Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 4843–4852
Bioorganic & Medicinal Chemistry

Dual 5-HT$_{1A}$ agonists and 5-HT re-uptake inhibitors by combination of indole-butyl-amine and chromenonyl-piperazine structural elements in a single molecular entity

Timo Heinrich,* Henning Böttcher, Kai Schiemann, Günter Hölzemann, Michael Schwarz, Gerd D. Bartoszyk, Christoph van Amsterdam, Hartmut E. Greiner and Christoph A. Seyfried

Preclinical Pharmaceutical Research, Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany

Received 27 May 2004; accepted 6 July 2004
Available online 3 August 2004

Abstract—The dual serotonin (5-HT) re-uptake inhibitor and 5-HT$_{1A}$ receptor agonist *vilazodone* was found to increase central serotonin levels in rat brain. In the course of structural modifications of *vilazodone* 3-{4-[4-(2-oxo-2H-1-benzopyran-6-yl)-1-piperazinyl]-butyl}-1H-indole-5-carbonitrile **8i** and its fluorine analogue 6-{4-[4-(5-fluor-3-indolyl)-butyl]-1-piperazinyl}-2H-1-benzopyran-2-one have been identified. These unsubstituted chromenones are equally potent at the 5-HT$_{1A}$ receptor and 5-HT transporter. The implementation of nitrogen functionalities in position 3 of the chromenones resulted in compounds acting as agonists at the 5-HT$_{1A}$ receptor and as 5-HT re-uptake inhibitors like *vilazodone*. Ex vivo 5-HT re-uptake inhibition and in vitro 5-HT agonism were determined in the PCA- and GTPγS-assay, respectively. The potential of these chromenones to increase central 5-HT levels was measured in microdialysis studies and especially the derivatives 3-{4-[4-(3-amino-2-oxo-2H-chromen-6-yl)-piperazin-1-yl]-butyl}-1H-indole-5-carbonitrile **8f**, ethyl (6-{4-[4-(5-cyano-1H-indol-3-yl)-butyl]-piperazin-1-yl}-2-oxo-2H-chromen-3-yl)-carbamate **8h** and *N*-(6-{4-[4-(5-cyano-1H-indol-3-yl)-butyl]-piperazin-1-yl}-2-oxo-2H-chromen-3-yl)-acetamide **8k** give rise to rapid development of increased serotonin levels in rat brain cortex, lasting longer than 3 h.

1. Introduction

Depression currently ranks as the world's fourth greatest cause of illness and is expected to rank second by the year 2020 according to WHO studies.[1] Tricyclic antidepressants, the monoamine oxidase inhibitors and the last generation of selective serotonin re-uptake inhibitors (SSRIs) although widely used lack sufficient influence on this development. Treatment difficulties often are compliance problems resulting from severe side effects, dietary restrictions or slow onset of action, respectively.[2] Hypothetical explanations of the SSRI's latency (time taken to desensitise 5-HT autoreceptors on cell bodies) are discussed in the literature[3–5] and are supported experimentally in compound combination approaches (e.g., 5-HT$_{1A}$ ligand + SSRI).[6–9]

As a consequence, there have been intense efforts on optimising a single compound with dual activity to reduce the time to on-set of action and to decrease side effects. For example, Laboratorios Vita discussed *VN-2222* as 5-HT$_{1A}$ antagonist and 5-HT re-uptake inhibitor exhibiting affinity for both targets with a K_i value of 20 nM.[10] Merck KGaA discovered *vilazodone*, a presynaptic 5-HT$_{1A}$ agonist (0.2 nM) and 5-HT re-uptake inhibitor (0.5 nM)[11] and both compounds increase rat hippocampal extra-cellular 5-HT level (Chart 1).[10,12]

We knew from our previous work that 5-cyano-indole-butyl-piperazines as potent 5-HT re-uptake inhibitors tolerate a variety of substituents in the aryl moiety attached to the piperazine. In addition, 2-carboxamido benzofurane-5-yl as piperazine residue introduced the desired 5-HT$_{1A}$ agonism, resulting in *vilazodone* that is a clinical candidate as novel putative antidepressant exhibiting both selective 5-HT re-uptake inhibition (5-HT RUI) and 5-HT$_{1A}$ receptor agonism. In vivo microdialysis revealed that a single treatment with *vilazodone*

Keywords: Serotonin; Microdialysis; Antidepressant.
* Corresponding author. Tel.: +49-0-6151-726589; fax: +49-0-6151-723129; e-mail: timo.heinrich@merck.de

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.07.014

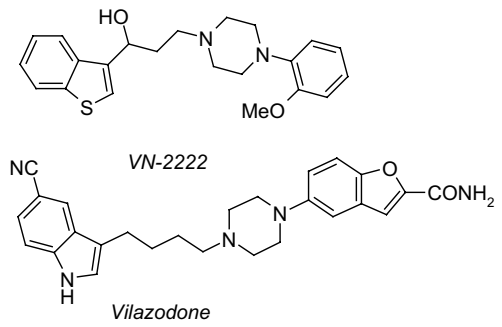


Chart 1. Dual 5-HT_{1A} ligands and 5-HT re-uptake inhibitors.

dose dependently increases extra-cellular 5-HT levels in rat frontal cortex and ventral hippocampus.[13] The selective serotonin re-uptake inhibitors *fluvoxamine*, *fluoxetine*, *citalopram* and *paroxetine* were also shown to significantly enhance 5-HT levels in certain brain areas of the rat (Chart 2).

However, the increase induced by *vilazodone* is significantly larger than that elicited by *fluoxetine*.[13] In the course of our research of *vilazodone*, we discovered equally potent aromatic systems like the unsubstituted chromenones 3-{4-[4-(2-oxo-2H-1-benzopyran-6-yl)-1-piperazinyl]-butyl}-1H-indole-5-carbonitrile **8i** ([IC_{50} nM] 5-HT_{1A} = 0.1; 5-HT RUI = 0.7; D_2 = 740) and its fluorine analogue 6-{4-[4-(5-fluor-3-indolyl)-butyl]-1-piperazinyl}-2H-1-benzopyran-2-one ([IC_{50} nM]

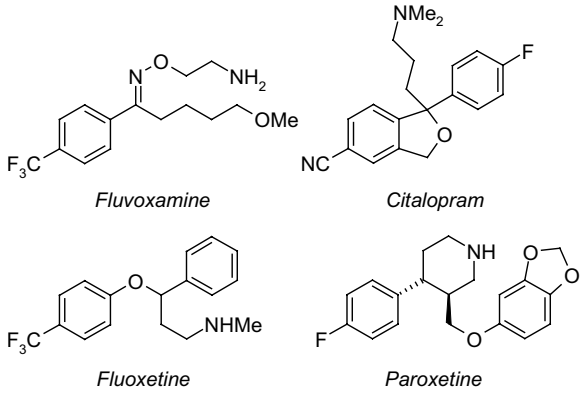


Chart 2. Established 5-HT re-uptake inhibitors.

5-HT_{1A} = 0.7; 5-HT RUI = 0.9; D_2 > 100).[11] The optimisation of the chromenone moiety to identify compounds with a superior increase of the central 5-HT level compared to *vilazodone* is described subsequently.

2. Results and discussion

2.1. Chemistry

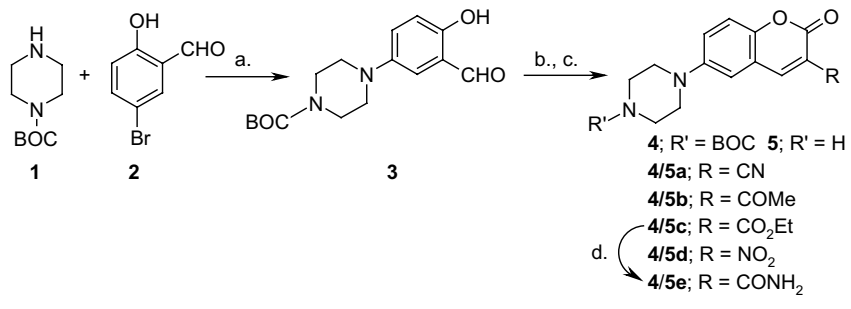
Founded on the above described re-uptake scaffold we synthesised a variety of novel substituted chromenones (Scheme 1).

