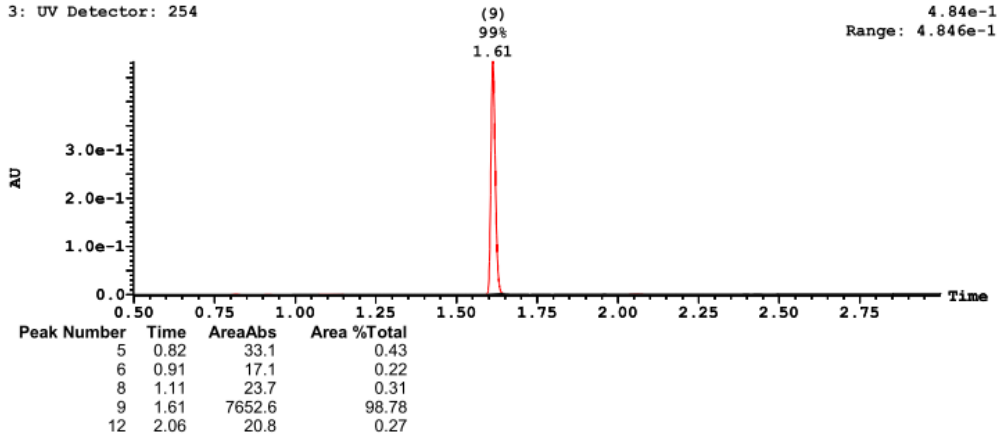
Peak Number	Time	AreaAbs	Area %Total
6	1.06	40.2	0.12
8	1.34	21.6	0.07
9	1.68	32096.6	99.19
10	1.83	20.3	0.06
11	2.12	179.1	0.55

Reactivity Assay of compound 22 with *N*-Acetyl Cysteine or Glutathione

General Protocol: 10 µL of compound **22** (10 mM stock solution in DMSO) and 10 µL of *N*-acetyl Cysteine (10 mM stock solution in DMSO) or Glutathione (10 mM stock solution in water) were diluted with 980 µL of a PBS/DMSO (2:1, v:v) and incubated at 25 °C for 24 h. The samples were analyzed by UPLC by injecting 7.5 µL of the standard solution after 0 and 24 h. Analytical Method: Method 1, HPLC/UV or HPLC/MS, without calibration curve.

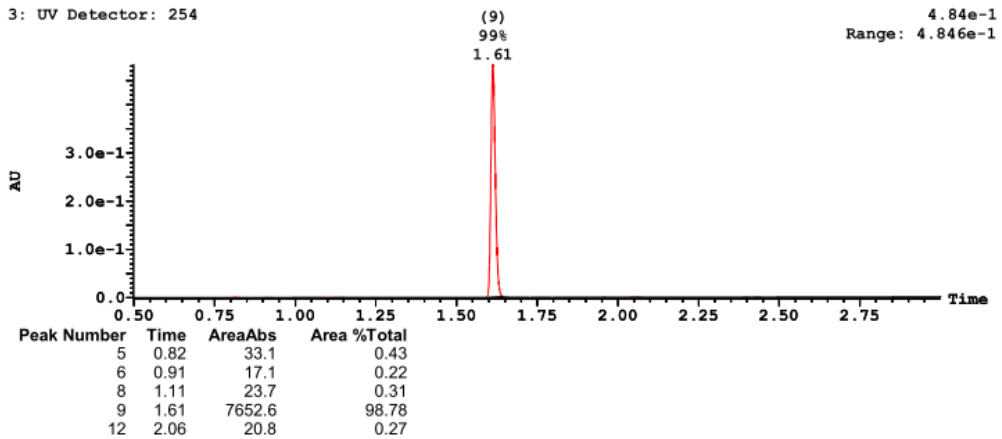
Data Analysis: The samples were evaluated by comparing the AUC of the test solution in comparison with a stock solution of compound **22** (100 µM) in PBS/DMSO (2:1, v:v) as well as by MS. As shown in Figures S1 and S2 only the peak of compound **22** in UV and MS was detected and no NAC or glutathione adduct was detectable under the experimental conditions.

A



Peak Number	Time	AreaAbs	Area %Total
5	0.82	33.1	0.43
6	0.91	17.1	0.22
8	1.11	23.7	0.31
9	1.61	7652.6	98.78
12	2.06	20.8	0.27

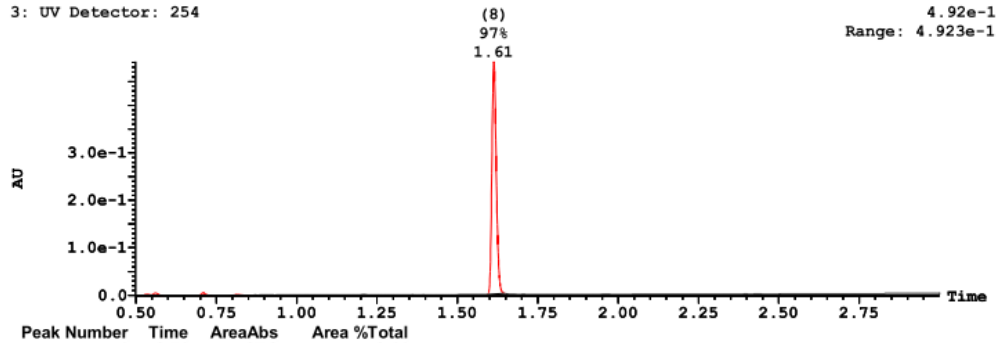
B



Peak Number	Time	AreaAbs	Area %Total
5	0.82	33.1	0.43
6	0.91	17.1	0.22
8	1.11	23.7	0.31
9	1.61	7652.6	98.78
12	2.06	20.8	0.27

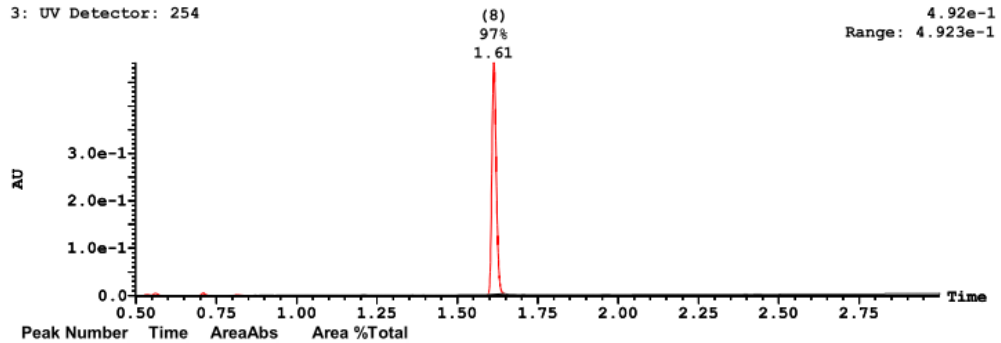
Figure S1 UPLC analyses (UV absorption at 254 nm) of compound **22** (chromatogram A) and compound **22** after 24h incubation with glutathione (chromatogram B; glutathione…..)

