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Original article

Pyrrolo[3,4-*c*]-β-carboline-diones as a novel class of inhibitors of the platelet-derived growth factor receptor kinase

Steffen Teller[a], Stella Eluwa[b], Markus Koller[b], Andrea Uecker[a], Thomas Beckers[c], Silke Baasner[c], Frank-D. Böhmer[a]*, Siavosh Mahboobi[b]**

[a]Research Unit Molecular Cell Biology, Medical Faculty, Friedrich Schiller University, D-07747 Jena, Germany
[b]Faculty of Chemistry and Pharmacy, Institute of Pharmacy, University Regensburg, D-93040 Regensburg, Germany
[c]ASTA Medica AG, D-60314 Frankfurt/Main, Germany

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Abstract – Members of the structurally diverse family of β-carbolines have previously been shown to exhibit a wide range of biological activities. A novel synthetic strategy for generation of β-carbolines was developed, allowing imido-β-carbolines to be created in three steps from known compounds. The compounds were screened for inhibition of platelet-derived growth factor (PDGF)-stimulated tyrosine phosphorylation in Swiss 3T3 fibroblasts. A number of the newly synthesized β-carbolines with moderate to potent inhibitory activity were revealed. The most active derivative, 2,3-dihydro-8,9-dimethoxy-5-(2-methylphenyl)-1*H*,6*H*-pyrrolo[3,4-*c*]pyrido[3,4-*b*]indole-1,3-dione **2ee**, inhibited purified PDGF receptor kinase and PDGF-receptor autophosphorylation in intact cells with IC_{50} values of 0.4 and 2.6 µM, respectively. Dione **2ee** also inhibited PDGF-stimulated DNA synthesis in Swiss 3T3 fibroblasts with an IC_{50} of 3.2 µM. The compound had no effect on Src or epidermal growth factor (EGF) receptor kinase activity and a six–seven-fold higher IC_{50} for inhibition of basic fibroblast growth factor (bFGF)-stimulated tyrosine phosphorylation or Kit/stem cell factor (SCF) receptor autophosphorylation, indicating a reasonable extent of kinase specificity. Thus, β-carbolines present a new lead of tyrosine kinase inhibitors with the capacity to selectively interfere with PDGF receptor signal transduction and PDGF-dependent cell growth. © 2000 Éditions scientifiques et médicales Elsevier SAS

β-carbolines / PDGF receptor / tyrosine kinase / signal transduction

1. Introduction

Platelet-derived growth factors (PDGFs) comprise a family of dimeric proteins with potent effects on proliferation and migration of mesenchymal cells such as fibroblasts and vascular smooth muscle cells. They exert their cellular actions by activating their cognate class III receptor tyrosine kinases, the PDGFα or PDGFβ receptors (for reviews see [1–3]). Aberrant activation of PDGF receptors has been implicated in various disease states. A well-investigated example is the autocrine activation of resident PDGF receptors by aberrantly expressed PDGF in human glioma cells [4–6]. Also, evidence for activation of PDGF receptors in situ in human meningioma was recently obtained [7]. An example of ligand-independent activation of PDGFβ-receptor in a human tumour is the TEL–PDGFR fusion protein, detected in human chronic myelomonocytic leukaemia [8, 9]. In other forms of human cancer, tumour-derived PDGF may be involved in stroma cell stimulation and thereby indirectly drive tumour growth [10]. A role of PDGF for the development of atherosclerotic lesions and restenosis following coronary balloon angioplasty and also for the development of several forms of fibrosis has been well documented [11–14]. Thus, inhibitors of PDGF receptor signalling activity are likely to be valuable for the treatment of these

* Correspondence regarding functional aspects
** Correspondence regarding chemical aspects and reprints: siavosh.mahboobi@chemie.uni-regensburg.de
Dedicated to Professor W. Schunack on the occasion of his 65th birthday.
Abbreviations: bFGF: basic fibroblast growth factor; DMSO: dimethylsulfoxide; EGF: epidermal growth factor; FCS: foetal calf serum; FRS2: FGF receptor substrate 2; c-KIT: stem cell factor; PDGF: platelet-derived growth factor; PMSF: phenylmethylsulfonyl fluoride; SCF: stem cell factor; TFA: trifluoroacetic acid; TMS: tetramethylsilane; THF: tetrahydrofurane; XTT: 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide.

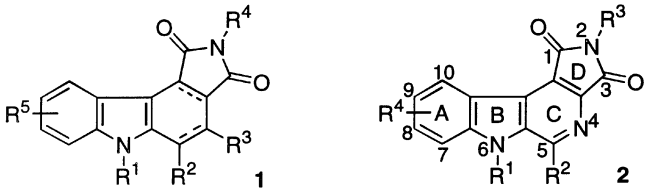

Figure 1. Imidocarbazoles **1** and imido-β-carbolines **2**.

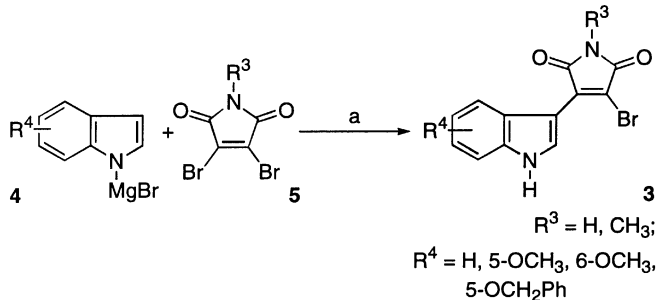

Figure 2. Synthesis of 3-bromo-4-(3-indolyl)pyrroline-2,5-diones **3**. Key: (a) toluene/THF/room temperature to reflux.

pathological conditions. Several classes of direct inhibitors of the PDGF receptor tyrosine kinase activity have been described, including quinoxalines [15–17], phenylaminopyrimidines [18], quinolines [19–22], pyridopyrimidines [23], phenylbenzimidazoles [24] and leflunomide [25]. They have been shown to suppress disease-relevant PDGFR signalling in experimental models. Given the potential medical importance of blockade of this signalling system, the development of novel leads for PDGF receptor blockers is highly warranted.

Alkaloids with a β-carboline nucleus possess widespread and potent biological activities [26–30]. The reported effects of this class of compounds comprise antineoplastic (tubulin binding) [28, 29, 31], anticonvulsive, hypnotic and anxiolytic (benzodiazepine receptor ligands) [26–28, 32, 33], antiviral [30], antimicrobial [29] as well as topoisomerase-II inhibition [34] and inhibition of cGMP-dependent processes [35]. On the other hand, the synthesis and evaluation of imidocarbazole derivatives related to structure **1** [36] have recently received much attention (*figure 1*). Compounds of this type act inter alia as inhibitors of protein kinase C and different protein tyrosine kinases [37–39].

Therefore, imido-β-carbolines **2** (*figure 1*) which combine structural features of both classes of compounds were synthesized and screened as effectors of PDGF receptor tyrosine kinase activity in intact cells. Several compounds of this class were found to exert inhibitory activity. Direct inhibition was demonstrated at the level of purified kinase and extended to inhibition of PDGF-stimulated cell growth. Further characterization revealed structure–activity relationships and defined selectivity in this novel group of PDGF receptor blockers.

2. Synthesis of pyrrolo[3,4-*c*]-β-carboline-diones (2)

Though there are several synthetic approaches to β-carbolines [27, 40], none of them are applicable to the preparation of the desired products of type **2**. One reported synthesis of compounds of structure **2** would lead to non-removable groups R^1 or R^3 [33]. However, in spite of several attempts, we were unable to apply these methods even to synthesis of the originally described compounds [40].

Starting materials for our β-carboline syntheses are the well-known 3-bromo-4-(3-indolyl)pyrroline-2,5-diones **3** [41, 42], which can be obtained easily by quenching excess indolylmagnesium bromides **4** with N-unsubstituted or N-substituted 3,4-dibromopyrroline-2,5-dione **5** in toluene/THF (*figure 2*).

In order to introduce the pyridine nitrogen of the target β-carbolines, we chose the azido group as a precursor of the activated imino group. After several unsuccessful attempts with several dipolar aprotic solvents, sodium azide in DMSO at ambient temperature yielded **6** as a precipitate on addition of H_2O. Due to their apparent instability (every attempt to obtain an analytically pure sample led to decomposition), the moist 3-azido-4-(3-indolyl)-pyrroline-2,5-diones **6** were dissolved in THF immediately without any purification and, after drying over Na_2SO_4, tri-*n*-butylphosphine was added at 0 °C. When evolution of nitrogen ceased, removal of the solvent afforded the 3-tributylphosphanimino-4-(3-indolyl)-pyrroline-2,5-diones **7**, which in principle should be more reactive than the triphenylphosphanimines which can only be cleaved by strong acids [28, 29]. However, compounds **7** are reactive enough to undergo a Staudinger reaction [30, 43] with benzaldehydes or phenylacetaldehyde R^2CHO in *o*-xylene under catalysis of Pd/C to furnish the desired pyrrolo[3,4-*c*]-β-carbolin-1,3-diones **2a**–**gg** (*figure 3*). The yields are low (relative to **3**, because compounds **7** as well as **6** did not provide satisfactory analytical data due to instability) and strongly depend on the nature and position of the substituents on the R^2 phenyl ring. A further reason is the formation of the 3-amino-4-(3-indolyl)pyrroline-2,5-diones **8** with yields of 10–15%, which are easily separable from **2** by column chromatography. Compound **2ee** could only be