Commercially available *N*-BOC piperazine **1** was coupled with 5-bromo-2-hydroxy-benzaldehyde **2** to the protected 2-hydroxy-5-piperazin-1-yl-benzaldehyde **3** in the presence of base and an appropriate palladium catalyst in THF on a 10 g scale with 97% yield. The subsequent tandem reactions, Knoevenagel and ring closure, with different CH acids yielded the corresponding chromenones **4**, which were deprotected under acidic conditions to the free amines **5a**–**d**. The ester **4c** was transferred into the corresponding amide using 25% aqueous ammonia and acidification of the crude product yielded the free piperazine **5e** in 54% over all. The 3-nitro-chromenone **4d** was used in further transformations as indicated in Scheme 2.

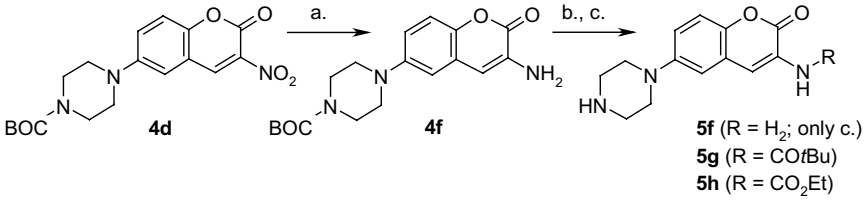
The nitro group was hydrogenated to the amine **4f** at a palladium charcoal contact in 70% yield. Deprotection of **4f** under acidic conditions yielded the 3-amino-6-piperazinyl chromenone **5f**. To generate the piperazines **5g** and **5h**, the amine **4f** was acylated with pivaloyl chloride or ethyl chloro formate, respectively, before acidic deprotection.

The chromenones **5i**, **5j** and **5k** were accessible in two steps (Scheme 3).

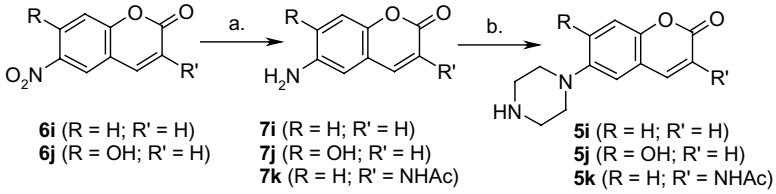
Commercially available 6-nitro chromenones (**6i**, **6j**) were hydrogenated to the corresponding amino derivatives using Raney Nickel in 53% (**7i**) and 73% (**7j**) yield, respectively. The piperazines were prepared in 48% (**5i**), 65% (**5j**) and 53% (**5k**)[14] yield by stirring the amine intermediate with bis-(2-chloro ethyl)-ammonium chloride in NMP (**7i**, **7j**) or chloro benzene (**7k**). The piperazines were used to substitute the halogen of 3-(4-halo butyl)-



Scheme 1. Reagents and conditions: (a) Sodium *tert*-butylat; chloro-(di-2-norbornyl phosphino)-(2′-dimethyl-amino-1,1′-biphenyl-2-yl)-palladium(II), toluene; (b) malononitrile or RCH_2CO_2Et piperidine, acetic acid; (c) HCl, EtOH; (d) Pd/C, H_2, EtOH; (d) (i) 25% NH_3/water, (ii) HCl, EtOH.



Scheme 2. Reagents and conditions: (a) 5% Pd/C, H_2, EtOH; (b) RCOCl, base, dichloromethane; (c) HCl, EtOH.



Scheme 3. Reagents and conditions: (a) Raney-Ni, H_2, MeOH; (b) bis-(2-chloro ethyl)-ammonium chloride, NMP.

1H-indole-5-carbonitrile as previously described.[15] Some piperazines (**5e**, **5i**) reacted sufficiently with the 4-chloro derivative to the corresponding products **8e** and **8i**, respectively. For the other piperazines **5a**, **5b**, **5f**, **5g**, **5h** and **5k** the 4-chloro building block had to be transferred into its 4-iodo analogue to succeed in the substitution reaction to **8a**, **8b**, **8f**, **8g**, **8h** and **8k**. The *trans*-halogenation was done under Finkelstein conditions.[16] In this special case, it proved to be crucial to add 6 equiv of sodium iodide in portions in 24 h intervals to the solution of the chloro-derivative in acetone and to complete the reaction quantitatively within 14 days. Elevated temperature to accelerate the reaction progress and attempts to purify the iodide caused product degradation.

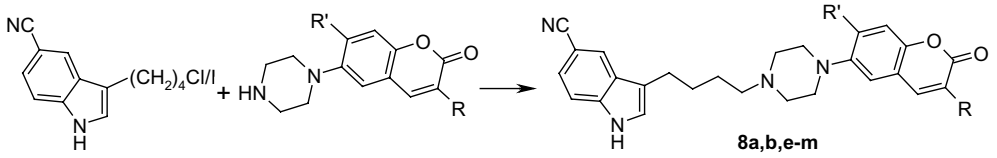
The amine **8f** was acylated with methyl chloroformate to the carbamate **8l** and the acetamide **8k** was hydrolysed within 3 h in refluxing 1 N HCl to the hydroxy chromenone **8j**. Scheme 4 summarises how all the chromenones but one (**8c**, vide infra) were prepared by coupling the chromenone moiety with the indole building block.

Because of the insufficient reactivity of **5c** with both of the two 4-halo-butyl-indoles the synthesis of **8c** was performed as indicated in Scheme 5.

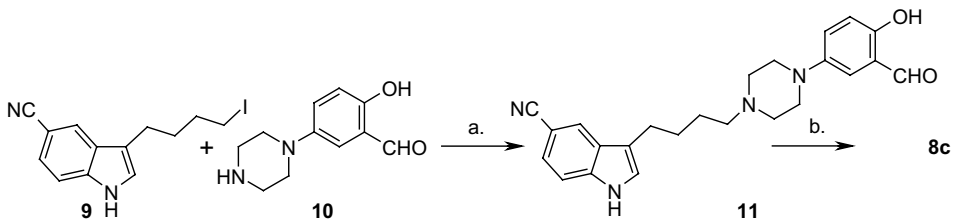
The iodide **9** was substituted with **10**, the deprotected piperazine of **3**, to the intermediate **11** which was cyclised to the chromenone **8c** under standard Knoevenagel conditions.

2.2. Biology

The affinity of the compounds towards the dopamine D_2-receptor subtype was evaluated by their ability to displace [^{3}H]spiperone (dopamine antagonist)[17] in rat striatal membranes or cloned human receptors. Serotonergic activity (5-HT_{1A}) of the compounds was measured in rat hippocampal membranes with [^{3}H]-8-OH-DPAT as ligand.[18] The intrinsic activity of the compounds such



Scheme 4. A generic visualisation of the synthesis of compounds **8**.



Scheme 5. Reagents and conditions: (a) acetonitrile, *N*-ethyl diisopropyl amine; (b) diethyl malonate, piperidine, glacial acetic acid.

as 5-HT_{1A} agonists was tested in GTPγS assays with recombinant human 5-HT_{1A} receptors stably expressed in membranes of CHO cells.[19] Re-uptake inhibition of [^{3}H]-5-HT was evaluated in rat synaptosomes in vitro[20] and ex vivo after *p*-chloro amphetamine induced 5-HT depletion (PCA assay) in rats.[21] The measurement of cortical serotonin levels in rat brain in vivo were done via microdialysis.[22] The 5-HT_{1A} agonistic in vivo activity was determined in the rat ultrasonic vocalisation test.[12,23]

The only compound with subnanomolar 5-HT_{1A} binding affinity described in this work is the unsubstituted chromenone **8i** but most other derivatives show acceptable single digit nanomolar affinity (Table 1).

The bulky *tert*-butyl amide **8g** shows the lowest 5-HT_{1A} receptor affinity. Concerning low D_2 binding and very strong re-uptake inhibition **8f**, **8i** and **8k** are the most interesting compounds. Of course the phenol **8m** should be considered cautiously because of its possible phase II metabolism and no further work was initiated. Because of their promising in vitro data a couple of compounds were measured ex vivo in the PCA assay and the intrinsic activity was validated for the chromenones as shown in Table 2.