A



Peak Number	Time	AreaAbs	Area %Total
1	0.54	35.3	0.44
2	0.56	76.2	0.96
4	0.71	77.4	0.97
6	0.82	29.9	0.38
8	1.61	7761.6	97.26

B



Peak Number	Time	AreaAbs	Area %Total
1	0.54	35.3	0.44
2	0.56	76.2	0.96
4	0.71	77.4	0.97
6	0.82	29.9	0.38
8	1.61	7761.6	97.26

Figure S2 UPLC analyses (UV absorption at 254 nm) of compound **22** (chromatogram A) and compound **22** after 24h incubation with N-acetyl-cystein (chromatogram B; N-acetyl-cystein…..)

***In Vitro* Pharmacology: Binding Assays**

Summary Results of compound **22** tested at 10 μM

Assay	% inhibition of control specific binding
A_1 *(h)* (antagonist radioligand)	40
A_{2A} *(h)* (agonist radioligand)	43
A_3 *(h)* (agonist radioligand)	13
α_1 (non-selective) (antagonist radioligand)	5
α_2 (non-selective) (antagonist radioligand)	-12
β_1 *(h)* (agonist radioligand)	6
β_2 *(h)* (agonist radioligand)	0
AT_1 *(h)* (antagonist radioligand)	-2
AT_2 *(h)* (agonist radioligand)	-6
BZD (central) (agonist radioligand)	3
B_1 *(h)* (agonist radioligand)	2
B_2 *(h)* (agonist radioligand)	-7
CB_1 *(h)* (agonist radioligand)	-6
CB_2 *(h)* (agonist radioligand)	-9
CCK_1 (CCK_A) *(h)* (agonist radioligand)	32
CCK_2 (CCK_B) *(h)* (agonist radioligand)	6
CRF_1 *(h)* (agonist radioligand)	13
D_1 *(h)* (antagonist radioligand)	-7
D_{2S} *(h)* (antagonist radioligand)	4
D_3 *(h)* (antagonist radioligand)	0
$D_{4.4}$ *(h)* (antagonist radioligand)	36
ET_A *(h)* (agonist radioligand)	-2
ET_B *(h)* (agonist radioligand)	12

GABA (non-selective) (agonist radioligand)	5
AMPA (agonist radioligand)	2
kainate (agonist radioligand)	13
NMDA (antagonist radioligand)	3
H_1 *(h)* (antagonist radioligand)	-6
H_2 *(h)* (antagonist radioligand)	18
H_3 *(h)* (agonist radioligand)	9
I_2 (antagonist radioligand)	-12
BLT_1 (LTB_4) *(h)* (agonist radioligand)	0
$CysLT_1$ (LTD_4) *(h)* (agonist radioligand)	10
MC_4 *(h)* (agonist radioligand)	-1
M (non-selective) (antagonist radioligand)	13
NK_1 *(h)* (agonist radioligand)	-8
NK_2 *(h)* (agonist radioligand)	6
NK_3 *(h)* (antagonist radioligand)	10
Y (non-selective) (agonist radioligand)	-3
N neuronal α-BGTX-insensitive ($\alpha4\beta2$) (agonist radioligand)	19
opioid (non-selective) (antagonist radioligand)	7
NOP (ORL1) *(h)* (agonist radioligand)	-11
PPARγ *(h)* (agonist radioligand)	-1
PCP (antagonist radioligand)	-6
EP_4 *(h)* (agonist radioligand)	4
IP (PGI_2) *(h)* (agonist radioligand)	-4
P2X (agonist radioligand)	-8
P2Y (agonist radioligand)	-3
5-HT (non-selective) (agonist radioligand)	18
σ (non-selective) (agonist radioligand)	4

GR *(h)* (agonist radioligand)	0
PR *(h)* (agonist radioligand)	-15
AR *(h)* (agonist radioligand)	-5
TRH_1 *(h)* (agonist radioligand)	-14
V_{1a} *(h)* (agonist radioligand)	8
V_2 *(h)* (agonist radioligand)	3
Ca^{2+} channel (L, dihydropyridine site) (antagonist radioligand)	7
Ca^{2+} channel (L, diltiazem site) (benzothiazepines) (antagonist radioligand)	-24
Ca^{2+} channel (L, verapamil site) (phenylalkylamine) (antagonist radioligand)	12
K_{ATP} channel (antagonist radioligand)	10
K_V channel (antagonist radioligand)	-3
SK_{Ca} channel (antagonist radioligand)	-8
Na^+ channel (site 2) (antagonist radioligand)	-1
Cl^- channel (GABA-gated) (antagonist radioligand)	10
norepinephrine transporter *(h)* (antagonist radioligand)	85
dopamine transporter *(h)* (antagonist radioligand)	18
GABA transporter (antagonist radioligand)	-5
choline transporter (CHT1) *(h)* (antagonist radioligand)	-4
5-HT transporter *(h)* (antagonist radioligand)	4

***In Vitro* Pharmacology: Enzyme Assays**

Summary Results of compound **22** tested at 10 μM

Assay	% Inhibition of Control Values
PDE1B *(h)*	2
PDE2A *(h)*	2
PDE3A *(h)*	-4
PDE4D *(h)*	-1

PDE5 *(h)* (non-selective)	-2
PKCα *(h)*	23
acetylcholinesterase *(h)*	1
COMT (catechol- O-methyl transferase)	8
GABA transaminase	2
MAO-A *(h)*	3
MAO-B *(h)*	-3
PNMT (phenylethanolamine N-methyl transferase)	-10
tyrosine hydroxylase	-8
ATPase (Na^+/K^+)	0

Synthesis and characterization of compounds prepared in analogy to the procedures described in the experimental part

(E)-N-(3-Chlorophenyl)-3-(4-hydroxyphenyl)-prop-2-enamide (5)

Cinnamic anilide **5** was obtained by coupling 4-hydroxycinnamic acid **1c** (500 mg, 3.05 mmol) and 3-chloroaniline 2a (0.27 mL, 2.57 mmol) according to the procedure described for the preparation of **3**. Purification by trituration in DCM afforded ((E)-N-(3-chlorophenyl)-3-(4-hydroxyphenyl)-prop-2-enamide **5** (370 mg 44%) as a white powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.30 (bs, 1H), 9.58 (bs, 1H), 7.94 (m, 1H), 7.42 (m, 5H), 7.11 (m, 1H), 6.83 (m, 2H), 6.58 (d, J = 15.6 Hz, 1H). m/z (ES+), (M + H)+ = 274.