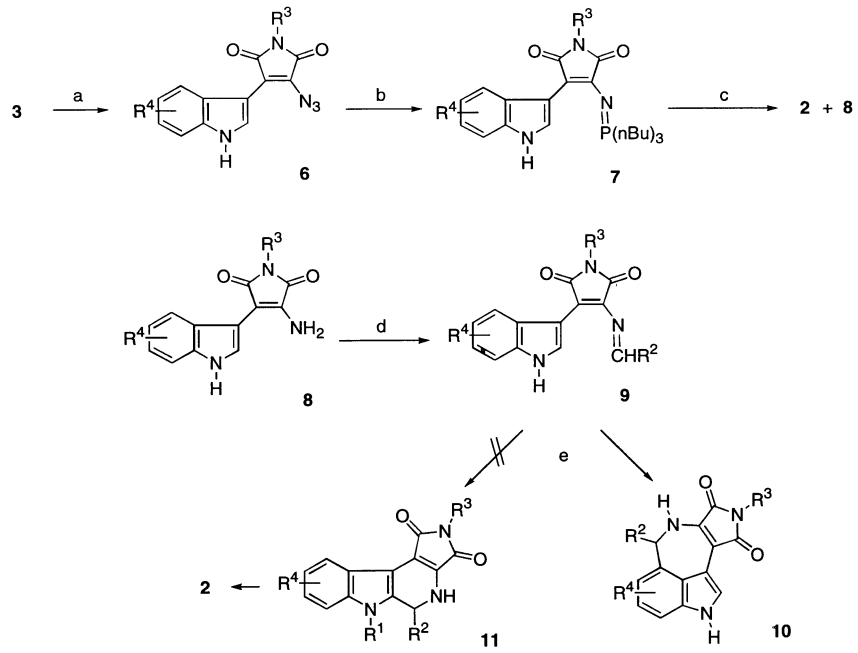

Figure 3. Synthesis of pyrrolo[3,4-*c*]-β-carboline-1,3-diones **2** and pyrrolo[3',4':2,3]-azepino[4,5,6-*cd*]indole-8,10-diones **10**. Keys: (a) NaN_3/DMSO/room temperature; (b) $(n\text{Bu})_3$P/THF/room temperature; (c) R^2CHO/Pd(C)/*o*-xylene/reflux; (d) R^2CHO/dichloromethane/methanol/room temperature; (e) TFA/dichloromethane/–78 °C.

obtained in satisfactory quantities by a modified procedure using higher temperature and shorter reaction time, but this method was not able to raise the yields of other β-carbolines (e.g. **2v** and **2z**). Condensation of **8** with various aldehydes gave the corresponding imines **9**. Under Pictet-Spengler conditions the latter did not cyclize to pyrrolo-β-carbolines **11**, which we intended to dehydrogenate to compounds **2**. Instead, pyrrolo[3',4':2,3]azepino[4,5,6-*cd*]indole-8,10-diones **10** are obtained [44].

The 9-benzyloxy derivatives **2ff** and **2gg** could be transformed to the 9-hydroxy compounds **2hh** and **2ii** by heating under reflux with ammonium formate and Pd/C in ethanol/THF [45] (*figure 4*). Compounds **2** are fluorescent yellow solids crystallizing from ethyl acetate. The various compounds prepared are shown in *table I*.

3. Characterization of pyrrolo[3,4-*c*]-β-carboline-diones as inhibitors of the PDGF receptor tyrosine kinase

The newly synthesized compounds were screened for PDGF receptor antagonist activity by measuring PDGF-stimulated tyrosine phosphorylation in intact Swiss 3T3 fibroblasts as the primary assay. Among the compounds listed in *table I*, compounds **2k**, **2t**, **2w** and **2bb** displayed inhibitory activity in this test (*table I*), whereas the other compounds had little effect ($IC_{50} > 30$ µM). When the inhibition was analysed qualitatively by immunoblotting, compounds **2w** and **2t** revealed inhibition of PDGF receptor autophosphorylation (*table II*, *figure 5A* and *B*) and inhibition of other PDGF-dependent tyrosine phosphorylations in these cells, whereas **2bb** and **2k** had comparatively weak effects on PDGF receptor autophosphorylation (not shown).

These results revealed that certain β-carbolines display inhibitory activity for the PDGF receptor. Although the

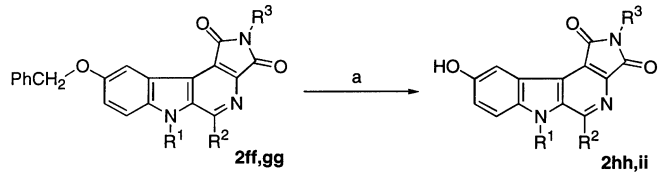

Figure 4. Synthesis of 9-hydroxy-pyrrolo[3,4-*c*]-β-carboline-1,3-diones **2hh–ii**. Key: (a) HCO_2NH_4/Pd(C)/ethanol/THF/reflux.

Table I. Structure of the β-carboline derivatives **2a–2ii** and effects on PDGF-stimulated phosphorylation in Swiss 3T3 cells (reference compound: AG 1295 [16]).

Number	R^{2a}	R^3	R^4	IC_{50}[b] (μM)	IC_{50}[c] (μM)
2a	Ph	H	H	> 30	> 30
2b	4-CN-Ph	H	H	> 30	> 30
2c	4-tert-BuO-Ph	H	H	> 30	> 30
2d	4-EtO-Ph	H	H	> 30	> 30
2e	4-MeO-Ph	H	H	> 30	> 30
2f	4-Me-Ph	H	H	> 30	> 30
2g	4-F-Ph	H	H	> 30	> 30
2h	CH_2-Ph	H	H	> 30	> 30
2i	2-OH-3-MeO-Ph	H	H	> 30	> 30
2j	2-MeO-Ph	H	H	> 30	> 30
2k	2-Me-Ph	H	H	3–10	3–10
2l	3-Me-Ph	H	H	> 30	> 30
2m	3-OH-4-MeO-Ph	H	H	> 30	> 30
2n	2,4-di-MeO-Ph	H	H	> 30	> 30
2o	3,4-di-MeO-Ph	H	H	> 30	> 30
2p	4-OH-3-MeO-Ph	H	H	> 30	> 30
2q	4-NMe_2-Ph	H	H	> 30	> 30
2r	3-NO_2-Ph	H	H	> 30	> 30
2s	3-MeO-Ph	H	H	> 30	> 30
2t	Ph	H	9-MeO	0.1–1	3–10
2u	4-MeO-Ph	H	9-MeO	> 30	> 30
2v	3-MeO-Ph	H	9-MeO	> 30	> 30
2w	2-MeO-Ph	H	9-MeO	3–10	3–10
2x	2,4-di-MeO-Ph	H	9-MeO	> 30	> 30
2y	3,4-di-MeO-Ph	H	9-MeO	> 30	> 30
2z	4-CN-Ph	H	9-MeO	> 30	> 30
2aa	Ph	Me	9-MeO	> 30	> 30
2bb	Ph	H	8,9-di-MeO	0.1–1	1–10[d]
2cc	Ph	Me	H	> 30	> 30
2dd	2-Me-Ph	H	9-MeO	–	1–10[d]
2ee	2-Me-Ph	H	8,9-di-MeO	–	1–3
2ff	2-MeO-Ph	H	9-$PhCH_2O$	–	> 30
2gg	2-Me-Ph	H	9-$PhCH_2O$	–	> 30
2hh	2-MeO-Ph	H	9-OH	–	10–30
2ii	2-Me-Ph	H	9-OH	–	> 30
AG 1295	–	–	–	–	0.5–1[e]

[a] R^1 = H; [b]PDGF-stimulated tyrosine phosphorylation in total cell lysates; [c]autophosphorylation of PDGF receptor evaluated by immunoblotting, ranges for the IC_{50} were defined based on at least three independent experiments; [d]very shallow dose–response curve; [e]taken from [16].

number of active compounds is limited, preliminary conclusions for SAR can be made: methoxy-substitution in R^4 at C-9 or at C-8 and C-9, respectively, seems favourable (compounds **2t** and **2bb** versus **2a**). Substitution of the imido NH affects activity negatively (compound **2aa** versus compound **2t**). Generally, substitution of the R^2 phenyl ring had negative effects, except in the R^2 *o*-position, where Me substitution is beneficial (compound **2k** versus **2a)**. On the basis of these considerations, further compounds were synthesized. To explore the effects of alternative substitutions at C-9 in R^4, OH- or benzyloxy- groups were introduced. This generally gave inactive or only weakly active compounds, regardless of the substitutions in R^2 (*table I*, compounds, **2ff–2ii**). In particular, the OH derivatives **2hh** and **2ii** or the benzyloxy- derivatives **2ff** and **2gg** had little or no detectable activity compared to their 9-methoxy-substituted counterparts **2w** or **2dd**. However, the combination of the favourable structural features identified in the first series of compounds, namely Me-substitution in *o*-position of ring R^2 and MeO-substitution in C-9 (**2dd**) or C-8 and C-9 (**2ee**) yielded active compounds, with **2ee**

Table II. Effect of the β-carbolines **2t**, **2w** and **2ee** on the phosphorylation of the PDGF receptor in intact cells and in vitro and on other tyrosine kinases.

Test	IC_{50} (μM)		
	2t	**2w**	**2ee**
PDGF receptor phosphorylation (Swiss 3T3 cells)[a]	7.4	4.8	2.6
PDGF receptor phosphorylation (Swiss 3T3 membranes)[a]	1.7	1.9	n.d.
PDGF receptor phosphorylation (purified human PDGFβ-receptor)	0.4	0.2	0.4
EGF receptor phosphorylation (A 431 cells)	> 30	> 30	> 30
Src-dependent tyrosine phosphorylation in *src*-transformed NIH3T3 cells	> 30	> 30	> 30
p60c-src kinase activity in vitro	> 30	11.8	> 30
FGF receptor-dependent tyrosine phosphorylation (FRS-2; Swiss 3T3 cells)	15.4	> 30	17.3
c-Kit/SCF receptor phosphorylation (CHRF cells)	> 30	> 30	16.4

[a] Quantification of IC_{50} values was based on the intensity of the signal for autophosphorylated PDGF receptor. Titration was done using four–eight inhibitor concentrations within a range of two orders of magnitude which were selected on the basis of preliminary experiments. Results of triplicate determinations were subjected to curve fitting.