Though the ethyl carbamate (**8h**) and the *tert*-butyl amide (**8g**) are slightly less active all compounds confirmed expectations concerning their intrinsic activity as full agonist. The unsubstituted chromenone **8i**, the amine **8f** and its acetamide analog **8k** were examined in more detail. The microdialysis results shown in the following figures indicate their potential to increase 5-HT levels in the cortex of freely moving rats.

Figure 1 shows that at a similar dose, the increase in extracellular cortical 5-HT levels induced by **8i** is less pronounced as observed with *vilazodone*. In contrast, the acetamide **8k** enhanced 5-HT comparably to *vilazo-*

Table 2. Ex vivo 5-HT up-take and in vitro intrinsic activity at the 5-HT_{1A} receptor

No	PCA [ED_{50} mg/kg][a]	GTPγS [EC_{50} ± SEM (*n*) (nM/L)][b]
8e	>3*	0.8 ± 0.2 (4)
8f	1.7**	1 ± 0.7 (4)
8g	>1*	30 ± 8 (4)
8h	1.1**	18 ± 11 (4)
8i	0.05*/0.49**	2.5 ± 0.9 (4)
8j	>9*	n.d.
8k	0.09*/0.39**	4.3 ± 0.2 (3)
8l	n.d.	6.8 ± 0.5 (3)

**s.c.*; ***p.o.*; n.d. = not determined.
[a] Results are the means of 4–8 rats per dose.
[b] Results are expressed as means ± SEM of (*n*) independent determinations.

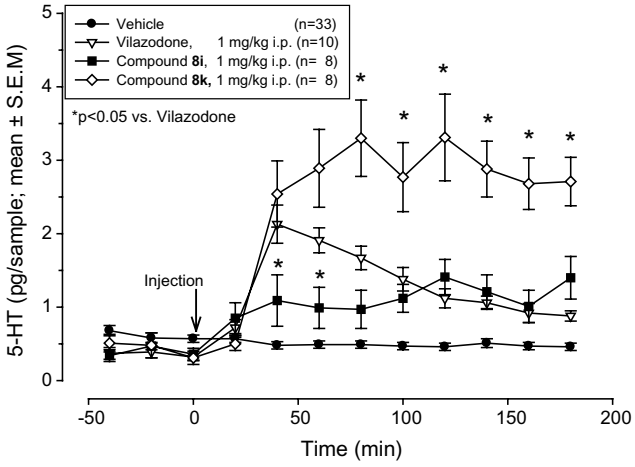
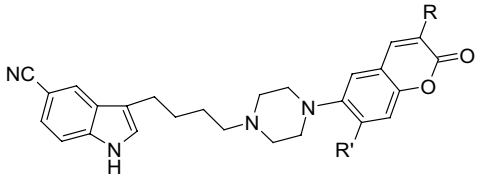


Figure 1. Microdialysis of *vilazodone* in comparison to **8i** and **8k**. pg/sample against time. * Marks statistical significance of these results.

Table 1. 5-HT re-uptake inhibition (5-HT RUI) and receptor binding [IC_{50} ± SEM (nM)]



No	R	R′	5-HT_{1A}	5-HT RUI	D_2
8a	CN	H	2.4 ± 0.8	5.7 ± 3.1	75 ± 37
8b	$COCH_3$	H	n.d.	3.9 ± 1.4	n.d.
8c	CO_2Et	H	3.9 ± 0.4	3.3 ± 0.3	n.d.
8e	$CONH_2$	H	2.9 ± 1.1	2.9 ± 0.7	180 ± 46
8f	NH_2	H	3.1 ± 1.2	6.5 ± 0.4	520 ± 242
8g	$NHCOC(CH_3)_3$	H	56 ± 32	19 ± 7	220 ± 69
8h	$NHCO_2Et$	H	9.1 ± 2.9	6 ± 4	130 ± 17
8i	H	H	0.1 ± 0.05	0.7 ± 0.5	740 ± 364
8j	OH	H	5.3 ± 2.5	2.1 ± 0.6	36 ± 15
8k	$NHCOCH_3$	H	2.5 ± 0.6	2.9 ± 1.6	950 ± 505
8l	$NHCO_2CH_3$	H	2.4 ± 0.8	4.5 ± 1.5	100 ± 29
8m	H	OH	3.1 ± 1.2	0.2 ± 0.1	560 ± 266

n.d. = not determined; IC_{50} values were obtained from 5 to 10 concentrations of the compound, each in triplicate, and are defined as the concentration (nM) resulting in 50% inhibition of binding.

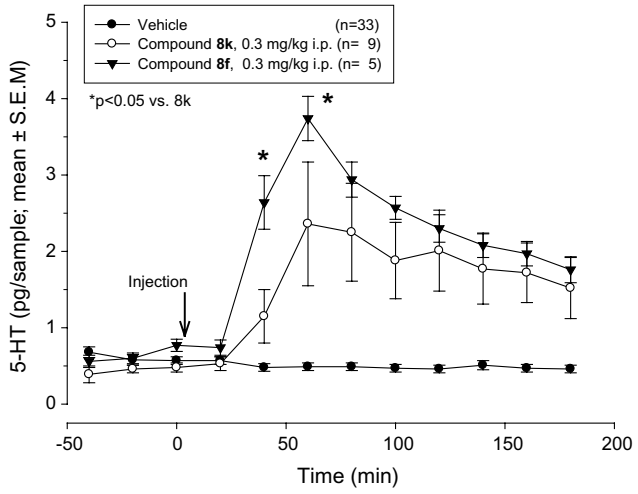


Figure 2. Microdialysis of **8f** and **8k**. pg/sample against time. * Marks statistical significance of these results.

done. However, the duration of the effect is longer. In comparison to *vilazodone*, 5-HT levels remain significantly increased to ~600% basal level at 180 min post-administration.

In line with the high 5-HT_{1A} agonistic activity of **8i**, the unsubstituted chromenone potently inhibited ultrasonic vocalisation in vivo after *s.c.* (ED_{50} 0.5 mg/kg) but was much less potent after *p.o.* (ED_{50} 9 mg/kg) administration indicating poor bio-availability. The acetamide **8k** lacked any activity in the ultrasonic vocalisation test up to 3 mg/kg *s.c.* though the compound binds with single digit nanomolar affinity to the 5-HT_{1A} receptor. The comparison in Figure 2 shows that at similar doses the unsubstituted amine **8f** is slightly more potent with regard to peak 5-HT levels than the amide **8k** already mentioned in Figure 1. In these experiments **8f** reached ~600% basal level 60 min post-treatment. The controversial results found for **8k** in the ultrasonic vocalisation assay and the microdialysis cannot be explained on the basis of the available information. It can just be speculated that some pharmacokinetic issues are responsible for the observed discrepancy.

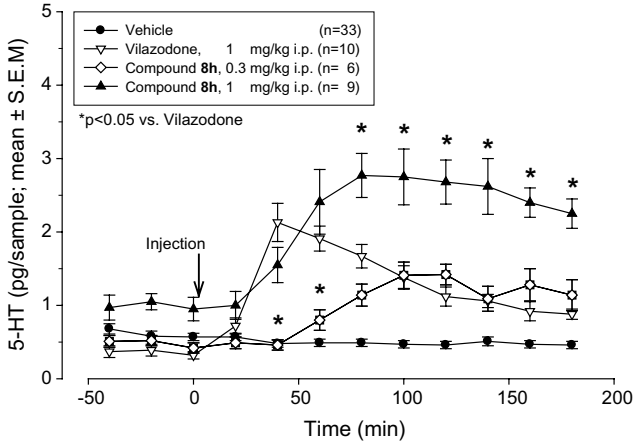


Figure 3. Different doses of **8h**. pg/sample against time. * Marks statistical significance of these results.

To extend our knowledge about the chromenones, the urethane **8h** was tested in the microdialysis (Fig. 3).

The ethyl carbamate **8h** does not cause such a fast serotonin increase like *vilazodone* or the other nitrogen derivatives discussed in Figures 1 and 2 but at a dose of 1 mg/kg it reaches a high (~280% bl) and long lasting 5-HT level (230% bl; 180 min).