(E)-N-(3-Chlorophenyl)-3-(3-hydroxy-4-methoxyphenyl)-prop-2-enamide (6)

Cinnamic anilide **6** was obtained by coupling 3-hydroxy-4-methoxycinnamic acid 1d (515 mg, 2.57 mmol) and 3-chloroaniline **2a** (0.27 mL, 2.57 mmol) according to the procedure described for the preparation of **4**. Purification by flash silica chromatography (n-hexane/ EtOAc 70:30) afforded (E)-N-(3-chlorophenyl)-3-(3-hydroxy-4-methoxy-phenyl)-prop-2-enamide **6** (280 mg 36%) as a white powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.28 (s, 1H), 9.26 (s, 1H), 7.93 (m, 1H), 7.50 (m, 1H), 7.46 (d, J = 15.4Hz, 1H), 7.35 (m, 1H), 7.12 (m, 1H), 7.10-6.97 (m, 3H), 6.57 (d, J = 15.4Hz, 1H), 3.81 (s, 3H). m/z (ES+), (2M + Na)+ = 629.

(E)-N-(3-Chlorophenyl)-3-(4-hydroxy-3-methoxyphenyl)-prop-2-enamide (7)

Cinnamic anilide **7** was obtained by coupling 4-hydroxy-3-methoxycinnamic acid acid **1e** (500 mg, 2.57 mmol) and 3-chloroaniline 2a (0.27 mL, 2.57 mmol) according to the procedure described for the preparation of **3**. Purification by flash silica chromatography (elution gradient: 10% to 30% EtOAc in n-hexane) afforded (E)-N-(3-chlorophenyl)-3-(4-hydroxy-3-methoxyphenyl)-prop-2-enamide **7** (391 mg 50%) as a white powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.26 (s, 1H), 9.54 (s, 1H), 7.94 (m, 1H), 7.42 (m, 2H), 7.35 (m, 1H), 7.20-7.09 (m, 3H), 6.83 (m, 1H), 6.59 (d, J = 15.6Hz, 1H), 3.83 (s, 3H). m/z (ES+), (M + Na)+ = 326.

(E)-N-(3-Chlorophenyl)-3-(3,4-dimethoxyphenyl)-prop-2-enamide (8)

Cinnamic anilide **8** was obtained by coupling 3,4-dimethoxycinnamic acid **1f** (500 mg, 2.4 mmol) and 3-chloroaniline **2a** (0.21 mL, 1.99 mmol) according to the procedure described for the preparation of **4**. Purification by flash silica chromatography (elution gradient: 30% to 40% EtOAc in n-hexane) afforded (E)-N-(3-chlorophenyl)-3-(3,4-dimethoxyphenyl)-prop-2-enamide **8** (409 mg 65%) as a white powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.32 (s, 1H), 7.94 (m, 1H), 7.57-7.51 (m, 2H), 7.35 (m, 1H), 7.23-7.19 (m, 2H), 7.13-7.10 (m, 1H), 7.02 (m, 1H), 6.67 (d, J = 15.6, 1H), 3.82 (s, 3H), 3.80 (s, 3H). m/z (ES+), (2M + Na)+ = 657.

(E)-N-(3-Chlorophenyl)-3-(3-fluorophenyl)-prop-2-enamide (9)

Cinnamic anilide **9** was obtained by coupling 3-fluorocinnamic acid **1g** (500 mg, 3.01 mmol) and 3-chloroaniline **2a** (0.32 mL, 3.01 mmol) according to the procedure described for the preparation of **4**. Purification by flash silica chromatography (n-hexane/EtOAc 85:15) afforded (E)-N-(3-chlorophenyl)-3-(3-fluorophenyl)-prop-2-enamide **9** (298 mg 36%) as a beige powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.44 (s, 1H), 7.94 (m, 1H), 7.62 (d, *J* = 15.76Hz, 1H), 7.64-7.47 (m, 4H), 7.38 (m, 1H), 7.29-7.23 (m, 1H), 7.15 (m, 1H), 6.84 (d, *J* = 15.76Hz, 1H). m/z (ES+), $(M + H)^+$ = 276.

(E)-N-(3-Chlorophenyl)-3-(3-nitrophenyl)-prop-2-enamide (10a)

Cinnamic anilide **10a** was obtained by coupling 3-nitrocinnamic acid **1h** (500 mg, 2.59 mmol) and 3-chloroaniline **2a** (0.27 mL, 2.59 mmol) according to the procedure described for the preparation of **4**. Purification by flash silica chromatography (n-hexane/EtOAc 80:20) afforded (E)-N-(3-chlorophenyl)-3-(3-nitrophenyl)-prop-2-enamide **10a** (152 mg 19%) as a light orange powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.49 (s, 1H), 8.41 (m, 1H), 8.26 (m, 1H), 8.09 (m, 1H), 7.95 (m, 1H), 7.72-7.78 (m, 2H), 7.53 (m, 1H), 7.39 (m, 1H), 7.15 (m, 1H), 7.00 (d, *J* = 15.6Hz, 1H). m/z (ES+), $(M + H)^+$ = 303.

(E)-N-(3-Chlorophenyl)-3-(3-hydroxy-4-methylphenyl)-prop-2-enamide (13)

Cinnamic anilide **13** was obtained by coupling 3-hydroxy-4-methylcinnamic acid **1l** (178 mg, 1 mmol) and 3-chloroaniline **2a** (0.425 mL, 4.00 mmol) according to the procedure described for the preparation of **4**. Purification by flash silica chromatography (elution gradient: 0% to 2% MeOH in DCM) afforded (E)-N-(3-chlorophenyl)-3-(3-hydroxy-4-methylphenyl)-prop-2-enamide **13** (238 mg 65%) as a white powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.35 (s, 1H), 9.56 (s, 1H), 7.93 (s, 1H), 7.49 (m, 2H), 7.36 (m, 1H), 7.13 (m, 2H), 6.98 (m, 2H), 6.65 (d, J = 16 Hz, 1H), 2.15 (s, 3H). m/z (ES+), $(M + H)^+$ = 288.

(E)-N-(3-Chlorophenyl)-3-(3-hydroxy-4-nitrophenyl)-prop-2-enamide (14a)

Cinnamic anilide **14a** was obtained by coupling 3-hydroxy-4-nitrocinnamic acid **1m** (1.050 g, 5 mmol) and 3-chloroaniline **2a** (2.12 ml, 20.00 mmol) according to the procedure described for the preparation of **4**. Purification by flash silica chromatography (elution gradient: 0% to 2.5% MeOH in DCM) afforded (E)-N-(3-chlorophenyl)-3-(3-hydroxy-4-nitrophenyl)-prop-2-enamide **14a** (1.255 g 79%) as a white powder.