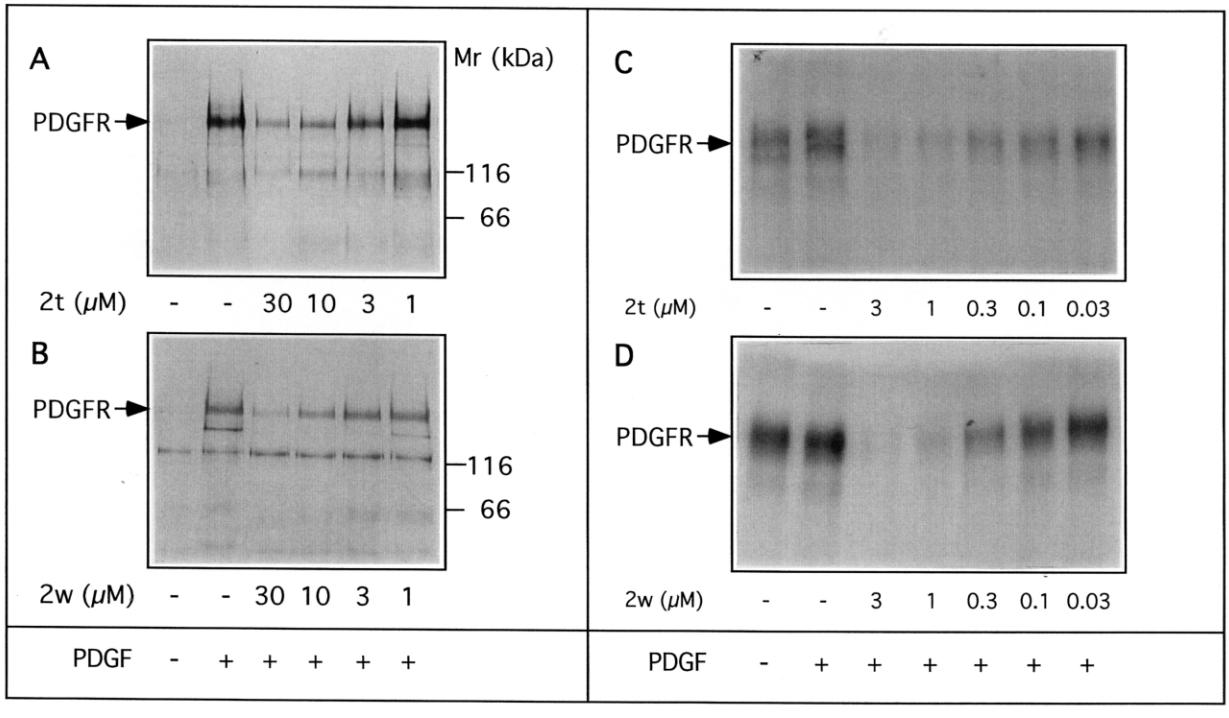

Figure 5. Inhibition of PDGF receptor (PDGFR) autophosphorylation in intact Swiss 3T3 cells and in vitro by β-carbolines **2t** and **2w**. Swiss cells were cultured in 24-well plates to confluence and made quiescent. Cells were then incubated with **2t** (**A**) or **2w** (**B**) for 2 h in serum free medium in the indicated concentrations, stimulated with PDGF (100 ng/mL) for 10 min and extracted. The extracts were subjected to SDS-PAGE and immunoblotting with anti-phosphotyrosine antibodies. Purified PDGFβ-receptor samples were pre-incubated with compound **2t** (**C**) or **2w** (**D**) for 15 min on ice. Then, PDGF was added for 20 min and receptor autophosphorylation was allowed to proceed for 3 min in the presence of 2 μM [γ-^{32}P]ATP. The reaction was quenched with SDS-PAGE sample buffer and the samples were subjected to electrophoresis and autoradiography.

Table III. Effect of the β-carbolines **2t** and **2w** and **2ee** on DNA synthesis in Swiss 3T3 cells stimulated by different agents.

DNA synthesis (Swiss 3T3 cells)[a]	IC_{50} (μM)		
	2t	**2w**	**2ee**
PDGF-stimulated	5.4	3.2	3.2
FGF-stimulated	2.7	21.4	11.4
EGF/insulin-stimulated	8.9	> 30	> 30
10 % FCS-stimulated	5.7	20	4.5

[a] Quiescent Swiss 3T3 cells were stimulated with the different growth-promoting agents in the absence or presence of inhibitors. Four–five inhibitor concentrations were used in the range of activity defined by preliminary experiments. Results are means of three independent experiments run with duplicate determinations. IC_{50} values were determined by curve-fitting.

being the most active compound identified among β-carboline structures so far (*tables II* and *III*, *figure 6A*). Compound **2dd** displayed very shallow dose–response curves (not shown), possibly because of low solubility and was therefore precluded from further analysis.

Compounds **2t**, **2w** and **2ee** were then subjected to a more detailed characterization. All three compounds inhibited PDGF receptor autophosphorylation in isolated Swiss 3T3 cell membranes (not shown) and PDGF receptor autokinase reaction with a purified PDGF receptor preparation (*figure 5C* and *D* and *figure 6B*). Thus, the inhibition of PDGF receptor phosphorylation observed in intact cells is based on a direct kinase inhibition. Inhibition of PDGF receptor autophosphorylation by **2ee** occurred with similar efficacy in the murine Swiss 3T3 cells (*figure 6A*) and in porcine aortic endothelial (PAE) cells, stably transfected with the human PDGFβ receptor (*figure 6C*).

We further evaluated the specificity of kinase inhibition by the active β-carboline derivatives. For comparison we chose, on the one hand, the epidermal growth factor (EGF) receptor tyrosine kinase and the cytoplasmic tyrosine kinase pp60src (Src kinase). These two enzymes are only very distantly related to PDGF receptors at the level of kinase domain sequence homology. They are widely expressed and represent tyrosine kinase families implicated in various types of cancer. For a more rigorous selectivity judgement we employed the fibroblast growth factor (FGF) receptor tyrosine kinase and the stem cell factor (SCF) receptor tyrosine kinase, also known as c-Kit. FGF receptors and PDGF receptors are moderately related and frequently expressed in the same type of cells, while the c-Kit/SCF receptor is a very close relative of the PDGF receptor. EGF receptor kinase activity was measured as receptor autophosphorylation in intact A431 cells, pp60src kinase activity was assayed with recombinant kinase in vitro or as pp60src-dependent tyrosine phosphorylation in *src*-transformed NIH3T3 cells, FGF receptor activity was measured as bFGF-stimulated phosphorylation of the 92 kDa FGF receptor substrate 2 (FRS2) in Swiss 3T3 cells and c-Kit/SCF receptor kinase activity was measured as SCF-stimulated autophosphorylation of c-Kit in CHRF cells (*table II*, *figure 7*), a haematopoietic cell line with high c-Kit/SCF receptor levels.

Compound **2t** did not show activity in any of these assays, whereas **2w** displayed some activity on pp60src kinase activity in vitro and moderately inhibited Src-dependent phosphorylation in intact cells (*table II*, *figure 6*).

Compound **2ee** partially inhibited bFGF-stimulated phosphorylation of FRS2 in Swiss 3T3 cells and Kit/SCF receptor phosphorylation at 30 μM but had no effect on these kinases at lower concentrations or on EGF receptor kinase or Src kinase (*table II*, *figure7*).

We further tested whether the compounds would affect PDGF-dependent cell growth. We observed inhibition of PDGF-dependent DNA synthesis (*table III*) by **2t**, **2w** and **2ee** in Swiss 3T3 cells, with **2ee** having the highest potency. Interestingly, **2t** exhibited little selectivity with respect to DNA synthesis stimulated by different growth promoting agents (*table III*), whereas **2w** had a quite selective effect on PDGF-stimulated growth. Compound **2ee** displayed intermediate selectivity, with similar inhibition of DNA synthesis stimulated by PDGF or FCS, moderate inhibition of FGF-stimulated DNA synthesis and no effect on DNA synthesis stimulated by the combination of EGF and insulin. Compounds **2t** and **2w** were also tested for their capacity to inhibit proliferation of *sis*-transformed NIH3T3 fibroblasts. Both compounds inhibited colony formation of *sis*-transformed NIH3T3 fibroblasts but not of *src*- or BxB *raf*-transformed NIH3T3 fibroblasts in semi-solid agar (*table IV*).

4. Discussion

A synthetic approach for the generation of imido-β-carbolines **2** via phosphanimines **7** is presented. Members

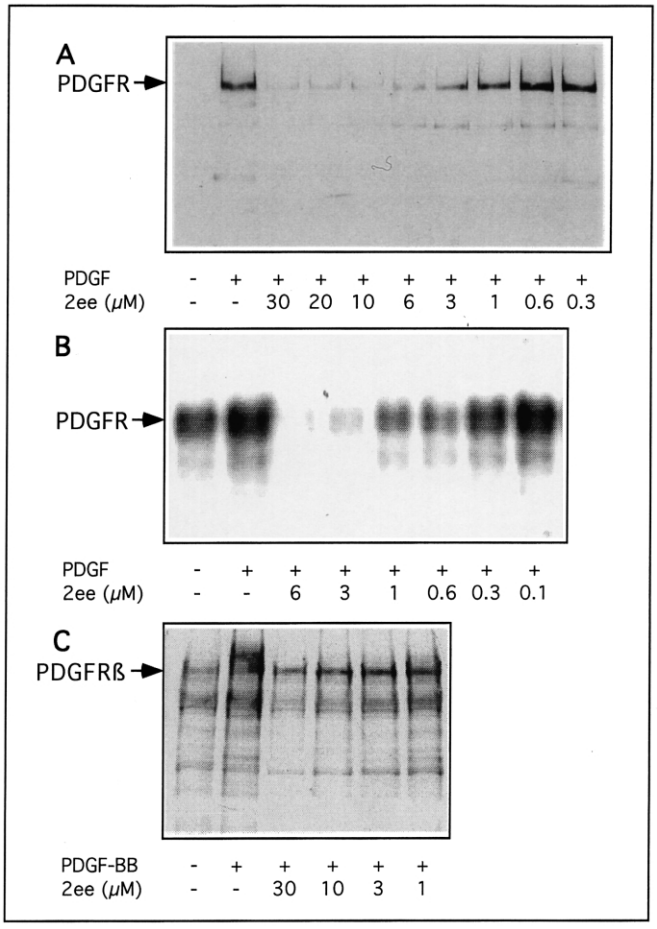

Figure 6. Inhibition of PDGF receptor autophosphorylation in intact cells and in vitro by β-carboline **2ee**. Inhibition of PDGF receptor (PDGFR) autophosphorylation in intact Swiss 3T3 cells (**A**) and with purified PDGFβ-receptor (**B**) by **2ee** was measured as described in the legend to *figure 5*. PAE cells stably expressing human PDGFβ-receptor were cultured in 6-well plates to confluence and made quiescent. Cells were then incubated with **2ee** in the indicated concentrations for 2 h in serum free medium, stimulated with PDGF (50 ng/mL) for 10 min and extracted. The extracts were subjected to an affinity precipitation with wheat germ agglutinin beads, SDS-PAGE and immunoblotting with anti-phosphotyrosine antibodies (**C**).