3. Conclusion

With these findings we can conclude that the chromenones, especially 3-piperazino chromenones linked to the 3-butyl-1H-indole-5-carbonitrile scaffold, are able to boost central serotonin to higher and longer lasting levels in rat brain than it was possible with *vilazodone*. Clinical evaluation of one of these compounds has to verify if the property of increasing serotonin levels as seen in certain rat brain areas results in a significant antidepressant effect in humans and can cause a faster onset of action compared to the established antidepressants.

4. Experimental

4.1. General methods

Melting points were determined on a Büchi 535 melting point apparatus and are uncorrected. IR, ^{1}H NMR and mass spectra are in agreement with the structures and were recorded on a Bruker IFS 48 IR spectrophotometer, a Bruker AMX 300 MHz NMR spectrometer (TMS as an internal standard), and vacuum generators VG 70-70 or 70-250 at 70 eV, respectively. Elemental analyses (obtained with a Perkin–Elmer 240 BCHN analyser) were within 0.4% of theoretical values. All reactions were followed by TLC carried out on Merck KGaA F254 silica gel plates. Solutions were dried over Na_2SO_4 and concentrated with a Büchi rotary evaporator at low pressure. The obtained crystalline material was recrystallised from EtOH.

4.2. Building block 3

Toluene (250 mL) was degassed and charged with 725 mg (3,2 mmol) Pd(II)acetate and 445 mg (2.2 mmol) tri *tert*-butyl phosphine. To the red suspension 25.5 g (125 mmol) 5-bromo-2-hydroxy-benzaldehyde **2**, 26.7 g (144 mol) *tert*-butyl 1-piperazine carboxylate **1** and 26.5 g (276 mmol) sodium *tert*-butylate were added leading to a colour change to yellow. After 2 h at 60 °C 500 mL of water were poured into the brown suspension. After adjusting the pH to 3 with acetic acid the organic phase was extracted three times with ethyl acetate. The combined organic phases were dried and after evaporation of the solvent the residue was purified over silica gel with ether/heptane to yield 19.3 g (50%) yellow crystals **3**

tert-butyl (4-(3-formyl-4-hydroxy-phenyl)-piperazine-1-carboxylate acid ester, 99.6% purity, calibrated HPLC); ^{1}H NMR (DMSO-d_6) δ 10.41 (s, 1H), 7.58 (br s, 1H), 7.32 (dd, 1H, J = 3.1 Hz and J = 9.1 Hz), 7.23 (d, 1H, J = 3.1 Hz), 7.06 (d, 1H, J = 9.1 Hz), 3.46 (m, 4H), 3.03 (m, 4H), 1.42 (s, 9H).

4.3. Chromenone intermediates

4.3.1. 2-Oxo-6-piperazin-1-yl-2H-chromene-3-carbonitrile (5a). A suspension of 20 g (65 mmol) **3** and 4.7 g (71 mmol) malononitrile in 325 mL aqueous 0.05 M $NaHCO_3$ were heated to 80 °C for 15 min. The resulting crystals were filtered at room temperature, washed with water and dried, yielding 21.8 g (94%) *tert*-butyl 4-(3-cyano-2-oxo-2H-chromen-6-yl)-piperazine-1-carboxylate acid ester **4a**. The urethane was deprotected without further purification.

A suspension of 1.78 g (5 mmol) **4a** in 50 mL ethanol and 2 mL HCl saturated ethanol were heated to 80 °C for 1 h. The resulting crystals were filtered, washed with ethanol and dried, yielding 1.33 g reddish crystals **5a** hydrochloride. Mp 210 °C (decomp.) ^{1}H NMR (DMSO-d_6) δ 9.45 (br s, 2H); 8.81 (s, 1H); 7.55 (dd, 1H, J = 2.8 Hz, J = 9.4 Hz); 7.43 (d, 1H, J = 9.4 Hz); 7.33 (d, 1H, J = 2.8 Hz); 3.42 (m, 4H); 3.24 (m, 4H); Anal. ($C_{14}H_{13}N_3O_2$*HCl) calcd C 57.6; H 4.8; N 14.4; found: C 54.1; H 5.0; N 13.7.

4.3.2. *tert*-Butyl 4-(3-acetyl-2-oxo-2H-chromen-6-yl)-piperazine-1-carboxylate (4b). A solution of 6.13 g (20 mmol) **3**, 2.38 mL (24 mmol) piperidine, 1.37 mL glacial acid and 3.04 mL (24 mmol) 3-oxo-butyrate acid ethyl ester in 100 mL acetonitrile was refluxed for 30 min when TLC indicated complete conversion. After the suspension had cooled to room temperature, the product was filtered and dried yielding 6.5 g (87%) red crystals **4b**. Mp 187–189 °C; ^{1}H NMR (DMSO-d_6) δ 8.54 (s, 1H), 7.47–7.32 (m, 3H), 3.48 (m, 4H), 3.13 (m, 4H), 2.54 (s, 3H), 1.43 (s, 9H); Anal. ($C_{20}H_{24}N_2O_5$) calcd C 64.5; H 6.4; N 7.5; found: C 64.2; H 6.3; N 7.5.

4.3.3. 3-Acetyl-6-piperazin-1-yl-chromen-2-one (5b). A suspension of the above prepared urethane **4b** (1.23 g; 3 mmol) in 30 mL ethanol was treated with 1.23 mL HCl-saturated ethanol for 2 h. The resulting crystals were filtered, washed with ethanol and ether and dried, yielding 0.8 g (86%) **5b** hydrochloride. Mp 308 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ 9.22 (br s, 2H); 8.57 (s, 1H); 7.51 (d, 1H, J = 2.0 Hz); 7.49 (d, 1H, J = 2.0 Hz); 7.42 (s, 1H); 3.33 (m, 8H); 2.58 (s, 3H); Anal. ($C_{15}H_{16}N_2O_3$*HCl) calcd C 58.4; H 5.5; N 9.1; found: C 57.9; H 5.8; N 8.8.

4.3.4. 6-Amino-chromen-2-one (7i). A solution of 41 g (0.21 mol) **6i** was dissolved in 450 mL methanol and added to 10 g methanol–wet Raney Nickel. After 20 h and the addition of 3 mol H_2, TLC indicated complete conversion. The solvent was partially removed and HCl-saturated ethanol was added. The precipitating salt was filtered and dried yielding 22.2 g (53%) yellow crystals **7i** hydrochloride. Mp >280 °C. Anal. ($C_9H_7NO_2$*HCl) calcd C 54.7; H 4.1; N 7.1; Cl 17.9; found: C 55.1; H 4.2; N 7.2; Cl 17.3.

4.3.5. 6-Piperazin-1-yl-chromen-2-one (5i). A solution of 49.9 g (0.28 mol) bis-(2-chloro ethyl)-ammonium chloride in 50 mL NMP was added dropwise at 130 °C to a solution of 22.1 g (0.11 mol) **7i** in 110 mL NMP. After 60 h, TLC indicated complete conversion and the solvent was removed. The residue was washed with ethyl ester and crystallised from 2-propanol. The green crystals were washed with acetone and diethyl ether and dried giving 14.4 g (48%) **5i** hydrochloride. Mp 293–295 °C. Anal. ($C_{13}H_{14}N_2O_2$*HCl) calcd C 58.5; H 5.7; N 10.5; Cl 13.3; found: C 58.4; H 5.9; N 12.7; Cl 13.7.

4.4. Indole intermediate

4.4.1. 3-(4-Iodo-butyl)-1H-indole-5-carbonitrile (9). Under nitrogen 600 g NaI was added to a solution of 150 g 3-(4-chloro-butyl)-1H-indole-5-carbonitrile[11] in 500 mL acetone in six portions in 24 h intervals. The insoluble part was filtered off and the solution evaporated to dryness. The crude product was partitioned between water and ethyl acetate, the organic phase was dried and again evaporated. The solid material was crystallised from hexane/diethyl ether, washed with diethyl ether and dried, yielding 184.1 g (88%) **9**. Mp 117–119 °C.