^{1}H NMR (400 MHz, CDCl3) δ 10.64 (s, 1H), 8.16 (m, 1H), 7.77 (bs, 1H), 7.73 (d, *J* = 16.0 Hz, 1H), 7.39 (m, 1H), 7.32 (s, 1H), 7.30 (m, 2H), 7.17 (m, 2H), 6.65 (m, 1H). m/z (ES+), $(M + H)^+$ = 319.

(E)-N-(3-Bromophenyl)-3-(3-hydroxy-4-methoxyphenyl)-prop-2-enamide (15)

Cinnamic anilide **15** was obtained by coupling 3-hydroxy-4-methoxycinnamic acid **1d** (500 mg, 2.57 mmol) and 3-bromoaniline **2b** (0.28 mL, 2.57 mmol) according to the procedure described for the preparation of **4**. Purification by flash silica chromatography (n-hexane/EtOAc 60:40) afforded (E)-N-(3-bromophenyl)-3-(3-hydroxy-4-methoxyphenyl)-prop-2-enamide **15** (469 mg, 52%) as a white powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.26 (s, 1H), 9.24 (s, 1H), 8.06 (m, 1H), 7.56-7.54 (m, 1H), 7.46 (d, *J* = 15.6 Hz, 1H), 7.31-7.22 (m, 2H), 7.06- 7.03 (m, 2H), 6.97 (m, 1H), 6.56 (d, *J* = 15.6 Hz, 1H), 3.81 (s, 3H). m/z (ES+), $(2M + Na)^+$ = 719.

(E)-3-(3-Hydroxy-4-methoxyphenyl)-N-(3-methoxyphenyl)-prop-2-enamide (16)

Cinnamic anilide **16** was obtained by coupling 3-hydroxy-4-methoxycinnamic acid **1d** (500 mg, 2.57 mmol) and *m*-anisidine **2c** (0.29 mL, 2.57 mmol) according to the procedure described for the preparation of **4**. Purification by flash silica chromatography (n-hexane/EtOAc 6:4) afforded (E)-3-(3-hydroxy-4-methoxyphenyl)-N-(3-methoxyphenyl)-prop-2-enamide **16** (429 mg, 56%) as a pale yellow powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.07 (s, 1H), 9.24 (s, 1H), 7.45-7.41 (m, 2H), 7.24-7.18 (m, 2H), 7.04-7.02 (m, 2H), 6.98-6.96 (m, 1H), 6.65-6.62 (m, 1H), 6.58 (d, *J* = 15.6 Hz, 1H), 3.81 (s, 3H), 3.74 (s, 3H). m/z (ES+), $(2M + Na)^+$ = 621.

(E)-3-(3-Hydroxy-4-methoxyphenyl)-N-(3-isopropoxyphenyl)-prop-2-enamide (17)

Cinnamic anilide **17** was obtained by coupling 3-hydroxy-4-methoxycinnamic acid **1d** (500 mg, 2.57 mmol) and 3-isopropoxyaniline **2d** (0.38 mL, 2.57 mmol) according to the procedure described for the preparation of **4**. Purification by flash silica chromatography (n-hexane/EtOAc 6:4) afforded (E)-3-(3-hydroxy-4-methoxy-phenyl)-N-(3-isopropoxyphenyl)-prop-2-enamide **17** (311 mg, 37%) as a pale yellow powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.04 (s, 1H), 9.23 (s, 1H), 7.45-7.39 (m, 2H), 7.21-7.13 (m, 2H), 7.04-7.02 (m, 2H), 6.97 (d, *J* = 8.8 Hz, 1H), 6.62-6.56 (m, 2H), 4.54 (ep, *J* = 6.0 Hz, 1H), 3.81 (s, 3H), 1.27 (d, *J* = 6.0 Hz, 6H). m/z (ES+), $(2M + Na)^+$ = 677.

(E)-3-(3-Hydroxy-4-methoxyphenyl)-N-(3-nitrophenyl)-prop-2-enamide (18a)

Cinnamic anilide **18a** was obtained by coupling 3-hydroxy-4-methoxycinnamic acid **1d** (500 mg, 2.57 mmol) and 3-nitroaniline **2e** (355 mg, 2.57 mmol) according to the procedure described for the preparation of **4**. Purification by trituration with DCM afforded (E)-3-(3-hydroxy-4-methoxyphenyl)-N-(3-nitrophenyl)-prop-2-enamide **18a** (434 mg, 54%) as a yellow powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.57 (s, 1H), 9.25 (s, 1H), 8.73 (t, *J* = 2.0 Hz, 1H), 8.00-7.97 (m, 1H), 7.93-7.90 (m, 1H), 7.63 (t, *J* = 8.0 Hz, 1H), 7.51 (d, *J* = 15.6 Hz, 1H), 7.08-7.05 (m, 2H), 7.00-6.97 (m, 1H), 6.58 (d, *J* = 15.6 Hz, 1H), 3.82 (s, 3H). m/z (ES+), $(M + H)^+$ = 315.

(E)-N-[3-(Dimethylamino)phenyl]-3-(3-hydroxy-4-methoxyphenyl)-prop-2-enamide (19)

Cinnamic anilide **19** was obtained by coupling 3-hydroxy-4-methoxycinnamic acid **1d** (500 mg, 2.57 mmol) and N,N-dimethyl-1,3-phenylenediamine dihydrochloride **2f** (537 mg, 2.57 mmol) according to the

procedure described for the preparation of **4**. Purification by flash silica chromatography (n-hexane/EtOAc 6:4) afforded (E)-N-[3-(dimethylamino)phenyl]-3-(3-hydroxy-4-methoxyphenyl)-prop-2-enamide **19** (420 mg, 52%) as a yellow powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 9.88 (s, 1H), 9.20 (s, 1H), 7.40 (d, *J* = 15.6 Hz, 1H), 7.12-7.08 (m, 2H), 7.03-7.00 (m, 3H), 6.97 (d, *J* = 8.0 Hz, 1H), 6.59 (d, *J* = 15.6 Hz, 1H), 6.44 (dd, *J* = 8.4 Hz, *J* = 2.0 Hz, 1H), 3.81 (s, 3H), 2.88 (s, 6H). m/z (ES+), $(2M + Na)^+$ = 647.