of this structural family exhibit inhibition of PDGF-receptor autophosphorylation in vitro and in intact cells and block PDGF-dependent cell growth. The most active compounds **2t**, **2w** and **2ee**, which are structurally rather similar, exhibit little differences in their potency towards the purified PDGFβ-receptor kinase (*table II*). In contrast, **2ee** is more potent than **2w** and even more than **2t** when cellular PDGF receptor phosphorylation assays are compared (*table II*). Under these conditions, cellular uptake behaviour and the capacity of the compounds to compete with high intracellular ATP concentrations contribute to activity, which may possibly explain the differential result. Even more obvious are the effects of the structural differences of the three compounds on selectivity in DNA synthesis assays (*table III*). While **2t** inhibits DNA synthesis stimulated with all agents tested, **2w** preferentially inhibits PDGF-stimulated DNA synthesis and **2ee** exhibits an intermediate selectivity. The low selectivity of **2t** in these assays does not match the results obtained when testing susceptibility of other tyrosine kinases. Thus, **2t** may have other cellular targets which are common to the pathways of mitogenic stimulation for the investigated mitogenic agents. Compound **2w** is rather selective in all assays including a lack of inhibition of other tested tyrosine kinases, in particular c-Kit/SCF receptor. Thus, β-carbolines may be superior to previously described compounds in that they provide selectivity of inhibition distinguishing even between these two closely related receptor tyrosine kinases.

5. Experimental protocols

Uncorrected melting points were measured on a Reichert 300419 hot-stage microscope. R_f values were determined by TLC on Merck Art. 5554 silica 60 F_{254} coated aluminium sheets with 254 nm UV detection. Elemental analyses were performed by Analytical Lab. Univ. Regensburg, and all were within ± 0.4% of the theoretical values. FT-IR spectra were recorded in KBr pellets on a Nicolet 510 spectrometer. ^{1}H-NMR were recorded in DMSO-d_6 at 250 MHz on a Bruker 250 spectrometer. Chemical shifts were expressed in parts per million downfield relative to TMS as an internal standard. Electron impact mass spectra were recorded at 70 eV on a Varian MAT 112S spectrometer. All reactions were carried out under nitrogen that had been dried over self-indicating silica gel, conc. H_2SO_4 and KOH. Anhydrous ethanol was prepared using sodium and diethyl phthalate. Other solvents were used in commercially available quality without further purification.

5.1. General procedure for compounds **2a**–**2dd**, **2ff** *and* **2gg**

A solution of the corresponding 3-bromo-4-(3-indolyl)pyrroline-2,5-dione **3** [35, 36] (17.18 mmol) and NaN_3 (1.23 g, 18.89 mmol) in anhydrous DMSO (50 mL) is stirred for 1 h at ambient temperature. After addition of ice/H_2O (500 mL), the resulting red precipitate is filtered and rinsed with H_2O. The solid **6** is dissolved in THF

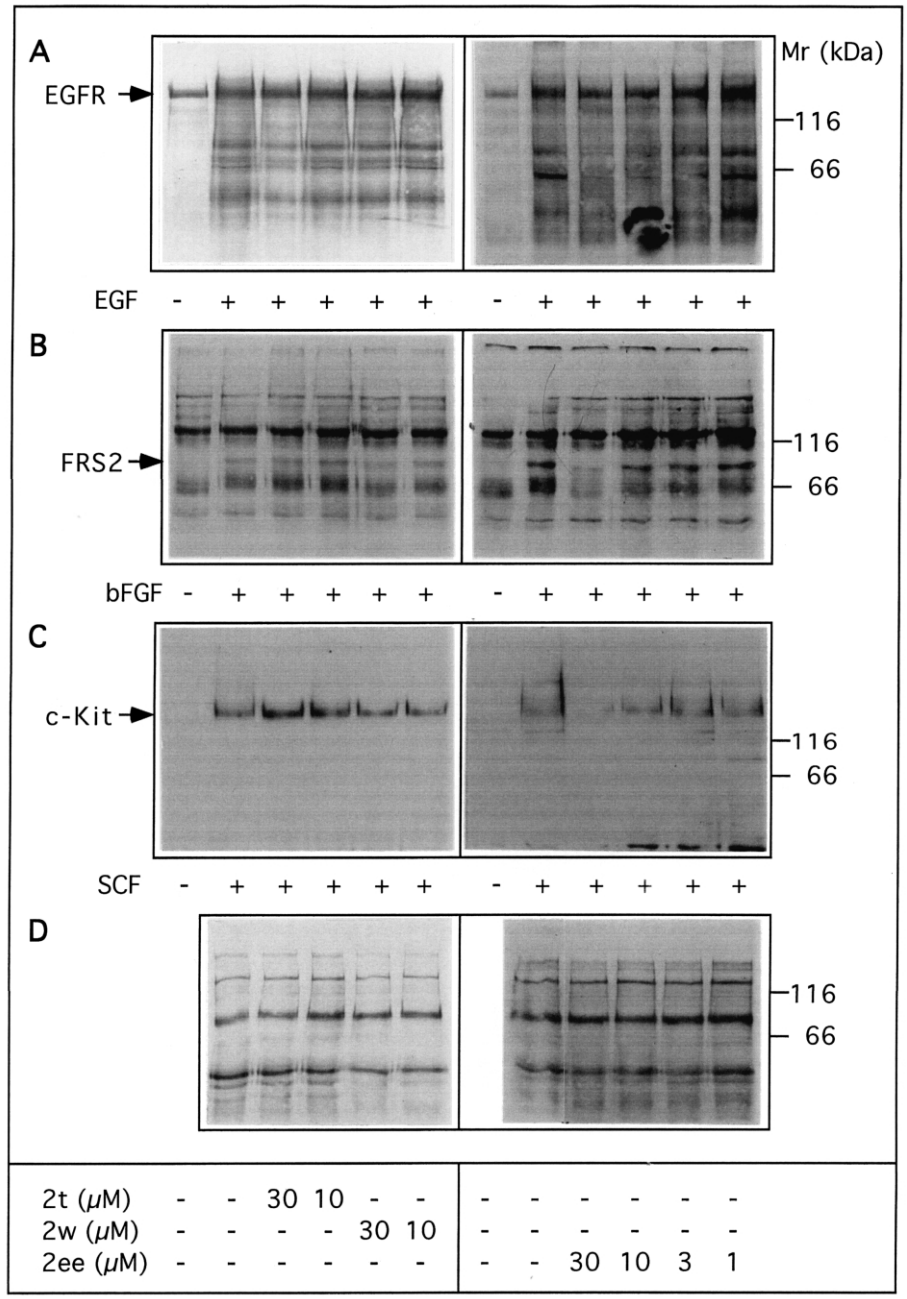

Figure 7. Effects of β-carbolines **2t**, **2w** and **2ee** on other tyrosine kinases. Effects on epidermal growth factor receptor (EGFR) (**A**), fibroblast growth factor (FGF) receptor (**B**) and c-Kit/stem cell factor (SCF) receptor (**C**) were measured in A431 cells, Swiss 3T3 cells and CHRF cells, respectively. Cells were treated with the inhibitors in the indicated concentrations as described in the legend to *figure 1* and were left unstimulated (–) or were stimulated (+) with 100 ng/mL EGF for 5 min, 100 ng/mL bFGF for 10 min or 40 ng/mL SCF for 5 min, respectively. Ligand-dependent autophosphorylation of EGFR was directly detectable in cell lysates. Autophosphorylation of c-Kit/SCF receptor was analysed in specific immunoprecipitates. Activity of FGF receptor was judged from phosphorylation of the 92kDa FGF receptor substrate 2 (FRS2), which was detectable in cell lysates. NIH3T3 cells transformed with a constitutively active variant of the *src* oncogene exhibit strongly elevated tyrosine phosphorylation, detectable in lysates of unstimulated cells. This was taken as a measure for effects on Src-kinase activity (**D**).

Table IV. Effect of the β-carbolines **2t** and **2w** on the soft agar colony formation and proliferation of cell lines transformed with different oncogenes.

Assay	Cell line	IC_{50} (μM)	
		2t	**2w**
Colony	*sis*-transformed NIH3T3	8.3	9
Formation	*src*-transformed NIH3T3	> 30	> 30
	BxB *raf*-transformed NIH3T3	> 30	> 30
Proliferation	*sis*-transformed NIH3T3	3.7	6.6

(100 mL) and dried over Na_2SO_4 for 1 h. The latter is filtered off, and tri-*n*-butylphosphine (4.30 mL, 17.18 mmol) is added to the filtrate, while cooling in an ice bath. After 1 h of stirring at ambient temperature the solvent is evaporated in vacuo. The resulting crude product **7** is dissolved in *o*-xylene (250 mL), the corresponding aldehyde (17.18 mmol) and 10% Pd/C (1 g) are added and the reaction mixture is refluxed for 48 h. The catalyst is removed by filtration through Celite, the solvent is removed in vacuo, and the oily residue is purified by flash column chromatography (column 5 cm × 10 cm, silica gel, ethyl acetate/hexane 1:1) to give yellow crystals (yields stated relative to **3**). Analytical samples were obtained from ethyl acetate.

5.1.1. 2,3-Dihydro-5-phenyl-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2a**

Yield 1.1 g (20%): m.p. > 350 °C; R_f 0.48 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 470, 3 380 (NH); 3 180–3 050 (CH); 1 765, 1 720 (C=O); 1 625, 1 560, 1 495 (C=C); ^{1}H-NMR: δ 7.42–7.46 (m; 1 H aromat., 7–H), 7.64–7.78 (m; 5 H phenyl), 8.01–8.07 (m; 2 H aromat., 8-H and 9-H), 8.79 (m; 1 H aromat., 10-H), 11.44 (br s; 1 H (exch.), NH imid), 12.27 (br s; 1 H (exch.), NH indole); MS: m/z (%): 313 (100) [$M^{+\cdot}$], 77 (3). Anal. ($C_{19}H_{11}N_3O_2$) C, H, N.