4.5. Indolyl-chromenones

4.5.1. 3-{4-[4-(3-Cyano-2-oxo-2H-chromen-6-yl)-piperazin-1-yl]-butyl}-1H-indole-5-carbonitrile (8a). A solution of 2 g (7 mmol) **5a**, 2.4 g (7 mmol) **9** and 3.1 mL (23 mmol) triethyl amine in 100 mL acetonitrile was refluxed for 12 h. The solvent was removed and the residue partitioned between ethyl ester and water. After the usual procedure, 2 g crude product were purified by chromatography yielding 0.7 g red crystals. These were dissolved in 120 mL acetone and 1 N HCl was added dropwise until pH 4 was reached. After 12 h, yellow crystals were filtered and washed with acetone and ether yielding 0.7 g (21%) **8a**. Mp 283–285 °C; ^{1}H NMR (DMSO-d_6) δ 11.48 (br s, 1H), 10.86 (br s, 1H), 8.81 (s, 1H), 8.10 (s, 1H), 7.56 (dd, 1H, J = 2.8 and J = 9.2 Hz), 7.52 (d, 1H, J = 8.4 Hz), 7.42 (m, 3H), 7.33 (d, 1H, J = 2.8 Hz), 3.82 (br d, 2H, J = 11.0 Hz), 3.58 (br d, 2H, J = 10.0 Hz), 3.25–3.15 (m, 7H), 2.78 (t, 2H, J = 7.4 Hz) 1.82 (m, 2H), 1.71 (m, 2H). Anal. ($C_{27}H_{25}N_5O_2$*HCl*0.75 H_2O) calcd C 64.7; H 5.5; Cl 7.1; N 14.0; found: C 64.7; H 5.2; Cl 7.1; N 13.8.

4.5.2. 3-{4-[4-(3-Acetyl-2-oxo-2H-chromen-6-yl)-piperazin-1-yl]-butyl}-1H-indole-5-carbonitrile (8b). A suspension of 1.54 g (5 mmol) **5b**, 1.78 g (5 mmol) **9** and 2.9 mL (17 mmol) triethyl amine in 150 mL acetonitrile was refluxed for 12 h. The solution was concentrated in vacuo and stirred with water and ethyl acetate. The organic phase was evaporated to dryness and after chromatography 0.8 g (33%) of the free base was isolated. The base was redissolved in 80 mL acetone and 1 N HCl was added until pH 4 was reached. The resulting yellow crys-

tals were filtered, washed with acetone and ether and dried yielding 0.8 g (95%) **8b** hydrochloride; Mp 245–247 °C; ^{1}H NMR (DMSO-d_6) δ 11.46 (s, 1H), 10.61 (s, 1H), 8.57 (s, 1H), 8.11 (s, 1H), 7.51 (m, 3H), 7.41 (m, 3H), 3.83 (br d, 2H, J = 10.8 Hz), 3.59 (br d, 2H, J = 10.8 Hz), 3.18 (m, 6H), 2.79 (t, 2H, J = 7.4 Hz) 1.81 (m, 2H), 1.71 (m, 2H); Anal. ($C_{28}H_{28}N_4O_3$*HCl) calcd C 66.6; H 5.8; N 11.1; Cl 7.0; found: C 65.4; H 5.7; N 11.1; Cl 7.4.

4.5.3. Ethyl 6-{4-[4-(5-cyano-1H-indol-3-yl)-butyl]-piperazin-1-yl}-2-oxo-2H-chromene-3-carboxylate (8c). A solution of 42.3 g (0.14 mol) **3** in 2-propanol (500 mL) was treated with saturated HCl solution in ethanol (40 mL) and refluxed overnight. The amount of solvent was reduced to 100 mL and the precipitating crude 2-hydroxy-5-piperazin-1-yl-benzaldehyde **10** hydrochloride was filtered off and dried. A solution of 33.4 g (0.14 mol) **10**, 44.7 g (0.14 mol) **9** and 58.7 mL (0.35 mol) diethyl isopropyl-amine in 1.5 L acetonitrile was refluxed for 5 h. The solvent was removed and the residue partitioned between ethyl acetate and water. After the usual procedure, the crude product was purified by chromatography and crystallised from ethyl acetate and *tert*-butyl methyl ether yielding 23.1 g (42%) 3-{4-[4-(3-formyl-4-hydroxy-phenyl)piperazin-1-yl]-butyl}-1H-indole-5-carbonitrile **11** as colourless crystals. ^{1}H NMR (DMSO-d_6) δ 11.36 (s, 1H), 10.21 (s, 1H), 10.18 (br s, 1H), 8.07 (s, 1H), 7.50 (d, 1H, J = 8.4), 7.40 (dd, 1H, J = 8.4 Hz, J = 1.5 Hz), 7.34 (d, 1H, J = 1.5 Hz), 7.26 (dd, 1H, J = 9.0 Hz, J = 3.0 Hz), 7.12 (d, 1H, J = 3.0 Hz), 6.91 (d, 1H, J = 9.0 Hz), 3.31 (br s, 2H), 3.04 (br s, 4H), 2.75 (t, 2H, J = 7.3 Hz), 2.68–2.53 (m, 4H), 1.68 (tt, 2H, J = 7.3 Hz), 1.53 (br s, 1H).

A mixture of 100 mg (0.25 mmol) **11**, 38 mL (0.25 mmol) diethyl malonate, 25 mL (0.25 mmol) piperidine and 14 mL (0.25 mmol) acetic acid in ethanol (5 mL) was refluxed for 18 h. Water (5 mL) was added and the formed precipitate was filtered off and dried. 110 mg (90%) of pure colourless solid of **8c** were obtained. Mp 103 °C; ^{1}H NMR (DMSO-d_6) δ 11.35 (s, 1H), 8.65 (s, 1H), 8.07 (s, 1H), 7.49 (d, 1H, J = 8.4), 7.43–7.35 (m, 3H), 7.33 (d, 1H, J = 1.7 Hz), 7.30 (d, 1H, J = 9.0 Hz), 4.29 (q, 2H, J = 7.1 Hz), 3.16 (m, 4H), 2.74 (t, 2H, J = 7.3 Hz), 2.54–2.48 (m, 4H), 2.38 (t, 2H, J = 7.3 Hz), 1.68 (tt, 2H, J = 7.2 Hz), 1.53 (tt, 2H, J = 7.2 Hz), 1.31 (t, 3H, J = 7.1 Hz); Anal. ($C_{29}H_{30}N_4O_4$) calcd C 69.9; H 6.1; N 11.2; found: C 69.6; H 6.3; N 11.0.

4.5.4. 6-{4-[4-(5-Cyano-1H-indol-3-yl)-butyl]-piperazin-1-yl}-2-oxo-2H-chromene-3-carboxamide (8e). A suspension of 6.36 g (16.4 mmol) **5e**, 7.64 g (32.84 mmol) 3-(4-chloro-butyl)-1H-indole-5-carbonitrile[11] and 6.4 g (49.2 mmol) ethyl diisopropyl-amine in 200 mL acetonitrile was refluxed for 12 h. The resulting precipitate was filtered off and the liquid phase evaporated. The crude product was purified via chromatography and the resulting colourless foam was transferred into the corresponding hydrochloride with HCl saturated ethanol yielding 800 mg (10%) of **8e** hydrochloride. Mp 280–282 °C; ^{1}H NMR (DMSO-d_6) δ 11.48 (br s, 1H), 10.50 (br s, 1H), 8.81 (s, 1H), 8.10 (s, 2H), 7.89 (br s, 1H), 7.52–7.49 (m, 3H), 7.44–7.39 (m, 3H), 3.84 (br s, 4H), 3.16 (m, 6H), 2.78 (m, 2H), 1.80 (m, 2H), 1.70 (m, 2H); Anal. ($C_{27}H_{27}N_5O_3$*HCl) calcd C 64.1; H 5.6; Cl 7.0; N 13.8; found: C 63.7; H 5.1; Cl 7.5; N 13.1.