(E)-N-(2-Chlorophenyl)-3-(3-hydroxy-4-methoxyphenyl)-prop-2-enamide (20)

Cinnamic anilide **20** was obtained by coupling 3-hydroxy-4-methoxycinnamic acid **1d** (770 mg, 3.96 mmol) and 2-chloroaniline **2g** (0.41 mL, 3.96 mmol) according to the procedure described for the preparation of **4**. Purification by flash silica chromatography (n-hexane/EtOAc 8:2) afforded (E)-N-(2-chlorophenyl)-3-(3-hydroxy-4-methoxyphenyl)-prop-2-enamide **20** (200 mg, 17%) as an off white powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 8.53 (m, 1H), 7.74 (m, 1H), 7.68 (d, J = 15, 1H), 7.39 (m, 1H), 7.30 (m, 1H), 7.19 (m, 1H), 7.06 (m, 2H), 6.44 (d, J = 15, 1H), 3.94 (s, 3H). m/z (ES+), $(M + H)^+$ = 304.

(E)-N-(4-Chlorophenyl)-3-(3-hydroxy-4-methoxyphenyl)-prop-2-enamide (21)

Cinnamic anilide **21** was obtained by coupling 3-hydroxy-4-methoxycinnamic acid **1d** (500 mg, 2.57 mmol) and 4-chloroaniline **2h** (328 mg, 2.57 mmol) according to the procedure described for the preparation of **4**. Purification by flash silica chromatography (elution gradient: 30% to 40% EtOAc in n-hexane) afforded (E)-N-(4-chlorophenyl)-3-(3-hydroxy-4-methoxyphenyl)-prop-2-enamide **21** (143 mg, 18%) as an off white powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.23 (s, 1H), 9.25 (s, 1H), 7.71 (AB system, 2H), 7.44 (d, *J* = 15.6, 1H), 7.38 (AB system, 2H), 7.05- 7.03 (m, 2H), 6.98-6.96 (m, 1H), 6.57 (d, *J* = 15.6, 1H), 3.81 (s, 3H). m/z (ES+), $(2M + Na)^+$ = 629.

(E)-3-(4-Fluoro-3-hydroxyphenyl)-N-(1-naphthyl)prop-2-enamide (22)

Cinnamic anilide **22** was obtained by coupling 4-fluoro-3-hydroxycinnamic acid **1m** (195 mg, 1.07 mmol) and 1-naphtylamine **2i** (328 mg, 2.57 mmol) according to the procedure described for the preparation of **4**. Purification by flash silica chromatography (n-hexane/EtOAc 7:3) afforded (E)-3-(4-fluoro-3-hydroxyphenyl)-N-(1-naphthyl)-prop-2-enamide **22** (120 mg, 36%) as an off white powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.13 (s, 2H), 8.16 (m, 1H), 7.98-7.90 (m, 2H), 7.79 (d, *J* = 8Hz, 1H), 7.61-7.52 (m, 4H), 7.24 (m, 2H), 7.18 (m,bs, 1H), 7.01 (d, *J* = 15.2Hz, 1H). m/z (ES+), $(M + H)^+$ = 308.

(E)-N-(3-Chlorophenyl)-3-(4-fluoro-3-hydroxy-phenyl)-prop-2-enamide (23)

Cinnamic anilide **23** was obtained by coupling 4-fluoro-3-hydroxycinnamic acid **1m** (159 mg, 0.87 mmol) and 3-chloroaniline **2a** (0.23 mL, 2.2 mmol) according to the procedure described for the preparation of **4**. Purification by sequential trituration with toluene, ethyl ether and DCM afforded (E)-N-(3-chlorophenyl)-3-(4-fluoro-3-hydroxyphenyl)-prop-2-enamide **23** (86 mg, 34%) as an off white powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.38 (s, 1H), 10.15 (s, 1H), 7.94 (bs, 1H), 7.54-7.49 (m, 2H), 7.39-7.35 (m, 1H), 7.22-7.20 (m, 2H), 7.15-7.08 (m, 2H), 6.64 (d, *J* = 15.6Hz, H). m/z (ES+), $(M + H)^+$ = 292.

(E)-N-(3,4-Dichlorophenyl)-3-(3-acetoxy-4-methoxyphenyl)prop-2-enamide (25a)

Cinnamic anilide **25a** was obtained by coupling 3-acetoxy-4-methoxycinnamic acid **1n** (354 mg, 1.5 mmol) and 3,4-dichloroaniline **2k** (292 mg, 1.8 mmol) according to the procedure described for the preparation of **24a**. Purification by flash silica chromatography (n-hexane/ethyl acetate 77:23) afforded (E)-N-(3,4-dichlorophenyl)-3-(3-acetoxy-4-methoxyphenyl)prop-2-enamide **25a** (200 mg, 44%) as a white powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.42 (s, 1H), 8.10 (d, *J* = 2.0 Hz, 1H), 7.60-7.53 (m, 4H), 7.40 (d, *J* = 2.0 Hz, 1H), 7.21 (d, *J* = 8.8 Hz, 1H), 6.65 (d, *J* = 16.0 Hz, 1H), 3.83 (s, 3H), 2.28 (s, 3H). m/z (ES+), $(M + H)^+$ = 380.

(E)-N-(3,4-Dichlorophenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-enamide (25)

Cinnamic anilide **25** was obtained by heating at reflux a suspension of (E)-N-(3,4-dichlorophenyl)-3-(3-acetoxy-4-methoxy-phenyl)prop-2-enamide **25a** (194 mg, 0.51 mmol) and NaOH (50% in water) (54 µL, 1.02 mmol) in MeOH (5 mL) according to the procedure described for the preparation of **24**. (E)-N-(3,4-dichlorophenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-enamide **25** (150 mg, 87%) was obtained as a white powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.39 (s, 1H), 9.26 (s, 1H), 8.10 (d, *J* = 2.0 Hz, 1H), 7.60-7.54 (m, 2H), 7.47 (d, *J* = 15.6 Hz, 1H), 7.06-7.04 (m, 2H), 6.98 (d, *J* = 8.8 Hz, 1H), 6.54 (d, *J* = 15.6 Hz, 1H), 3.81 (s, 3H). m/z (ES+), $(2M + Na)^+$ = 699.

(E)-N-(3-Chloro-4-methoxyphenyl)-3-(3-acetoxy-4-methoxyphenyl)prop-2-enamide (26a)

Cinnamic anilide **26a** was obtained by coupling 3-acetoxy-4-methoxycinnamic acid **1n** (354 mg, 1.5 mmol) and 3-chloro-4-methoxy-aniline **2l** (331 mg, 2.1 mmol) according to the procedure described for the preparation of **24a**. Purification by flash silica chromatography (n-hexane/ethyl acetate 6:4) afforded (E)-N-(3-chloro-4-methoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-enamide **26a** (390 mg, 69%) as a beige powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.14 (s, 1H), 7.90 (d, *J* = 2.0 Hz, 1H), 7.53-7.49 (m, 3H), 7.37 (d, *J* = 2.4 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 1H), 7.13 (d, *J* = 9.2 Hz, 1H), 6.63 (d, *J* = 15.6 Hz, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 2.28 (s, 3H). m/z (ES+), $(M + H)^+$ = 376.