5.1.2. 5-(4-Cyanophenyl)-2,3-dihydro-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2b**

Yield 0.40 g (7%): m.p. > 350 °C; R_f 0.47 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 385, 3 230 (NH); 3 090–3 025 (CH); 2 245 (C≡N); 1 775, 1 740 (C=O); 1 625, 1 585, 1 495, 1 450 (C=C); ^{1}H-NMR: δ 7.41–8.80 (m; 4 H aromat.), 8.15; 8.23 (AA'BB', J_{AB} = 8.4 Hz; 4 H phenyl), 11.54 (br s; 1 H (exch.), NH imid), 12.33 (br s; 1 H (exch.), NH indole); MS: m/z (%): 338 (100) [$M^{+\cdot}$]. Anal. ($C_{20}H_{10}N_4O_2$) C, H, N.

5.1.3. 5-(4-tert-Butoxyphenyl)-2,3-dihydro-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2c**

Yield 1.79 g (27%): m.p. 247 °C; R_f 0.50 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 450 (NH); 3 050–2 960 (CH); 1 735, 1 715 (C=O); 1 610, 1 580, 1 565, 1 450 (C=C); ^{1}H-NMR: δ 0.88 (s; 9 H, $C(CH_3)_3$), 7.22; 8.03 (AA'BB', J_{AB} = 8.7 Hz; 4 H phenyl), 7.38–8.80 (m; 4 H aromat.), 11.35 (br s; 1 H (exch.), NH imid), 12.12 (br s; 1 H (exch.), NH indole); MS: m/z (%): 385 (0.11) [$M^{+\cdot}$], 357 (100), 329 (37). Anal. ($C_{23}H_{19}N_3O_3$) C, H, N.

5.1.4. 5-(4-Ethoxyphenyl)-2,3-dihydro-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2d**

Yield 1.76 g (29%): m.p. 244 °C; R_f 0.43 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 370 (NH); 3 070–2 975 (CH); 1 735, 1 715 (C=O); 1 625, 1 605, 1 580, 1 450 (C=C); ^{1}H-NMR: δ 0.88 (t, J = 7.0 Hz; 3 H, CH_3CH_2), 4.19 (q, J = 7.0 Hz; 2 H, CH_3CH_2), 7.22; 8.02 (AA'BB', J_{AB} = 8.8 Hz; 4 H phenyl), 7.38–8.79 (m; 4 H aromat.), 11.38 (br s; 1 H (exch.), NH imid), 12.20 (br s; 1 H (exch.), NH indole); MS: m/z (%): 357 (80) [$M^{+\cdot}$], 329 (100). Anal. ($C_{21}H_{15}N_3O_3$) C, H, N.

5.1.5. 2,3-Dihydro-5-(4-methoxyphenyl)-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2e**

Yield 0.64 g (11%): m.p. 289 °C; R_f 0.40 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 200 (NH); 3 050–2 960 (CH); 1 765, 1 725 (C=O); 1 630, 1 610, 1 580, 1 455 (C=C); ^{1}H-NMR: δ 3.91 (s; 3 H, OCH_3), 7.24; 8.08 (AA'BB', J_{AB} = 8.8 Hz; 4 H phenyl), 7.38–8.79 (m; 4 H aromat.), 11.40 (br s; 1 H (exch.), NH imid), 12.11 (br s; 1 H (exch.), NH indole); MS: m/z (%): 343 (100) [$M^{+\cdot}$]. Anal. ($C_{20}H_{13}N_3O_3$) C, H, N.

5.1.6. 2,3-Dihydro-5-(4-methylphenyl)-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2f**

Yield 0.38 g (7%): m.p. > 350 °C; R_f 0.51 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 330 (NH); 3 035–2 990 (CH); 1 775, 1 725 (C=O); 1 630, 1 585, 1 505, 1 465 (C=C); ^{1}H-NMR: δ 2.51 (s; 3 H, CH_3), 7.50; 7.95 (AA'BB', J_{AB} = 8.1 Hz; 4 H phenyl), 7.38–8.79 (m; 4 H aromat.), 11.40 (br s; 1 H (exch.), NH imid), 12.19 (br s; 1 H (exch.), NH indole); MS: m/z (%): 327 (100) [$M^{+\cdot}$]. Anal. ($C_{20}H_{13}N_3O_2$) C, H, N.

5.1.7. 5-(4-Fluorophenyl)-2,3-dihydro-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2g**

Yield 10 mg (0.2%): m.p. 271 °C; R_f 0.45 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 365, 3 210 (NH); 3 170–3 020 (CH); 1 765, 1 720 (C=O); 1 640, 1 610, 1 535, 1 450 (C=C); ^{1}H-NMR: δ 7.43–8.89 (m; 8 H aromat.), 11.43 (br s; 1 H (exch.), NH imid), 12.25 (br s; 1 H (exch.), NH indole); MS: m/z (%): 331 (100) [$M^{+\cdot}$]. Anal. ($C_{19}H_{10}N_3O_2F$) C, H, N.

5.1.8. 5-Benzyl-2,3-dihydro-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2h**

Yield 0.10 g (2%): m.p. 306 °C; R_f 0.51 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 450 (NH); 3 190–2 960 (CH); 1 760, 1 720 (C=O); 1 630, 1 580, 1 495, 1 460 (C=C); ^{1}H-NMR: δ 4.63 (s; 2 H, CH_2), 7.16–8.73 (m; 9 H aromat.), 11.31 (br s; 1 H (exch.), NH imid), 12.55 (br s; 1 H (exch.), NH indole); MS: m/z (%): 327 (100) [$M^{+\cdot}$], 326 (81) [$M - {}^{\cdot}H]^+$. Anal. ($C_{20}H_{13}N_3O_2$) C, H, N.

5.1.9. 2,3-Dihydro-5-(2-hydroxy-3-methoxyphenyl)-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]-indole-1,3-dione **2i**

Yield 1.20 g (19%): m.p. 335 °C; R_f 0.30 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 340 (NH, OH); 3 050–2 960 (CH); 1 765, 1 720 (C=O); 1 625, 1 580, 1 490, 1 475 (C=C); ^{1}H-NMR: δ 3.92 (s; 3 H, OCH_3), 6.98–8.78 (m; 7 H aromat.), 9.53 (br s; 1 H (exch.), OH), 11.39 (br s; 1 H (exch.), NH imid), 11.82 (br s; 1 H (exch.), NH indole); MS: m/z (%): 359 (100) [$M^{+\cdot}$]. Anal. ($C_{20}H_{13}N_3O_4$) C, H, N.

5.1.10. 2,3-Dihydro-5-(2-methoxyphenyl)-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2j**

Yield 0.51 g (9%): m.p. 264 °C; R_f 0.46 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 335, 3 210 (NH); 3 050–2 970 (CH); 1 765, 1 720 (C=O); 1 630, 1 605, 1 580, 1 500 (C=C); ^{1}H-NMR: δ 3.78 (s; 3 H, OCH_3), 7.20–8.78 (m; 8 H aromat.), 11.38 (br s; 1 H (exch.), NH imid), 11.86 (br s; 1 H (exch.), NH indole); MS: m/z (%): 343 (100) [$M^{+\cdot}$]. Anal. ($C_{20}H_{13}N_3O_3$) C, H, N.

5.1.11. 2,3-Dihydro-5-(2-methylphenyl)-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2k**

Yield 0.41 g (7%): m.p. 314 °C; R_f 0.50 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 275 (NH); 3 070–2 950 (CH); 1 765, 1 715 (C=O); 1 625, 1 580, 1 500, 1 455 (C=C); ^{1}H-NMR: δ 2.18 (s; 3 H, CH_3), 7.39–8.79 (m; 8 H aromat.), 11.43 (br s; 1 H (exch.), NH imid), 11.98 (br s; 1 H (exch.), NH indole); MS: m/z (%): 327 (100) [$M^{+\cdot}$]. Anal. ($C_{20}H_{13}N_3O_2$) C, H, N.

5.1.12. 2,3-Dihydro-5-(3-methylphenyl)-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2l**

Yield 0.27 g (5%): m.p. 321 °C; R_f 0.52 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 400, 3 230 (NH); 3 060–2 970 (CH); 1 770, 1 715 (C=O); 1 605, 1 580, 1 570, 1 455 (C=C); ^{1}H-NMR: δ 2.52 (s; 3 H, CH_3), 7.39–8.80 (m; 8 H aromat.), 11.51 (br s; 1 H (exch.), NH imid), 12.18 (br s; 1 H (exch.), NH indole); MS: m/z (%): 327 (100) [$M^{+\cdot}$], 312 (8) [$M - {}^{\cdot}CH_3]^+$. Anal. ($C_{20}H_{13}N_3O_2$) C, H, N.

5.1.13. 2,3-Dihydro-5-(3-hydroxy-4-methoxyphenyl)-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]-indole-1,3-dione **2m**

Yield 0.10 g (2%): m.p. 329 °C; R_f 0.33 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 285 (NH, OH); 3 050–3 960 (CH); 1 765, 1 740 (C=O); 1 630, 1 580, 1 495, 1 460 (C=C); ^{1}H-NMR: δ 3.90 (s; 3 H, OCH_3), 7.18–8.79 (m; 7 H aromat.), 9.47 (br s; 1 H (exch.), OH), 11.31 (br s; 1 H (exch.), NH imid), 12.13 (br s; 1 H (exch.), NH indole); MS: m/z (%): 359 (100) [$M^{+\cdot}$]. Anal. ($C_{20}H_{13}N_3O_4$) C, H, N.

5.1.14. 5-(2,4-Dimethoxyphenyl)-2,3-dihydro-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2n**

Yield 0.43 g (7%): m.p. 308 °C; R_f 0.37 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 370, 3 205 (NH); 3 060–2 980 (CH); 1 770, 1 735 (C=O); 1 615, 1 580, 1 495, 1 450 (C=C); ^{1}H-NMR: δ 3.78 (s; 3 H, OCH_3), 3.91 (s; 3 H, OCH_3), 6.76–8.77 (m; 7 H aromat.), 11.34 (s; 1 H (exch.), NH imid), 12.13 (s; 1 H (exch.), NH indole); MS: m/z (%): 373 (100) [$M^{+\cdot}$]. Anal. ($C_{21}H_{15}N_3O_4$) C, H, N.