4.5.5. 3-{4-[4-(3-Amino-2-oxo-2H-chromen-6-yl)-piperazin-1-yl]-butyl}-1H-indole-5-carbonitrile (8f). A solution of 100 mg (0.31 mmol) **5f** dihydrochloride, 102 mg (0.31 mmol) **9** and 122 mg (0.94 mmol) ethyl diisopropyl-amine in 10 mL acetonitrile was refluxed for 18 h. The mixture was evaporated to dryness and the crude product was purified by chromatography directly to obtain a colourless solid **8f** (33 mg, 24%). Mp 154 °C; ^{1}H NMR (DMSO-d_6) δ 11.35 (s, 1H), 8.07 (s, 1H), 7.49 (d, 1H, J = 8.4), 7.39 (d, 1H, J = 8.4 Hz), 7.33 (s, 1H), 7.12 (d, 1H, J = 8.6 Hz), 6.90–6.83 (m, 2H), 6.65 (s, 1H), 5.58 (s, 2H), 3.09 (br s, 4H), 2.74 (t, 2H, J = 7.5 Hz), 2.39 (br s, 4H), 2.36 (t, 2H, J = 7.5 Hz), 1.68 (tt, 2H, J = 7.5 Hz), 1.52 (tt, 2H, J = 7.5 Hz); Anal. ($C_{26}H_{27}N_5O_2$) calcd C 70.7; H 6.2; N 15.9; found: C 70.2; H 6.4; N 15.5.

4.5.6. *N*-(6-{4-[4-(5-Cyano-1H-indol-3-yl)-butyl]-piperazin-1-yl}-2-oxo-2H-chromen-3-yl)-2,2-dimethyl-propionamide hydrochloride (8g). A solution of 244 mg (0.74 mmol) **5g**, 360 mg (1.11 mmol) **9** and 287 mg (2.22 mmol) ethyl diisopropyl-amine in 15 mL NMP was heated at 110 °C for 2 days. The mixture was poured into ice water and the precipitate was filtered off. The crude product was purified by chromatography. The obtained colourless solid was transferred to the hydrochloride salt using a hydrochloric acid isopropanol solution, yielding 36 mg (9%) **8g** hydrochloride as colourless solid. Mp 100 °C; ^{1}H NMR (DMSO-d_6) δ 11.43 (s, 1H), 9.98 (br s, 1H), 8.57 (s, 1H), 8.49 (s, 1H), 8.10 (s, 1H), 7.51 (d, 1H, J = 8.4), 7.41 (dd, 1H, J = 8.4 Hz, J = 2.9 Hz), 7.38 (d, 1H, J = 2.0 Hz), 7.35 (d, 1H, J = 9.0 Hz), 7.30 (d, 1H, J = 2.8 Hz), 7.23 (dd, 1H, J = 9.2 Hz, J = 2.8 Hz), 3.84 (d, 2H, J = 12.0 Hz), 3.57 (d, 2H, J = 12.0 Hz), 3.22–3.04 (m, 6H), 2.78 (t, 2H, J = 7.2 Hz) 1.81–1.66 (m, 4H), 1.25 (s, 9H); Anal. ($C_{31}H_{35}N_5O_3$*HCl*H_2O) calcd C 64.2; H 6.6; N 12.1; Cl 6.1; found: C 64.1; H 6.7; N 12.0.

4.5.7. Ethyl (6-{4-[4-(5-cyano-1H-indol-3-yl)-butyl]-piperazin-1-yl}-2-oxo-2H-chromen-3-yl)-carbamate, (8h). A solution of 162 mg (0.51 mmol) **5h** (the dihydrochloride salt was dissolved in 2 N NaOH solution and extracted with ethyl acetate three times, the combined organic layers were dried with sodium sulfate, filtered and evaporated to dryness), 166 mg (0.51 mmol) **9** and 200 mg (1.54 mmol) ethyl diisopropyl-amine in 10 mL acetonitrile was refluxed for 2 days. The solvent was removed and the residue partitioned between ethyl ester and water. The organic layer was purified by chromatography. The obtained colourless solid was transferred to the hydrochloride salt using a hydrochloric acid isopropanol solution, yielding 181 mg (64%) **8h** hydrochloride as colourless solid. Mp 220 °C; ^{1}H NMR (DMSO-d_6) δ 11.45 (s, 1H), 10.41 (br s, 1H), 8.89 (s, 1H), 8.20 (s, 1H), 8.10 (s, 1H), 7.51 (d, 1H, J = 8.4), 7.41 (dd, 1H, J = 8.4 Hz, J = 1.5 Hz), 7.39 (d, 1H, J = 1.5 Hz), 7.32 (d, 1H, J = 9.0 Hz), 7.28 (d, 1H, J = 2.8 Hz), 7.20 (dd,

1H, $J = 9.0$ Hz, $J = 2.8$ Hz), 4.17 (q, 2 H. $J = 7.1$ Hz), 3.82 (d, 2H, $J = 8.5$ Hz), 3.55 (d, 2H, $J = 8.5$ Hz), 3.21–3.10 (m, 6H), 2.78 (t, 2H, $J = 7.2$ Hz) 1.83–1.66 (m, 4H), 1.25 (t, 3H, $J = 7.1$ Hz); Anal. ($C_{29}H_{31}N_5O_4$*HCl) calcd C 63.3; H 5.9; N 12.7; Cl 6.4; found: C 63.1; H 6.1; N 12.8; Cl 6.4.

4.5.8. 3-{4-[4-(2-Oxo-2H-1-benzopyran-6-yl)-1-piperazinyl]-butyl}-indol-5-carbonitrile (8i). A suspension of 2.33 g (10 mmol) 3-(4-chloro-butyl)-1H-indole-5-carbonitrile,[11] 66 g (10 mmol) **5i**, 2.8 mL (20 mmol) triethyl amine and 1.38 g (10 mmol) K_2CO_3 in 1 L acetonitrile was refluxed for 36 h. The solvent was removed and the residue partitioned between ethyl ester and water. After extraction of the aqueous phase the organic phase was dried and concentrated. The residue was purified by chromatography and crystallised from diethyl ether yielding 1.1 g yellow crystals **8i**. Mp 135–137 °C; ^{1}H NMR (DMSO-d_6) δ 10.64 (br s, 1H), 9.91 (br s, 1H), 7.35 (s, 1H), 7.24 (d, 1H, $J = 8.7$ Hz), 6.83 (d, 1H, $J = 7.6$ Hz), 6.72 (m, 2H), 6.69 (s, 2H), 6.62 (s, 1H), 5.87 (d, 1H, $J = 8.7$ Hz), 3.45 (br d, 2H, $J = 10.3$ Hz), 3.23 (br d, 2H, $J = 10.3$ Hz), 2.88 (m, 6H), 2.52 (t, 2H, $J = 6.4$ Hz), 1.74–1.52 (m, 4H); Anal. ($C_{26}H_{24}N_4O_2$) calcd C 73.3; H 6.1; N 13.1; found: C 73.4; H 6.1; N 13.1.

4.5.9. 3-{4-[4-(3-Hydroxy-2-oxo-2H-chromen-6-yl)-piperazin-1-yl]-butyl}-1H-indole-5-carbonitrile (8j). One hundred milligrams (0.19 mmol) of **8k** were treated with 1 N HCl solution (5 mL) and refluxed for 3 h. The solution was neutralised with aqueous ammonia, the precipitate was collected and purified by chromatography directly. A colourless solid **8j** was obtained (20 mg, 24%). Mp 160 °C; ^{1}H NMR (DMSO-d_6) δ 11.39 (s, 1H), 8.07 (s, 1H), 7.49 (d, 1H, $J = 8.4$), 7.39 (dd, 1H, $J = 8.4$ Hz, $J = 1.3$ Hz), 7.33 (d, 1H, $J = 1.3$ Hz), 7.13 (d, 1H, $J = 9.5$ Hz), 6.93–6.87 (m, 2H), 6.82 (s, 1H), 3.09 (t, 4H, $J = 4.6$ Hz), 2.74 (t, 2H, $J = 7.4$ Hz), 2.50–2.46 (m, 4H), 2.36 (t, 2H, $J = 7.4$ Hz), 1.67 (tt, 2H, $J = 7.3$ Hz), 1.52 (tt, 2H, $J = 7.3$ Hz); Anal. ($C_{26}H_{26}N_4O_3$) calcd C 70.6; H 5.9; N 12.7; found: C 70.2; H 6.2; N 12.5.