(E)-N-(3-Chloro-4-methoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-enamide (26)

Cinnamic anilide **26** was obtained by heating at reflux a suspension of (E)-N-(3-chloro-4-methoxy-phenyl)-3-(3-acetoxy-4-methoxy-phenyl)prop-2-enamide **26a** (376 mg, 1 mmol) and NaOH (50% in water) (106 µL, 2 mmol) in MeOH (6 mL) according to the procedure described for the preparation of **24**. (E)-N-(3-chloro-4-methoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-enamide **26** (310 mg, 93%) was obtained as a pale yellow powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.11 (s, 1H), 9.24 (s, 1H), 7.89 (d, *J* = 2.4 Hz, 1H), 7.50 (dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 1H), 7.42 (d, *J* = 15.6 Hz, 1H), 7.13 (d, *J* = 9.2 Hz, 1H), 7.04-7.02 (m, 2H), 6.97 (d, *J* = 8.8 Hz, 1H), 6.53 (d, *J* = 15.6 Hz, 1H), 3.82 (s, 3H), 3.81 (s, 3H). m/z (ES+), $(2M + Na)^+$ = 689.

(E)-N-(3-Chloro-2-methoxyphenyl)-3-(3-acetoxy-4-methoxyphenyl)prop-2-enamide (27a)

Cinnamic anilide **27a** was obtained by coupling 3-acetoxy-4-methoxycinnamic acid **1n** (354 mg, 1.5 mmol) and 3-chloro-2-methoxy-aniline **2m** (284 mg, 1.8 mmol) according to the procedure described for the preparation of **24a**. Purification by flash silica chromatography (eluent: n-hexane/ethyl acetate 8:2) afforded (E)-N-(3-chloro-2-methoxyphenyl)-3-(3-acetoxy-4-methoxyphenyl)prop-2-enamide **27a** (180 mg, 32%) as a pale yellow powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 9.51 (s, 1H), 8.23 (dd, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H), 7.55-7.51 (m, 2H), 7.40 (d, *J* = 2.4 Hz, 1H), 7.22-7.19 (m, 2H), 7.16-7.11 (m, 2H), 3.83 (s, 3H), 3.80 (s, 3H), 2.29 (s, 3H). m/z (ES+), $(M + H)^+$ = 376.

(E)-N-(3-Chloro-2-methoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-enamide (27)

Cinnamic anilide **27** was obtained by heating at reflux a suspension of (E)-N-(3-chloro-2-methoxy-phenyl)-3-(3-acetoxy-4-methoxy-phenyl)prop-2-enamide **27a** (175 mg, 0.465 mmol) and NaOH (50% in water) (49 µL, 0.93 mmol) in MeOH (4 mL) according to the procedure described for the preparation of **24**. (E)-N-(3-chloro-2-methoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-enamide **27** (115 mg, 74%) was obtained as a beige powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 9.56 (s, 1H), 9.20 (s, 1H), 8.21-8.19 (m, 1H), 7.45 (d, *J* = 15.6 Hz, 1H), 7.20 (dd, *J* = 8.4 Hz, *J* = 2.0 Hz, 1H), 7.13 (t, *J* = 8.4 Hz, 1H), 7.08-6.97 (m, 4H), 3.82 (s, 3H), 3.80 (s, 3H). m/z (ES+), $(2M + Na)^+$ = 689.

(E)-3-(3-Acetoxy-4-methoxyphenyl)-N-indan-1-yl-acrylamide (28a)

Cinnamic anilide **28a** was obtained by coupling 3-acetoxy-4-hydroxycinnamic acid **1n** (236 mg, 1 mmol) and indan-1-amine **2n** (0.13 mL, 1.0 mmol) according to the procedure described for the preparation of **4**. Purification by trituration in diethyl ether afforded (E)-3-(3-acetoxy-4-methoxyphenyl)-N-indan-1-yl-acrylamide **28a** (290 mg, 82%) as a dark brown powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 8.39 (m, 1H),7.46-7.41 (m, 2H), 7.30 (m, 1H), 7.28-7.26 (m, 1H), 7.24-7.16 (m, 4H), 6.54 (d, *J* = 15.6 Hz, 1H), 5.40 (q, *J* = 7.6 Hz, 1H), 3.81 (s, 3H), 2.99-2.92 (m, 1H), 2.87-2.79 (m, 1H), 2.48-2.40 (m, 1H), 2.27 (s, 3H), 1.86-1.82 (m, 1H) . m/z (ES+), $(M + H)^+$ = 352.

(E)-N-(3-Chloro-4-methoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-enamide (26)

Cinnamic anilide **26** was obtained by heating at reflux a suspension of (E)-N-(3-chloro-4-methoxy-phenyl)-3-(3-acetoxy-4-methoxy-phenyl)prop-2-enamide **26a** (376 mg, 1 mmol) and NaOH (50% in water) (106 µL, 2 mmol) in MeOH (6 mL) according to the procedure described for the preparation of **24**. (E)-N-(3-chloro-4-methoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-enamide **26** (310 mg, 93%) was obtained as a pale yellow powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.11 (s, 1H), 9.24 (s, 1H), 7.89 (d, *J* = 2.4 Hz, 1H), 7.50 (dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 1H), 7.42 (d, *J* = 15.6 Hz, 1H), 7.13 (d, *J* = 9.2 Hz, 1H), 7.04-7.02 (m, 2H), 6.97 (d, *J* = 8.8 Hz, 1H), 6.53 (d, *J* = 15.6 Hz, 1H), 3.82 (s, 3H), 3.81 (s, 3H). m/z (ES+), $(2M + Na)^+$ = 689.

(E)-N-(3-Chloro-2-methoxyphenyl)-3-(3-acetoxy-4-methoxyphenyl)prop-2-enamide (27a)

Cinnamic anilide **27a** was obtained by coupling 3-acetoxy-4-methoxycinnamic acid **1n** (354 mg, 1.5 mmol) and 3-chloro-2-methoxy-aniline **2m** (284 mg, 1.8 mmol) according to the procedure described for the preparation of **24a**. Purification by flash silica chromatography (eluent: n-hexane/ethyl acetate 8:2) afforded (E)-N-(3-chloro-2-methoxyphenyl)-3-(3-acetoxy-4-methoxyphenyl)prop-2-enamide **27a** (180 mg, 32%) as a pale yellow powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 9.51 (s, 1H), 8.23 (dd, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H), 7.55-7.51 (m, 2H), 7.40 (d, *J* = 2.4 Hz, 1H), 7.22-7.19 (m, 2H), 7.16-7.11 (m, 2H), 3.83 (s, 3H), 3.80 (s, 3H), 2.29 (s, 3H). m/z (ES+), $(M + H)^+$ = 376.