5.1.15. 5-(3,4-Dimethoxyphenyl)-2,3-dihydro-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2o**

Yield 0.55 g (9%): m.p. 288 °C; R_f 0.36 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 300, (NH); 3 070–2 960 (CH); 1 765, 1 735 (C=O); 1 630, 1 605, 1 520, 1 460 (C=C); ^{1}H-NMR: δ 3.91 (s; 3 H, OCH_3), 3.92 (s; 3 H, OCH_3), 7.24–8.80 (m; 7 H aromat.), 11.41 (br s; 1 H (exch.), NH imid), 12.21 (br s; 1 H (exch.), NH indole); MS: m/z (%): 373 (100) [$M^{+\cdot}$]. Anal. ($C_{21}H_{15}N_3O_4$) C, H, N.

5.1.16. 2,3-Dihydro-5-(4-hydroxy-3-methoxyphenyl)-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]-indole-1,3-dione **2p**

Yield 0.10 g (2%): m.p. 345 °C; R_f 0.29 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 415, 3 310 (NH, OH); 3 050–2 980 (CH); 1 765, 1 715, (C=O); 1 625, 1 610, 1 575, 1 520 (C=C); ^{1}H-NMR: δ 3.93 (s; 3 H, OCH_3), 7.03–7.78 (m; 7 H aromat.), 9.63 (br s; 1 H (exch.), OH), 11.32 (br s; 1 H (exch.), NH imid), 12.17 (br s; 1 H (exch.), NH indole); MS: m/z (%): 359 (100) [$M^{+\cdot}$]. Anal. ($C_{20}H_{13}N_3O_4$) C, H, N.

5.1.17. 5-(4-N,N-Dimethylaminophenyl)-2,3-dihydro-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]-indole-1,3-dione **2q**

Yield 1.08 g (18%): m.p. 184 °C; R_f 0.30 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 450 (NH); 3 050–2 960 (CH); 1 765, 1 735 (C=O); 1 610, 1 565, 1 535, 1 495 (C=C); ^{1}H-NMR: δ 3.07 (s; 6 H, $N(CH_3)_2$), 6.98; 7.99 (AA'BB', J_{AB} = 8.9 Hz; 4 H phenyl), 7.37–8.78 (m; 4 H aromat.), 11.30 (br s; 1 H (exch.), NH imid), 12.11 (br s; 1 H (exch.), NH indole); MS: m/z (%): 356 (100) [$M^{+\cdot}$]. Anal. ($C_{21}H_{16}N_4O_2$) C, H, N.

5.1.18. 2,3-Dihydro-5-(3-nitrophenyl)-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2r**

Yield 0.1 g (2%): m.p. 322 °C; R_f 0.46 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 370 (NH); 3 070–2 980 (CH); 1 770, 1 720 (C=O); 1 630, 1 585 (C=C); 1 535, 1 350 (NO_2); ^{1}H-NMR: δ 7.42–8.81 (m; 8 H aromat.), 11.53 (br s; 1 H (exch.), NH imid), 12.41 (br s; 1 H (exch.), NH indole); MS: m/z (%): 358 (100) [$M^{+\cdot}$], 312 (70) [M – $^{\cdot}NO_2$]$^+$, 241 (44) [312–(HNCO + CO)]$^+$, 214 (25) [241 – HCN]$^+$. Anal. ($C_{19}H_{10}N_4O_4$) C, H, N.

5.1.19. 2,3-Dihydro-5-(3-methoxyphenyl)-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2s**

Yield 0.63 g (11%): m.p. 256 °C; R_f 0.34 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 350, 3 240 (NH); 3 040–2 980 (CH); 1 765, 1 740 (C=O); 1 650, 1 625, 1 600, 1 580 (C=C); ^{1}H-NMR: δ 3.91 (s; 3 H, OCH_3), 7.18–8.80 (m; 8 H aromat.), 11.41 (br s; 1 H (exch.), NH imid), 12.20 (br s; 1 H (exch.), NH indole); MS: m/z (%): 343 (100) [$M^{+\cdot}$], 342 (78) [M – $^{\cdot}H$]$^+$. Anal. ($C_{20}H_{13}N_3O_3$) C, H, N.

5.1.20. 2,3-Dihydro-9-methoxy-5-phenyl-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2t**

Yield 0.94 g (16%): m.p. 288 °C; R_f 0.40 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 450, 3 360 (NH); 3 060–2 835 (CH); 1 770, 1 710 (C=O); 1 630, 1 570, 1 490 (C=C); ^{1}H-NMR: δ 3.90 (s; 3 H, OCH_3), 7.35 (dd, J_o = 8.9 Hz, J_m = 2.0 Hz; 1 H, 8–H), 7.58–7.72 (m; 3 H, 3',4',5'–H), 7.66 (d, J_o = 8.9 Hz; 1 H, 7–H), 8.03 (d, J_o = 6.9 Hz; 2 H, 2',6'-H), 8.25 (δ, J_m = 1.9 Hz; 1 H, 10–H), 11.39 (br s; 1 H (exch.), NH imid), 12.07 (br s; 1 H (exch.), NH indole); MS: m/z (%): 343 (100) [$M^{+\cdot}$], 328 (67) [M – $^{\cdot}CH_3$]$^+$, 300 (43) [328 – CO]$^+$, 171.5 (3) M^{2+}. Anal. ($C_{20}H_{13}N_3O_3$) C, H, N.

5.1.21. 2,3-Dihydro-9-methoxy-5-(4-methoxyphenyl)-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]-indole-1,3-dione **2u**

Yield 0.90 g (14%): m.p. 341 °C; R_f 0.30 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 450, 3 365 (NH); 3 120–2 835 (CH); 1 770, 1 735 (C=O); 1 610, 1 580, 1 570 (C=C); ^{1}H-NMR: δ 3.89 (s; 3 H, OCH_3), 3.91 (s; 3 H, OCH_3), 7.22; 8.01 (AA'BB', J_{AB} = 8.9 Hz; 4 H, 2',3',5',6'-H), 7.33 (dd, J_o = 9.0 Hz, J_m = 2.6 Hz; 1 H, 8–H), 7.65 (d, J_o = 8.9 Hz; 1 H, 7–H), 8.24 (d, J_m = 2.5 Hz; 1 H, 10–H), 11.42 (br s; 1 H (exch.), NH imid), 11.98 (br s; 1 H (exch.), NH indole); MS: m/z (%): 373 (100) [$M^{+\cdot}$], 358 (51) [M – $^{\cdot}CH_3$]$^+$. Anal. ($C_{21}H_{15}N_3O_4$) C, H, N.

5.1.22. 2,3-Dihydro-9-methoxy-5-(3-methoxyphenyl)-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]-indole-1,3-dione **2v**

Yield 0.45 g (7%): m.p. 309 °C; R_f 0.38 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 360, 3 285 (NH); 3 075–2 835 (CH); 1 765, 1 740 (C=O); 1 630, 1 610, 1 570 (C=C); ^{1}H-NMR: δ 3.90 (s; 3 H, OCH_3), 3.91 (s; 3 H, OCH_3), 7.15–7.23 (m; 1 H, phenyl), 7.36 (dd, J_o = 9.0 Hz, J_m = 2.6 Hz; 1 H, 8–H), 7.53–7.63 (m; 3 H, phenyl), 7.67 (d, J_o = 9.0 Hz; 1 H, 7-H), 8.27 (d, J_m = 2.5 Hz; 1 H, 10-H), 11.42 (br s; 1 H (exch.), NH imid), 12.08 (br s; 1 H (exch.), NH indole); MS: m/z (%): 373 (100) [$M^{+\cdot}$], 358 (39) [M – $^{\cdot}CH_3$]$^+$. Anal. ($C_{21}H_{15}N_3O_4$) C, H, N.

5.1.23. 2,3-Dihydro-9-methoxy-5-(2-methoxyphenyl)-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]-indole-1,3-dione **2w**

Yield 0.96 g (15%): m.p. 307 °C; R_f 0.27 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 330, 3 200 (NH); 3 070–2 835 (CH); 1 775, 1 730 (C=O); 1 635, 1 605, 1 575 (C=C); ^{1}H-NMR: δ 3.77 (s; 3 H, 2'-OCH_3), 3.90 (s; 3 H, 9-OCH_3), 7.19 (m; 1 H, phenyl), 7.30 (d, J = 8.1 Hz; 1 H, phenyl), 7.34 (dd, J_o = 8.9 Hz, J_m = 2.5 Hz; 1 H, 8-H), 7.52–7.64 (m; 2 H, phenyl), 7.60 (d; J_o = 8.6 Hz; 1 H, 7–H), 8.26 (d, J_m = 2.5 Hz; 1 H, 10-H), 11.36 (br s; 1 H (exch.), NH imid), 11.71 (br s; 1 H (exch.), NH indole); MS: m/z (%): 373 (100) [$M^{+\cdot}$], 358 (9) [M – $^{\cdot}CH_3$]$^+$, 355 (19) [M – H_2O]$^{+\cdot}$, 340 (21) [355 – $^{\cdot}CH_3$]$^+$. Anal. ($C_{21}H_{15}N_3O_4$) C, H, N.

