# New Anilinophthalazines as Potent and Orally Well Absorbed Inhibitors of the VEGF Receptor Tyrosine Kinases Useful as Antagonists of Tumor-Driven Angiogenesis

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The sprouting of new blood vessels, or angiogenesis, is necessary for any solid tumor to grow large enough to cause life-threatening disease. Vascular endothelial growth factor (VEGF) is one of the key promoters of tumor induced angiogenesis. VEGF receptors, the tyrosine kinases Flt-1 and KDR, are expressed on vascular endothelial cells and initiate angiogenesis upon activation by VEGF. 1-Anilino-(4-pyridylmethyl)-phthalazines, such as CGP 79787D (or PTK787 / ZK222584), reversibly inhibit Flt-1 and KDR with $IC_{50}$ values $< 0.1\ \mu M$. CGP 79787D also blocks the VEGF-induced receptor autophosphorylation in CHO cells ectopically expressing the KDR receptor ($ED_{50} = 34$ nM). Modification of the 1-anilino moiety afforded derivatives with higher selectivity for the VEGF receptor tyrosine kinases Flt-1 and KDR compared to the related receptor tyrosine kinases PDGF-R and c-Kit. Since these 1-anilino-(4-pyridylmethyl)phthalazines are orally well absorbed, these compounds qualify for further profiling and as candidates for clinical evaluation.

## Introduction

Most solid tumors cannot grow beyond a certain critical size unless they establish their own blood supply by inducing formation of new vessels sprouting from existing host capillaries.[1] Growth of blood vessels, or *angiogenesis*, also promotes metastasis by providing an avenue for transmission of the cancer cells to other sites.[2] Specific inhibition of tumor-induced angiogenesis should prevent growth of many types of solid tumors, thereby providing a novel approach for the treatment of cancer.[3]

*Vascular endothelial growth factor (VEGF)*, an inducer of endothelial cell proliferation, migration, and survival, is considered to play a key role in angiogenesis[3a] and has been shown to be secreted by tumor cells and macrophages.[4] VEGF interacts with cell surface receptors, in humans KDR (kinase domain-containing receptor or VEGFR-2) and Flt-1 (fms-like tyrosine kinase or VEGFR-1),[5] expressed almost exclusively on vascular endothelial cells. These receptors have intracellular tyrosine kinase domains, which upon activation by bound VEGF undergo autophosphorylation, starting the transmission of the signal to the nucleus. As a consequence, the induction of endothelial cell proliferation and migration initiates sprouting of new blood vessels toward the tumor tissue. Tumors have the ability to upregulate the expression of the ligand as well as the receptor in endothelial cells of adjacent vessels. In keeping with this observation, inhibition of VEGF-induced angiogenic signaling selectively targets the tumor-associated vessels, since proliferation of endothelial cells in the normal vasculature is a rare event. Therefore, antiangiogenic therapy through inhibition of VEGF-mediated signaling, is expected to be safe and well-tolerated in cancer patients. Because host tissues are genetically more stable than tumor cells, targeting the host endothelial cells should provide a cancer treatment modality that is less prone to resistance development.[6] Outside the indication cancer, antiangiogenic drugs also have potential as new therapy for other neovascularization-related diseases, for example the treatment of diabetic retinopathy or age-related maculopathy, the major causes of human blindness[7] and rheumatoid arthritis. In addition, VEGF is a potent inducer of vascular permeability (and thus also known as vascular permeability factor, VPF[4a]) and may also play a key role in ascites formation and edema associated with malignant disease.

Recently, 3-substituted indolin-2-ones as inhibitors of the mouse VEGF-receptor tyrosine kinase Flk-1 (fms-like kinase) have been disclosed.[8] These inhibitors bind in the ATP binding pocket of the VEGF receptor tyrosine kinases. One compound from this series, **SU 5416** (see Chart 1), has been selected for clinical trials in cancer patients.

In this paper we will describe the 1-anilino-(4-pyridylmethyl)phthalazines,[9] which represent a new class of kinase inhibitors with high selectivity for the human VEGF-receptor tyrosine kinases and, in contrast to the previously described inhibitors, have favorable pharmacokinetic properties following their oral administration to animals and men. One of these compounds, **CGP**