(E)-N-(3-Chloro-2-methoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-enamide (27)

Cinnamic anilide **27** was obtained by heating at reflux a suspension of (E)-N-(3-chloro-2-methoxy-phenyl)-3-(3-acetoxy-4-methoxy-phenyl)prop-2-enamide **27a** (175 mg, 0.465 mmol) and NaOH (50% in water) (49 µL, 0.93 mmol) in MeOH (4 mL) according to the procedure described for the preparation of **24**. (E)-N-(3-chloro-2-methoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-enamide **27** (115 mg, 74%) was obtained as a beige powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 9.56 (s, 1H), 9.20 (s, 1H), 8.21-8.19 (m, 1H), 7.45 (d, *J* = 15.6 Hz, 1H), 7.20 (dd, *J* = 8.4 Hz, *J* = 2.0 Hz, 1H), 7.13 (t, *J* = 8.4 Hz, 1H), 7.08-6.97 (m, 4H), 3.82 (s, 3H), 3.80 (s, 3H). m/z (ES+), $(2M + Na)^+$ = 689.

(E)-3-(3-Acetoxy-4-methoxyphenyl)-N-indan-1-yl-acrylamide (28a)

Cinnamic anilide **28a** was obtained by coupling 3-acetoxy-4-hydroxycinnamic acid **1n** (236 mg, 1 mmol) and indan-1-amine **2n** (0.13 mL, 1.0 mmol) according to the procedure described for the preparation of **4**. Purification by trituration in diethyl ether afforded (E)-3-(3-acetoxy-4-methoxyphenyl)-N-indan-1-yl-acrylamide **28a** (290 mg, 82%) as a dark brown powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 8.39 (m, 1H),7.46-7.41 (m, 2H), 7.30 (m, 1H), 7.28-7.26 (m, 1H), 7.24-7.16 (m, 4H), 6.54 (d, *J* = 15.6 Hz, 1H), 5.40 (q, *J* = 7.6 Hz, 1H), 3.81 (s, 3H), 2.99-2.92 (m, 1H), 2.87-2.79 (m, 1H), 2.48-2.40 (m, 1H), 2.27 (s, 3H), 1.86-1.82 (m, 1H) . m/z (ES+), $(M + H)^+$ = 352.

(E)-3-(3-Acetoxy-4-methoxyphenyl)-N-(2-naphthyl)-prop-2-enamide (29a)

Cinnamic anilide **29a** was obtained by coupling 3-acetoxy-4-hydroxycinnamic acid **1n** (211 mg, 0.8 mmol) and 2-naphthylamine **2o** (100 mg, 0.7 mmol) according to the procedure described for the preparation of **4**. Purification by flash silica chromatography (elution gradient: 1% to 7% EtOAc in n-hexane) afforded (E)-3-(3-acetoxy-4-methoxyphenyl)-N-(2-naphthyl)-prop-2-enamide **29a** (81.5 mg, 33%) as a light brown powder.

^{1}H NMR (500 MHz, DMSO-d6) δ 10.34 (s, 1 H), 8.45 - 8.33 (m, 1 H), 7.91 - 7.81 (m, 3 H), 7.69 - 7.63 (m, 1 H), 7.59 - 7.52 (m, 2 H), 7.50 - 7.44 (m, 1 H), 7.44 - 7.36 (m, 2 H), 7.25 - 7.19 (m, 1 H), 6.76 (d, J = 15.7 Hz, 1 H), 3.83 (s, 3 H), 3.80 - 3.76 (m, 1 H), 2.29 (s, 3 H). m/z (ES+), $(M + H)^+$ = 362.

(E)-3-(3-Acetoxy-4-methoxyphenyl)-N-(1-naphthyl)prop-2-enamide (30a)

Cinnamic anilide **30a** was obtained by coupling 3-acetoxy-4-methoxycinnamic acid **1n** (354 mg, 1.5 mmol) and naphthalen-1-amine **2p** (215 mg, 1.5 mmol) according to the procedure described for the preparation of **24a**. The reaction mixture was stirred at room temperature for 4 days. Purification by flash silica chromatography (DCM/MeOH 100:1) afforded (E)-3-(3-acetoxy-4-methoxyphenyl)-N-(1-naphthyl)prop-2-enamide **30a** (255 mg, 47%) as a pale yellow powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.03 (s, 1H), 8.17-8.15 (m, 1H), 7.97-7.92 (m, 2H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.60-7.50 (m, 5H), 7.42 (d, *J* = 1.6 Hz, 1H), 7.23 (d, *J* = 8.4 Hz, 1H), 7.03 (d, *J* = 15.6 Hz, 1H), 3.84 (s, 3H), 2.30 (s, 3H). m/z (ES+), $(M + H)^+$ = 362.

(E)-3-(3-Hydroxy-4-methoxyphenyl)-N-(1-naphthyl)prop-2-enamide (30)

Cinnamic anilide **30** was obtained by heating at reflux a suspension of (E)-3-(3-acetoxy-4-methoxyphenyl)-N-(1-naphthyl)prop-2-enamide **30a** (175 mg, 0.465 mmol) and NaOH (50% in water) (49 µL, 0.93 mmol) in MeOH (4 mL) according to the procedure described for the preparation of **24**. (E)-3-(3-hydroxy-4-methoxyphenyl)-N-(1-naphthyl)prop-2-enamide **30** (213 mg, 95%) was obtained as a white powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.04 (s, 1H), 9.24 (s, 1H), 8.16 (d, *J* = 8.4 Hz, 1H), 7.96-7.94 (m, 1H), 7.90 (d, *J* = 7.2 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.60-7.47 (m, 4H), 7.10-7.06 (m, 2H), 6.99 (d, *J* = 8.0 Hz, 1H), 6.93 (d, *J* = 15.6 Hz, 1H), 3.83 (s, 3H). m/z (ES+), $(2M + Na)^+$ = 661.

(E)-N-(1,3-Benzoxazol-4-yl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-enamide (31)

Cinnamic anilide **31** was obtained by heating at reflux a suspension of (E)-N-Benzoxazol-4-yl-3-(3-acetoxy-4-methoxyphenyl)prop-2-enamide **31a** (50 mg, 0.14 mmol) and NaOH (50% in water) (15 µL, 0.28 mmol) in MeOH (1.5 mL) according to the procedure described for the preparation of **24**. Purification by trituration in diethyl ether afforded (E)-N-(1,3-benzoxazol-4-yl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-enamide **31** (12 mg, 27%) as a beige powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.37 (s, 1H), 9.30 (s, 1H), 8.66 (s, 1H), 8.33 (m, 1H), 7.51 (m, 3H), 7.12 (m, 4H), 3.88 (s, 3H). m/z (ES+), (M + H)+ = 311.