5.1.24. 5-(2,4-Dimethoxyphenyl)-2,3-dihydro-9-methoxy-1H,6H-pyrrolo[3,4-c]pyrido-[3,4-b]indole-1,3-dione **2x**

Yield 0.83 g (12%): m.p. 308 °C; R_f 0.20 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 395, 3 280 (NH); 3 090–2 840 (CH); 1 770, 1 745 (C=O); 1 615, 1 575, 1 515 (C=C); ^{1}H-NMR: δ 3.77 (s; 3 H, 2'-OCH_3), 3.90 (s; 3 H, OCH_3), 3.91 (s; 3 H, OCH_3), 6.77 (dd, J_o = 8.4 Hz, J_m = 2.3 Hz; 1 H, 5'-H), 6.82 (d, J_m = 2.3 Hz; 1 H, 3'-H), 7.33 (dd, J_o = 9.0 Hz, J_m = 2.6 Hz; 1 H, 8-H), 7.48 (d, J_o = 8.4 Hz; 1 H, 6'-H), 7.60 (dd, J_o = 9.0 Hz, J_p = 0.6 Hz; 1 H, 7-H), 8.25 (dd, J_m = 2.6 Hz, J_p = 0.6 Hz; 1 H, 10-H), 11.32 (br s; 1 H (exch.), NH imid), 11.65 (br s; 1 H (exch.), NH indole); MS: m/z (%): 403 (100) [$M^{+\cdot}$], 388 (7) [M – $^{\cdot}CH_3$]$^+$, 385 (16) [M – H_2O]$^{+\cdot}$, 373 (8) [M – $H_2C{=}O$]$^{+\cdot}$, 370 (17) [385 – $^{\cdot}CH_3$]$^+$, 201.5 (6) M^{2+}. Anal. ($C_{22}H_{17}N_3O_5$) C, H, N.

5.1.25. 5-(3,4-Dimethoxyphenyl)-2,3-dihydro-9-methoxy-1H,6H-pyrrolo[3,4-c]pyrido-[3,4-b]indole-1,3-dione **2y**

Yield 0.55 g (8%): m.p. 228 °C; R_f 0.36 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 435, 3 275 (NH); 3 060–2 870 (CH); 1 750, 1 700 (C=O); 1 630, 1 610, 1 585 (C=C); ^{1}H-NMR: δ 3.90 (s; 3 H, OCH_3), 3.90 (s; 3 H, OCH_3), 3.91 (s; 3 H, OCH_3), 7.24 (d, J_o = 8.3 Hz; 1 H, 5'-H), 7.35 (dd, J_o = 9.0 Hz, J_m = 2.7 Hz; 1 H, 8–H), 7.57 (d, J_m = 2.1 Hz; 1 H, 2'-H), 7.61 (dd, J_o = 8.3 Hz, J_m = 2.1 Hz; 1 H, 6'-H), 7.67 (dd, J_o = 9.0 Hz, J_p = 0.5 Hz; 1 H, 7–H), 8.26 (dd, J_m = 2.7 Hz, J_p = 0.5 Hz; 1 H, 10–H), 11.34 (br

s; 1 H (exch.), NH imid), 12.06 (br s; 1 H (exch.), NH indole); MS: m/z (%): 403 (100) [$M^{+\cdot}$], 388 (40) [M – $^{\cdot}CH_3$]$^+$. Anal. ($C_{22}H_{17}N_3O_5$) C, H, N.

5.1.26. 5-(4-Cyanophenyl)-2,3-dihydro-9-methoxy-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]-indole-1,3-dione **2z**

Yield 0.13 g (2%): m.p. 335 °C (dec.); R_f 0.39 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 455, 3 375 (NH); 3 100–2 835 (CH); 2 230 (C$^{\cdot}$ N); 1 770, 1 720 (C=O); 1 655, 1 630, 1 575, 1 510 (C=C); ^{1}H-NMR: δ 3.90 (s; 3 H, OCH_3), 6.88 (dd, J_o = 8.8 Hz, J_m = 2.4 Hz; 1 H, 8–H), 7.41 (d, J_o = 8.8 Hz; 1 H, 7–H), 8.14; 8.21 (AA'BB', J_{AB} = 8.0 Hz; 4 H, 2',3',5',6'-H), 8.30 (d, J_m = 2.5 Hz; 1 H, 10–H), 11.49 (br s; 1 H (exch.), NH imid), 12.21 (br s; 1 H (exch.), NH indole); MS: m/z (%): 368 (100) [$M^{+\cdot}$], 353 (79) [M – $^{\cdot}CH_3$]$^+$, 325 (36) [353 – CO]$^+$. Anal. ($C_{21}H_{12}N_4O_3$) C, H, N.

5.1.27. 2,3-Dihydro-9-methoxy-2-methyl-5-phenyl-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]-indole-1,3-dione **2aa**

Yield 0.74 g (12%): m.p. 305 °C; R_f 0.62 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 400 (NH); 3 100–2 835 (CH); 1 760, 1 705 (C=O); 1 635, 1 570, 1 495 (C=C); ^{1}H-NMR: δ 3.13 (s; 3 H, NCH_3), 3.88 (s; 3 H, OCH_3), 7.32 (dd, J_o = 9.0 Hz, J_m = 2.6 Hz; 1 H, 8-H), 7.59–7.70 (m; 3 H, 3',4',5'-H), 7.62 (d, J_o = 8.9 Hz; 1 H, 7–H), 8.01 (dd, J_o = 7.6 Hz, J_m = 7.7 Hz; 2 H, 2',6'-H), 8.19 (d, J_m = 2.6 Hz; 1 H, 10-H), 12.05 (br s; 1 H (exch.), NH indole); MS: m/z (%): 357 (100) [$M^{+\cdot}$], 342 (48) [M – $^{\cdot}CH_3$]$^+$, 314 (20) [342 – CO]$^+$. Anal. ($C_{21}H_{15}N_3O_3$) C, H, N.

5.1.28. 2,3-Dihydro-8,9-dimethoxy-5-phenyl-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2bb**

Yield 0.11 g (2%): m.p. > 350 °C; R_f 0.14 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 465, 3 310 (NH); 3 090–2 835 (CH); 1 770, 1 715 (C=O); 1 660, 1 590, 1 490 (C=C); ^{1}H-NMR: δ 3.90 (s; 3 H, OCH_3), 3.92 (s; 3 H, OCH_3), 7.18 (s; 1 H, 7–H), 7.58–7.70 (m; 3 H, 3',4',5'-H), 8.02 (dd, J_o = 8.0 Hz, J_m = 1.6 Hz; 2 H, 2',6'-H), 8.17 (s; 1 H, 10-H), 11.32 (br s; 1 H (exch.), NH imid), 11.99 (br s; 1 H (exch.), NH indole); MS: m/z (%): 373 (100) [$M^{+\cdot}$], 358 (50) [M – $^{\cdot}CH_3$]$^+$. Anal. ($C_{21}H_{15}N_3O_4$) C, H, N.

5.1.29. 2,3-Dihydro-2-methyl-5-phenyl-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]indole-1,3-dione **2cc**

Yield 0.05 g (1%): m.p. 317 °C; R_f 0.66 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 385 (NH); 3 060–2 940 (CH); 1 765, 1 705 (C=O); 1 630, 1 605, 1 585 (C=C); ^{1}H-NMR: δ 3.15 (s; 3 H, CH_3), 7.39–8.78 (m; 9 H aromat.), 12.23 (s; 1 H (exch.), NH indole); MS: m/z (%) 327 (100) [$M^{+\cdot}$]. Anal. ($C_{20}H_{13}N_3O_2$) C, H, N.

5.1.30. 2,3-Dihydro-9-methoxy-5-(2-methylphenyl)-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]-indole-1,3-dione **2dd**

Yield 0.74 g (12%): m.p. 243 °C; R_f 0.45 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 375, 3 225 (NH); 3 085–2 835 (CH); 1 770, 1 735 (C=O); 1 570, 1 495, 1 465 (C=C); ^{1}H-NMR: δ 2.17 (s; 3 H, CH_3), 3.90 (s; 3 H, OCH_3), 7.35 (dd, J_o = 9.0 Hz, J_m = 2.7 Hz; 1 H, 8-H), 7.40–7.56 (m; 4 H phenyl), 7.59 (dd, J_o = 9.0 Hz, J_p = 0.6 Hz; 1 H, 7–H), 8.26 (dd, J_m = 2.7 Hz, J_p = 0.6 Hz; 1 H, 10-H), 11.40 (br s; 1 H (exch.), NH imid), 11.84 (br s; 1 H (exch.), NH indole); MS: m/z (%): 357 (100) [$M^{+\cdot}$], 342 (29) [M – $^{\cdot}CH_3$]$^+$. Anal. ($C_{21}H_{15}N_3O_3$) C, H, N.

5.1.31. 2,3-Dihydro-8,9-dimethoxy-5-(2-methylphenyl)-1H,6H-pyrrolo[3,4-c]pyrido-[3,4-b]indole-1,3-dione **2ee**

Prepared analogous to the previous general method, but heating under reflux for 1 h in tetralin instead of *o*-xylene as solvent gives yellow crystals. Yield 0.67 g (10%): m.p. > 350 °C (ethyl acetate); R_f 0.21 (ethyl acetate/hexane 1:1); IR: ν (cm^{-1}) 3 300 (NH); 3 085–2 840 (CH); 1 770, 1 730 (C=O); 1 630, 1 575, 1 490 (C=C); ^{1}H-NMR: δ 2.17 (s; 3 H, CH_3), 3.89 (s; 3 H, OCH_3), 3.90 (s; 3 H, OCH_3), 7.11 (s; 1 H, 8-H), 7.39–7.55 (m; 4 H phenyl), 8.17 (s; 1 H, 10-H), 11.32 (br s; 1 H (exch.), NH imid), 11.75 (br s; 1 H (exch.), NH indole); MS: m/z (%): 387 (100) [$M^{+\cdot}$], 372 (24) [M – $^{\cdot}CH_3$]$^+$. Anal. ($C_{22}H_{17}N_3O_4$) C, H, N.

5.1.32. 9-Benzyloxy-2,3-dihydro-5-(2-methoxyphenyl)-1H,6H-pyrrolo[3,4-c]pyrido-[3,4-b]indole-1,3-dione **2ff**

Yield 1.39 g (18%): m.p. 279 °C; R_f 0.68 (dichloromethane/methanol 10:1); IR: ν (cm^{-1}) 3 415 (NH); 3 040–2 890 (CH); 1 775, 1 730 (C=O); 1 600, 1 570, 1 490, 1 460 (C=C); ^{1}H-NMR: δ 3.77 (s; 3 H, OCH_3), 5.24 (s; 2 H, CH_2), 7.16–7.22 (m; 1 H aromat.), 7.28–7.64 (m; 10 H aromat.), 8.35 (d, J = 2.5 Hz; 1 H, 10-H), 11.36 (s; 1 H (exch.), NH imid), 11.72 (s; 1 H (exch.), NH indole); MS: m/z (%): 449 (49) [$M^{+\cdot}$], 358 (100) [M – $^{\cdot}C_7H_7$]$^+$, 91 (51) [C_7H_7]$^+$. Anal. ($C_{27}H_{19}N_3O_4$) C, H, N.