4.5.10. *N*-(6-{4-[4-(5-Cyano-1H-indol-3-yl)-butyl]-piperazin-1-yl}-2-oxo-2H-chromen-3-yl)-acetamide (8k). A solution of 12.4 g (43 mmol) **5k**, 13.9 g (43 mmol) **9** and 11.1 g (86 mmol) ethyl diisopropyl amine in 150 mL NMP was heated at 120 °C for 3 days. The mixture was poured into ice water and the precipitate was filtered off. The crude product was purified by chromatography. The obtained colourless solid was transferred to the hydrochloride salt using a hydrochloric acid isopropanol solution, yielding 3.4 g (15%) **8k** hydrochloride as colourless solid. Mp 206 °C; ^{1}H NMR (DMSO-d_6) δ 11.44 (s, 1H), 10.35 (br s, 1H), 9.69 (s, 1H), 8.59 (s, 1H), 8.10 (s, 1H), 7.51 (d, 1H, $J = 8.4$), 7.41 (dd, 1H, $J = 8.4$ Hz, $J = 1.5$ Hz), 7.39 (d, 1H, $J = 1.5$ Hz), 7.30 (d, 1H, $J = 9.0$ Hz), 7.28 (d, 1H, $J = 2.5$ Hz), 7.20 (dd, 1H, $J = 9.0$ Hz, $J = 2.5$ Hz), 3.85 (br s, 2H), 3.55 (br s, 2H), 3.21–3.10 (m, 6H), 2.78 (t, 2H, $J = 7.2$ Hz), 2.17, (s, 3H), 1.82–1.66 (m, 4H); Anal. ($C_{28}H_{29}N_5O_3$*HCl*2 H_2O) calcd C 60.6; H 6.2; N 12.6; Cl 6.4; found: C 61.0; H 6.1; N 12.4; Cl 6.6.

4.5.11. Methyl (6-{4-[4-(5-cyano-1H-indol-3-yl)-butyl]-piperazin-1-yl}-2-oxo-2H-chromen-3-yl)-carbamate (8l). To a solution of 518 mg (1.17 mmol) **8f** and 0.11 mL (1.41 mmol) pyridine in 10 mL dichloromethane was added 0.11 mL (1.41 mmol) methyl chloroformate dropwise at 0 °C. The mixture was stirred at 0 °C for 1 h and 12 h at rt, poured into ice water and the colourless precipitate was filtered off yielding 57 mg (10%) **8l**. Mp 107 °C; ^{1}H NMR (DMSO-d_6) δ 11.35 (s, 1H), 8.97 (s, 1H), 8.17 (s, 1H), 8.07 (s, 1H), 7.49 (d, 1H, $J = 8.4$), 7.40 (d, 1H, $J = 8.4$ Hz), 7.33 (s, 1H), 7.25 (d, 1H, $J = 9.0$ Hz), 7.17–7.10 (m, 2H), 3.71 (s, 3H), 3.19–3.12 (m, 4H), 2.74 (t, 2H, $J = 7.5$ Hz), 2.52–2.46 (m, 4H), 2.40–2.33 (m, 2H), 1.68 (tt, $J = 7.5$ Hz), 1.53 (tt, $J = 7.5$ Hz); Anal. ($C_{28}H_{29}N_5O_4$) calcd C 67.3; H 5.9; N 14.0; found: C 66.9; H 6.3; N 13.7.

4.6. Pharmacological methods

4.6.1. Receptor binding assays. The affinity of compounds for dopamine receptors were determined in a total volume of 2 mL containing 2–3 nM [^{3}H]ADTN or 0.1 nM [^{3}H]spiperone resp., and 0.-3-1.0 mg of protein of rat striatal membranes per mL. Assay buffer was 50 mol/L Tris/HCl, pH 7.1, 120 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, and 0.1% ascorbic acid in the case of ADTN and 50 mmol Tris/HCl, pH 7.7 and 0.1% ascorbic acid in the case of spiperone. Incubations were carried out at 37 °C for 15 min and terminated by rapid filtration (Whatman GF/B) and three washes with ice-cold buffer. Nonspecific binding was determined in the presence of 1 mmol/L (+)-butaclamol.[17,24]

4.7. Microdialysis

Male rats (250–300 g body weight) were anesthetised with pentobarbital (50 mg/kg intraperitoneally (*i.p.*)) and placed in a stereotaxic frame (Stoelting Co., IL, USA). Body temperature was maintained at 37 °C with a heated pad and a temperature controller (CMA/105; CMA/Microdialysis, Stockholm, Sweden). A CMA/11 guide cannula plugged with an obturator was implanted into the frontal cortex (AP + 3.2, L + 2.5 from bregma, V−5.0; Paxinos and Watson, 1986). By using dental cement and anchoring screws, the guide was fixed to the skull. Post-operatively, the rats were left to recover from surgery 24 h with free access to food and water. Then the obturator was removed from the guide cannula and a microdialysis probe (CMA/11, membrane length 4 mm, O.D. 0.25 mm) was inserted.

The day after inserting of the microdialysis probe, the rats were placed in a CMA/120 microdialysis system. The probes were perfused with filtered Dulbecco's phosphate buffered saline (composition in mM: $CaCl_2 \times 2$ H_2O 0.9, $MgCl_2 \times 6$ H_2O 0.5, KCl 2.7, K_2HPO_4 1.5, NaCl 138 and Na_2HPO_4 8.1, pH 7.4) and ascorbic acid (10 μM) at a rate of 1.2 μL/min using a microdialysis pump (CMA/100). The samples were automatically collected by a CMA/140 microfraction collector in 300 μL borosilicate vials and frozen until HPLC analysis. A 2 h stabilisation period was allowed following start of

the microdialysis procedure. A 20 min sampling regimen was used throughout the experimental period. After three basal samples were collected, drugs were administered *i.p.* at a volume of 0.2 mL/100 g body weight and the response was followed for another 3 h. At the end of the experiment, brains were removed and cut into slices following formalin fixation. Probe placement was verified by visual inspection.

Dialysate concentration of 5-HT was determined using a reversed phase/ion pair HPLC method with electrochemical detection. The dialysate was injected automatically into the HPLC system by a HTC-PAL (CTC Analytics AG, Zwingen, Switzerland) refrigerated autosampler programmed to inject 20 μL of dialysate. The degassed (CMA/260 degasser) mobile phase consisting of 50 mM sodium citrate, 27 μM disodium-EDTA, 10 mM diethylamine–HCl, 10 mM NaCl, 2 mM decane–sulfonic acid (sodium salt) and 17.5% (v/v) acetonitrile was delivered at a flow rate of 60 μL/min by a Rheos 2000 LC/MS (Flux Instruments AG, Basel, Switzerland) pump. Analytes were separated on a Ultracarb RP-18 ODS 30 5 μm column (150 × 1 mm, Phenomenex, CA, USA), maintained at ambient temperature. For electrochemical detection, a BAS LC4C detector (Bioanalytical Systems, IN, USA) with a glassy carbon electrode set at a potential of +650 mV versus a Ag/AgCl electrode was used. Retention time (9–11 min for 5-HT), peak areas and peak heights were measured with a data management software system (ChemServer Target Software, Falcon Integrator, Hewlett–Packard Company, NE, USA). Dialysate concentrations of 5-HT were calculated by comparing peak areas with those of a range of standard concentrations. Data are presented as pg/sample (mean ± S.E.M.). Values were compared using two-way ANOVA followed by Bonferroni post-hoc analysis. Significance levels were set at $p < 0.05$.

4.7.1. PCA assay. Effects of drugs on PCA (*p*-chloroamphetamine) induced 5-HT depletion in rat hypothalamus were carried out as previously described.[25] Male rats (135–160 g body weight) were used. Brain regions were dissected out on ice[26] and immediately processed for HPLC analysis. PCA (5 mg/kg *i.p.* in saline) was given 3 h and drugs 3.5 or 5 h prior to decapitation. Brain tissue 5-HT was determined by an automated reversed phase/ion pair, direct injection HPLC method[24] within a 25 min run. *N*-Methyldopamine or *N*-ω-methylserotonin was used as internal standard and the recovery was >95%.