(E)-3-(3-Acetoxy-4-methoxyphenyl)-N-(1-methyl-1H-benzimidazol-2-yl)prop-2-enamide (33a)

Cinnamic anilide **33a** was obtained by coupling 3-acetoxy-4-hydroxycinnamic acid **1n** (236 mg, 1 mmol) and 2-amino-1-methyl-benzoimidazole **2s** (0.15 mL, 1.0 mmol) according to the procedure described for the preparation of **4**. Purification by flash silica chromatography (DCM/MeOH 99:1) afforded (E)-3-(3-acetoxy-4-methoxyphenyl)-N-(1-methyl-1H-benzoimidazol-2-yl)prop-2-enamide **33a** (65 mg, 18%) as a light yellow powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 7.60-7.48 (m, 5H), 7.26-7.17 (m, 3H), 6.66 (d, *J* = 15.2 Hz, 1H), 3.82 (s, 3H), 3.64 (s, 3H), 2.28 (s, 3H). m/z (ES+), $(M + H)^+$ = 366.

(E)-3-(3-Hydroxy-4-methoxyphenyl)-N-(1-methyl-1H-benzimidazol-2-yl)prop-2-enamide hydrochloride (33)

Cinnamic anilide **33** was obtained by hydrolysis of (E)-3-(3-acetyloxy-4-methoxyphenyl)-N-(1-methyl-1H-benzoimidazol-2-yl)prop-2-enamide **33a** (40 mg, 0.11 mmol) using 3N HCl in MeOH (2ml) according to the procedure described for the preparation of **28**. Purification by trituration in diethyl ether afforded (E)-3-(3-Hydroxy-4-methoxyphenyl)-N-(1-methyl-1H-benzoimidazol-2-yl)prop-2-enamide hydrochloride **33** (31 mg, 78%) as a beige powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 9.42 (bs, 1H), 7.78-7.72 (m, 3H), 7.51-7.44 (m, 2H), 7.19-7.11 (m, 3H), 7.03 (d, *J* = 8.4 Hz, 1H), 3.96 (s, 3H), 3.84 (s, 3H). m/z (ES+), $(M + H)^+$ = 324.

(E)-N-(3-Chlorophenyl)-3-(3-acetoxy-4-methoxyphenyl)-N-methyl-prop-2-enamide (34a)

Cinnamic anilide **34a** was obtained by coupling 3-acetoxy-4-methoxycinnamic acid **1n** (472 mg, 2 mmol) and 3-chloro-N-methyl-aniline **2t** (252 mg, 2.0 mmol) according to the procedure described for the preparation of **24a**. The reaction mixture was stirred at room temperature overnight and then at reflux for 18 h. Purification by flash silica chromatography (n-hexane/ethyl acetate 7:3) afforded (E)-N-(3-chlorophenyl)-3-(3-acetoxy-4-methoxyphenyl)-N-methyl-prop-2-enamide **34a** (450 mg, 62%) as a brown oil.

^{1}H NMR (400 MHz, DMSO-d6) δ 7.63 (d, *J* = 15.6 Hz, 1H), 7.41-7.35 (m, 2H), 7.23 (dd, *J* = 8.4 Hz, *J* = 2.0 Hz, 1H), 7.14-7.12 (m, 1H), 7.03 (d, *J* = 2.0 Hz, 1H), 6.91 (d, *J* = 8.8 Hz, 1H), 6.21 (d, *J* = 15.6 Hz, 1H), 4.72 (t, *J* = 5.6 Hz, 1H), 3.84 (s, 3H), 3.40 (s, 3H), 2.31 (s, 3H). m/z (ES+), $(M + H)^+$ = 360.

(E)-N-(3-Chlorophenyl)-3-(3-hydroxy-4-methoxyphenyl)-N-methyl-prop-2-enamide (34)

Cinnamic anilide **34** was obtained by heating at reflux a suspension of (E)-N-(3-chlorophenyl)-3-(3-acetoxy-4-methoxy-phenyl)-N-methyl-prop-2-enamide **34a** (435 mg, 1.21 mmol) and NaOH (50% in water) (128 µL, 2.42 mmol) in MeOH (5 mL) according to the procedure described for the preparation of **24**. (E)-N-(3-

chlorophenyl)-3-(3-hydroxy-4-methoxy-phenyl)-N-methyl-prop-2-enamide **34** (360 mg, 93%) was obtained as a yellow powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 9.14 (s, 1H), 7.51-7.45 (m, 3H), 7.39 (d, *J* = 15.6 Hz, 1H), 7.31 (d, *J* = 7.6 Hz, 1H), 6.88 (s, 2H), 6.80 (s, 1H), 6.19 (d, *J* = 15.6 Hz, 1H), 3.76 (s, 3H), 3.29 (s, 3H). m/z (ES+), $(2M + Na)^+$ = 657.

Plasma concentration of compound 22 in the rat telemetry study

The plasma concentration was also determined using a satellite group (n=3) treated at the same dose of 30 mg/kg i.v. At this dose **22** showed a plasma concentration after 5 min of 74 μM that reduced to 0.05 μM after 240 min from the i.v. injection and lasted up to 24 h at a level of 0.01 μM.

The compound was cleared less rapidly than in the rabbit showing a $t_{½}$ of about 300 min. Notwithstanding the very high plasma concentration, which reached values well above those observed in the efficacy experiment, compound **22** at the dose of 30 mg/kg i.v. in the rat did not alter the monitored cardiovascular parameters.

Increase of the calcium retention capacity of mouse liver mitochondria after incubation with different concentrations of inhibitor

Inhibitor concentration (µM)	0.1	0.5	1	5
compd.	CRC_i / CRC_0 %			
3	109	131	155	190
4	102	120	136	110
5	89	84	87	85
6	121	161	186	172
7	97	98	93	92
8	79	91	75	83
9	84	91	90	94
10	93	89	94	95
11	91	90	91	89
12	81	86	87	89
13	108	125	133	182
14	90	97	102	154
15	115	119	139	188
16	128	146	170	270
17	114	114	116	119
18	114	108	106	124
19	111	109	114	144
20	126	156	165	266
21	132	163	194	300
22	266	340	413	557
23	114	156	175	141
24	115	115	115	108
25	127	160	216	343
26	132	173	196	320
27	128	146	158	248
28	155	188	219	338
29	132	170	201	305
30	228	295	358	547
31	116	138	147	231
32	104	113	115	154
33	132	150	176	285
34	105	100	103	99
37	108	109	106	109
41	110	112	107	103
44	na	na	106	na
46	104	108	108	108