5.1.33. 9-Benzyloxy-2,3-dihydro-5-(2-methylphenyl)-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]-indole-1,3-dione **2gg**

Yield 0.52 g (7%): m.p. 264 °C; R_f 0.64 (dichloromethane/methanol 10:1); IR: ν (cm^{-1}) 3 450 (NH); 3 095–2 965 (CH); 1 770, 1 700 (C=O); 1 620, 1 570, 1 485, 1 465 (C=C); ^{1}H-NMR: δ 2.17 (s; 3 H, CH_3), 5.24 (s; 2 H, CH_2), 7.34–7.61 (m; 11 H aromat.), 8.35 (d, J = 2.4 Hz; 1 H, 10-H), 11.37 (br s; 1 H (exch.), NH imid), 11.84 (br s; 1 H (exch.), NH indole); MS: m/z (%): 433 (33) [$M^{+\cdot}$], 342 (80) [M – $^{\cdot}C_7H_7$]$^+$, 91 (100) [C_7H_7]$^+$. Anal. ($C_{27}H_{19}N_3O_3$) C, H, N.

5.2. General procedure for compounds **2hh** *and* **2ii**

To a solution of the 9-benzyloxy compound (0.12 mmol) in anhydrous ethanol (5 mL) and THF (5 mL) are added ammonium formate (15.8 mg, 0.22 mmol) and 10% Pd/C (10 mg). This mixture is heated under reflux for 3 h. After cooling to room temperature the catalyst is removed by filtration through Celite, the solvent is removed in vacuo, and the oily residue is purified by column chromatography (column 1 cm × 2 cm, silica gel, dichloromethane/methanol 10:1) to yield yellow crystals. Analytical samples were obtained as yellow crystals from ethyl acetate.

5.2.1. 2,3-Dihydro-9-hydroxy-5-(2-methoxyphenyl)-1H, 6H-pyrrolo[3,4-c]pyrido[3,4-b]-indole-1,3-dione **2hh**

Yield 30 mg (70%): m.p. 262 °C; R_f 0.60 (dichloromethane/methanol 10:1); IR: ν (cm^{-1}) 3 425 (NH, OH); 3 070–2 960 (CH); 1 765, 1 705 (C=O); 1 640, 1 605, 1 570, 1 500 (C=C); ^{1}H-NMR: δ 3.77 (s; 3 H, OCH_3), 7.15–7.20 (m; 2 H aromat.), 7.29 (d, J = 8.2 Hz; 1 H aromat.), 7.48–7.63 (m; 3 H aromat.), 8.16 (d, J = 2.4 Hz; 1 H, 10-H), 9.45 (s; 1 H (exch.), OH), 11.31 (s; 1 H (exch.), NH imid), 11.57 (s; 1 H (exch.), NH indole); MS: m/z (%): 359 (100) [$M^{+\cdot}$]. Anal. ($C_{20}H_{13}N_3O_4$) C, H, N.

5.2.2. 2,3-Dihydro-9-hydroxy-5-(2-methylphenyl)-1H,6H-pyrrolo[3,4-c]pyrido[3,4-b]-indole-1,3-dione **2ii**

Yield 25 mg (61%): m.p. > 350 °C; R_f 0.41 (dichloromethane/methanol 10:1); IR: ν (cm^{-1}) 3 320, 3 265 (NH, OH); 3 080–2 975 (CH); 1 780, 1 705 (C=O); 1 640, 1 570, 1 510, 1 460 (C=C); ^{1}H-NMR: δ 2.17 (s; 3 H, CH_3), 7.19 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz; 1 H, 8–H), 7.39–7.55 (m; 5 H aromat.), 8.17 (d, J = 2.4 Hz; 1 H, 10-H), 9.49 (s; 1 H (exch.), OH), 11.35 (s; 1 H (exch.), NH imid), 11.70 (s; 1 H (exch.), NH indole); MS: m/z (%): 343 (100) [$M^{+\cdot}$]. Anal. ($C_{20}H_{13}N_3O_3$) C, H, N.

5.3. Assays for tyrosine kinase inhibition

Swiss 3T3 fibroblasts (ATCC CCL 92) were cultivated in DMEM/10% FCS (Life Technologies). The effect on PDGF-stimulated tyrosine phosphorylation was measured by subjecting quiescent cultures in 24-well plates (NUNC) to a medium change into serum-free DMEM (0.4 mL per well) and treating them with the test compounds in DMSO (final DMSO concentration 1%) or solvent for 2 h. The cells were subsequently stimulated with 100 ng/mL human recombinant PDGF-BB (TEBU/Peprotech) or mock-treated for 10 min at room temperature, washed twice with PBS and extracted with lysis buffer containing Hepes, pH 7.5, 1% Triton X-100, phosphatase and protease inhibitors as described [16]. Equal amounts of lysate were diluted 1:10 in 10 mM Tris/HCl, pH 7.6, and were applied to a nitrocellulose membrane in a dot-blot apparatus (BioRad). The membrane was blocked with 1% BSA and developed with anti-phosphotyrosine antibodies (RC20–HRP conjugate, Dianova, Transduction Laboratories) and the ECL reagent (Amersham). The films were scanned, and the phosphorylation signals were quantified using the program NIH Image 1.54. IC_{50} values were calculated referring to the difference of non-PDGF stimulated samples (0%) and DMSO treated PDGF stimulated samples (100%). Ranges for the IC_{50} values were defined based on at least three independent experiments. For a qualitative analysis of the effects on tyrosine phosphorylation, 10 µg protein of identically treated cell extracts were subjected to SDS-PAGE with 7.5% gels and immunoblotting with anti-phosphotyrosine antibodies. To evaluate PDGF receptor levels, total cell lysates of one well were subjected to incubation with wheat germ agarose beads (Pharmacia, 30 µL 1:1 suspension per extract) for 1 h at 4 °C with end-over-end rotation. The beads were washed once with lysis buffer and extracted with SDS-PAGE sample buffer. SDS-PAGE and immunoblotting were performed as above except that the blots were developed with anti-PDGF receptor antibodies DIG 1 [16]. Quantification of IC_{50} values was based on the intensity of the signal for autophosphorylated PDGF receptor. Titration was done using four–eight inhibitor concentrations within a range of two orders of magnitude which was selected on the basis of preliminary experiments. Results of triplicate determinations were subjected to curve fitting using the program Sigma Plot 2.0 (Jandel Corporation). Porcine aortic endothelial (PAE) cells expressing human PDGFb receptors were kindly provided by Dr L. Claesson-Welsh (Uppsala). Testing inhibition of human PDGFb receptor kinase in these cells was done as with Swiss 3T3 cells except that the cells were starved in 0.5% FCS-containing medium for 24 h before the experiment. In vitro kinase reactions with isolated Swiss 3T3 cell membranes or purified PDGF receptor were performed as described earlier [16, 17]. To measure effects on EGF receptor phosphorylation, either in vitro kinase reactions with Swiss 3T3 membranes were performed [16], or A431 cells (ATCC CRL 1555) were starved overnight in serum-free medium, treated for 2 h with the compounds, thereafter stimulated with 100 ng/mL EGF (TEBU/Peprotech) for 10 min and subjected to extraction and immunoblotting as described above. FGF-dependent tyrosine phosphorylation was measured in Swiss 3T3 cells, cultivated and treated with compounds as described above and stimulated for 10 min with 100 ng/mL basic FGF (TEBU/Peprotech). Cell extracts were analysed by

immunoblotting with anti-phosphotyrosine antibodies. A prominent phosphoprotein band of 90–92 kDa, probably representing FRS2 [45-46], appeared only in FGF-stimulated cells and was taken as a measure of FGF receptor activity. pp60src Kinase activity and Src-dependent tyrosine phosphorylation in *src*-transformed NIH-fibroblasts were determined as described elsewhere [47]. SCF-dependent c-Kit phosphorylation was measured in CHRF cells [48] (generously provided by Dr M. Lieberman, Cincinnati), cultivated in DMEM/10% HS (Life Technologies). For the assay, CHRF cells were treated for 2 h in serum-free medium with inhibitors, then stimulated with 40 ng/mL human SCF (TEBU/Peprotech) for 5 min, washed twice with buffer containing 50 mM Tris, pH 8.0, 250 mM NaCl, 100 mM NaF and 10%(v/v) glycerol, and followed by centrifugation at 1 500 rpm for 5 min. The cells were extracted by lysis buffer containing 50 mM Tris, pH 8.0, 250 mM NaCl, 100 mM NaF, 10% (v/v) glycerol, 1% (v/v) NP-40, 1 mM PMSF, 1 µM leupeptin, 1 µM aprotinin and 1 mM Na-orthovanadate. The lysates were subjected to an immunoprecipitation with anti-c-Kit antibodies (Santa Cruz). Thereafter, the receptor was immunoblotted with antiphosphotyrosine antibodies and re-blotted with anti-c-Kit antibodies to check the unchanged receptor content.

5.4. Cell growth parameters

DNA synthesis in Swiss 3T3 fibroblasts stimulated by different agents and PDGF-dependent cell proliferation of Swiss 3T3 fibroblasts were determined as described [16]. Colony formation in semi-solid agar was measured similarly as described in [49]. Different cell lines (*sis* NIH3T3, *src* NIH3T3 and BxB *raf* NIH3T3; 20 000 cells/mL) were cultured in 0.25% agar in the presence of DMEM/10% FCS on a base layer of 0.5% agar in the presence or absence of inhibitors. After 1 week (*sis* NIH3T3, BxB *raf* NIH3T3) or 2 weeks (*src* NIH3T3) the formed colonies were counted. For XTT proliferation assay, cells were treated with test compounds in medium containing 0.25% FCS for 48 h. Proliferation was measured using a cell proliferation kit (Boehringer, Mannheim, Germany) according to the instructions of the manufacturer.

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