4.7.2. Ultrasonic vocalisation test. Male rats weighing 180–280 g from Charles River (Sulzfeld, Germany) were used. Ultrasonic vocalisation was measured in a sound-attenuated test chamber (W 24 cm, L 22 cm, H 22 cm) with a grid floor for delivery of foot-shock (scrambled shock of 0.2 mA for 0.5 s, shocker Getra BN 2002). Ultrasonic vocalisation was recorded (microphone 4004, Bruel and Kjær) and processed by an interface (developed at Merck, Darmstadt) to select 22 kHz ± 4 kHz signals and to digitise the resulting signals for automatic processing in a personal computer. In a priming phase, each rat was placed in the test chamber. After a 2 min time period, a series of at most 10 shocks (trials), 1.8 mA for 0.3 s, separated by 20 s shock-free intervals, was delivered via the grid floor of the test chamber. In the shock-free intervals, the occurrence of ultrasonic vocalisation was automatically recorded, and the duration of ultrasonic vocalisation was calculated immediately. The priming session was terminated either when the rat constantly vocalised at least for 10 s on three consecutive trials or after the 10th trial. Rats which did not respond with ultrasonic vocalisation on three consecutive trials were excluded from further testing. In the actual test performed on the next day, each rat received 5 initial shocks (1.8 mA for 0.3 s, separated by 20 s shock-free intervals) in the test chamber, and the duration of ultrasonic vocalisation was recorded during the following 3 min period. Animals were tested 30 min after *s.c.* or *p.o.* administration of compounds, respectively. The given values are the mean of 6–10 animals per dose. The half-maximum effect was determined from the dose–response curve.

4.7.3. Stimulation of [^{35}S]GTPγS binding at cloned 5-HT$_{1A}$ receptors. The effects of different compounds tested on [^{35}S]GTPγS binding were evaluated according to a method of Newman-Tancredi et al.[27] with modifications. Membranes of CHO cells stably expressing the recombinant human 5-HT$_{1A}$-receptor were obtained from NEN (Catalog No. CRM035, GenBank No. X13556). The membranes were stored at −70 °C. Prior to use, membranes were thawed and rehomogenised in assay buffer ($MgCl_2$, NaCl and EDTA in Tris–HCl, pH 7.4). Membranes (~10 μg protein) were incubated at 37 °C for 30 min (shaking water bath) in duplicate in a total volume of 800 μL of buffer containing $MgCl_2$ (3 mM), NaCl (120 mM), EDTA (0.2 mM), GDP (10 μM), [^{35}S]GTPγS (0.1 nM), Tris (50 mM) and test compounds. Prior to adding to the incubation mixture, the test compounds were dissolved in bi-distilled water. DMSO was used to aid in solubilising certain compounds. Nonspecific binding was defined with 0.1 μM GTPγS. 5-HT was tested as standard in each experiment at concentrations of 100, 30 and 10 nM. Experiments were terminated by rapid filtration through Whatman GF/B filters using a Brandel cell harvester. Subsequently, the filters were rinsed twice with 5 mL ice cold Tris–HCl and placed in scintillation vials. Radioactivity was extracted in 4 mL scintillation fluid (Ultima Gold^R, Packard Instruments, Frankfurt, Germany) and determined by liquid scintillation counting. Binding isotherms were analysed by nonlinear regression. Agonist efficacy ($=E_{max}$) is expressed relative to that of 5-HT (=100%), which was tested at a maximally active concentration (0.1 mM) in each experiment. EC_{50} values were defined as the concentration of the compound at which 50% of its own maximal stimulation was obtained.

Acknowledgements

We thank Jürgen Eckstein, Uwe Eckert and Kurt Schuster for the preparation of the compounds and Herbert Ziegler for performing pharmacological tests.

References and notes

1. Murray, C. J. L.; Lopez, A. D. *Science* **1996**, *274*, 740.
2. (a) Wong, M.-L.; Licinio, J. *Nature* **2004**, *427*, 136; (b) Pacher, P.; Kohegyi, E.; Kecskemeti, V.; Furst, S. *Curr. Med. Chem.* **2001**, *8*, 89; (c) Nemeroff, C. B. *Sci. Am.* **1998**, *278*, 28; (d) Blier, P. *J. Clin. Psychiat.* **2001**, *62*, 12; (e) Spinks, D.; Spinks, G. *Curr. Med. Chem.* **2002**, *9*, 799.
3. Asberg, M.; Eriksson, B.; Mertensson, B.; Trakman-Bendz, L.; Wagner, A. *J. Clin. Psychiat.* **1986**, *47*, 23.
4. Thompson, C. *Human Psychopharmacol.* **2002**, *17*(Sup.1), S27.
5. Rutter, J. J.; Gundlach, C.; Auerbach, S. B. *Synapse* **1995**, *20*, 225.
6. Hjorth, S. *J. Neurochem.* **1993**, *60*, 776.
7. Artigas, F.; Perez, V.; Alvarez, E. *Arch. Gen. Psychiat.* **1994**, *51*, 248.
8. Gartside, S. E.; Umbers, V.; Hajos, M.; Sharp, T. *Br. J. Pharmacol.* **1995**, *115*, 1064.
9. Dreshfield, L. J.; Wong, D. T.; Perry, K. W.; Engleman, E. A. *Neurochem. Res.* **1996**, *21*, 557.
10. Tordera, R. M.; Monge, A.; Del Rio, J.; Lasheras, B. *Eur. J. Pharmacol.* **2002**, *442*, 63.
11. Heinrich, T.; Böttcher, H.; Gericke, R.; Bartoszyk, G. D.; Anzali, S.; Seyfried, C. A.; Greiner, H. E.; van Amsterdam, C. *J. Med. Chem.*, in press.
12. Bartoszyk, G. D.; Hegenbart, R.; Ziegler, H. *Eur. J. Pharmacol.* **1997**, *322*, 147.
13. Page, M. E.; Cryan, J. F.; Sullivan, A.; Salvi, A.; Saucy, B.; Manning, D. R.; Lucki, I. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 1220.
14. *N*-(6-Amino-2-oxo-2H-chromen-3-yl)-acetamide **5k** is commercially available (Maybridge).
15. Böttcher, H.; Gericke, R. *Liebigs Ann. Chem.* **1988**, 749.
16. Finkelstein, H. *Ber. Dtsch. Chem. Ges.* **1910**, *43*, 1528.
17. Urwyler, S.; Markstein, R. *J. Neurochem.* **1986**, *46*, 1058.
18. Matzen, L.; van Amsterdam, C.; Rautenberg, W.; Greiner, H. E.; Harting, J.; Seyfried, C. A.; Böttcher, H. *J. Med. Chem.* **2000**, *43*, 1149.
19. Functional in vitro assays for inhibition of 5-HT stimulated [^{32}S]GTPγS accumulation in CHO cells transfected with h5-HT_{1A} receptor: adapted from (a) Newman-Tancredi, A.; Conte, C.; Chaput, C.; Spedding, M.; Millan, M. J. *Br. J. Pharmacol.* **1997**, *120*, 737; (b) Newman-Tancredi, A.; Chaput, C.; Verrièle, L.; Millan, M. J. *Eur. J. Pharmacol.* **1996**, *307*, 107.
20. Wong, D. T.; Bymaster, F. P.; Mayle, D. A.; Reid, L. R.; Krushinski, J. H.; Robertson, D. W. *Neuropsychopharmacol.* **1993**, *8*, 22.
21. Fuller, R. W.; Snoddy, H. D.; Snoddy, A. M.; Hemrick, S. K.; Wong, D. T.; Molloy, B. B. *J. Pharmacol. Exp. Ther.* **1980**, *212*, 115.
22. (a) Anderson, J.; DiMicco, J. *Life Sci.* **1992**, *51*, 623; (b) Di Chiara, G. *Trends Pharmacol. Sci.* **1990**, *11*, 116.
23. De Vry, J.; Benz, U.; Schreiber, R.; Traber, J. *Eur. J. Pharmacol.* **1993**, *249*, 331.
24. Crees, I.; Schneider, R.; Snyder, S. H. *Eur. J. Pharmacol.* **1977**, *46*, 377.
25. Seyfried, C. A.; Greiner, H. E.; Haase, A. F. *Eur. J. Pharmacol.* **1989**, *160*, 31.
26. Glowinski, J.; Iversen, L. L. *J. Neurochem.* **1966**, *13*, 655.
27. Newman-Tancredi, A.; Chaput, C.; Verrièle, L.; Millan, M. J. *Eur. J. Pharmacol.* **1996**, *307*, 107.