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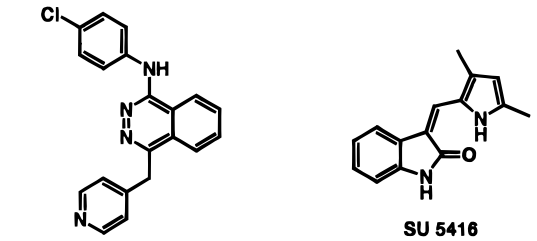
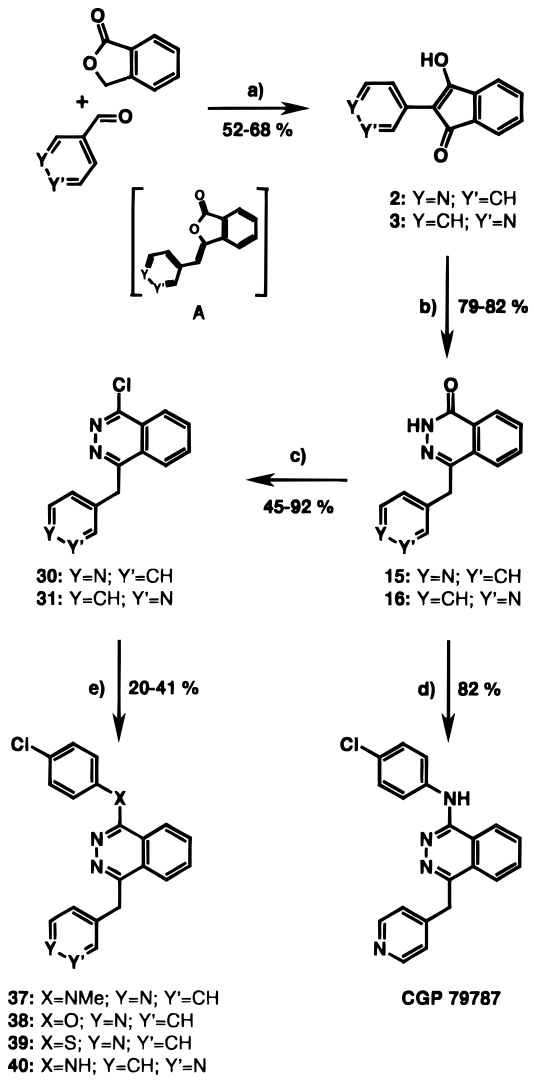
**Chart 1**




dihydrochloride: **1**
free base: **CGP 79787**
succinate: **CGP 79787D**
or **PTK787 / ZK222584**

**Scheme 1.** Synthesis of the Phthalazine Derivatives **CGP 79787**, **37**−**40**[a]




[a] Conditions: (a) MeONa/MeOH, EtCOOEt, 0 °C → ↑↓; (b) $H_2N-NH_2 \cdot H_2O$, ↑↓; (c) $POCl_3$, $CH_3CN$, HCl, 50 °C; or $POCl_3$, $CH_3CN$, 100 °C; (d) $P_2O_5$, $Et_3N \cdot HCl$, 4-chloroaniline, 170 °C. (e) *N*-methyl-4-chloroaniline, DMPU, 100 °C; or 4-chlorophenol or 4-chlorothiophenol, $K_2CO_3$, DMSO, 90 °C; or 4-chloroaniline neat, 110 °C.

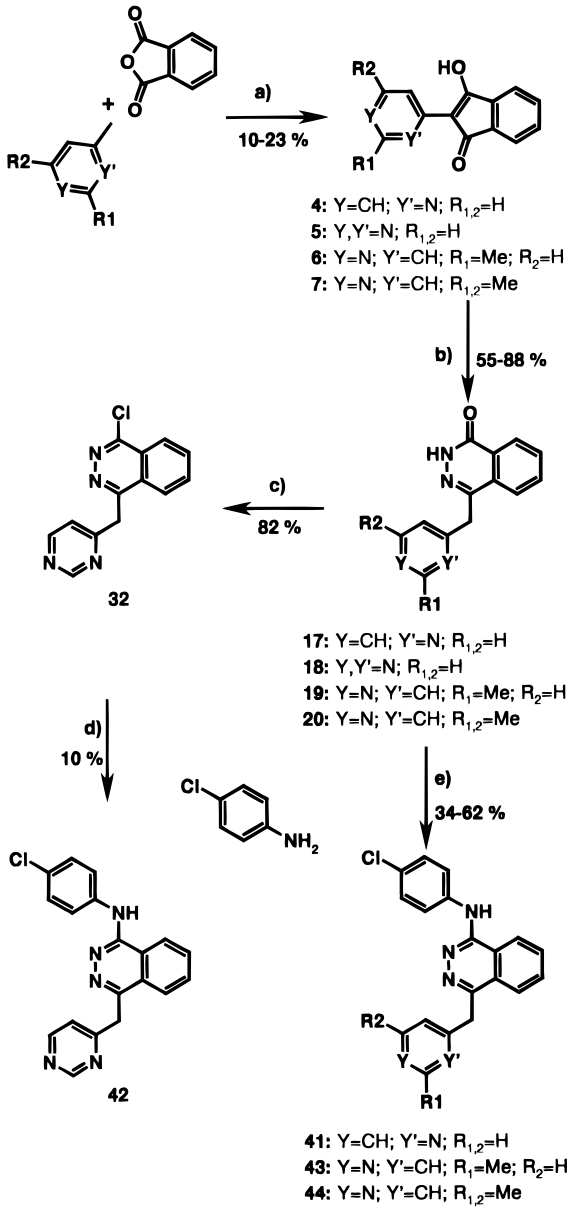
**79787D** (also know as **PTK787** / **ZK222584**), is currently in phase I trials in patients with advanced cancer.

## Chemistry

Generally, the phthalazines were synthesized from 2-aryl-3-hydroxyinden-1-ones by condensation with hydrazine hydrate. Thus, either base-catalyzed condensation of phthalides or tetrahydrophthalides with the corresponding arylcarbaldehydes (Schemes 1, 4, and 5) or melting of phthalic anhydrides with 2- or 4-methylnitrogen heterocycles (Schemes 2 and 3) led to intermediates of type **A** (Scheme 1). This intermediate rearranged to the required 2-aryl-3-hydroxyinden-1-ones[10] **2**−**9**, the 7-hydroxypyrindin-5-ones **10** and **11**, or the tetrahydroinden-1-one **13**, respectively. The tetrasubstituted phthalide intermediates, for example, **12**, do not rearrange and can be isolated as such.

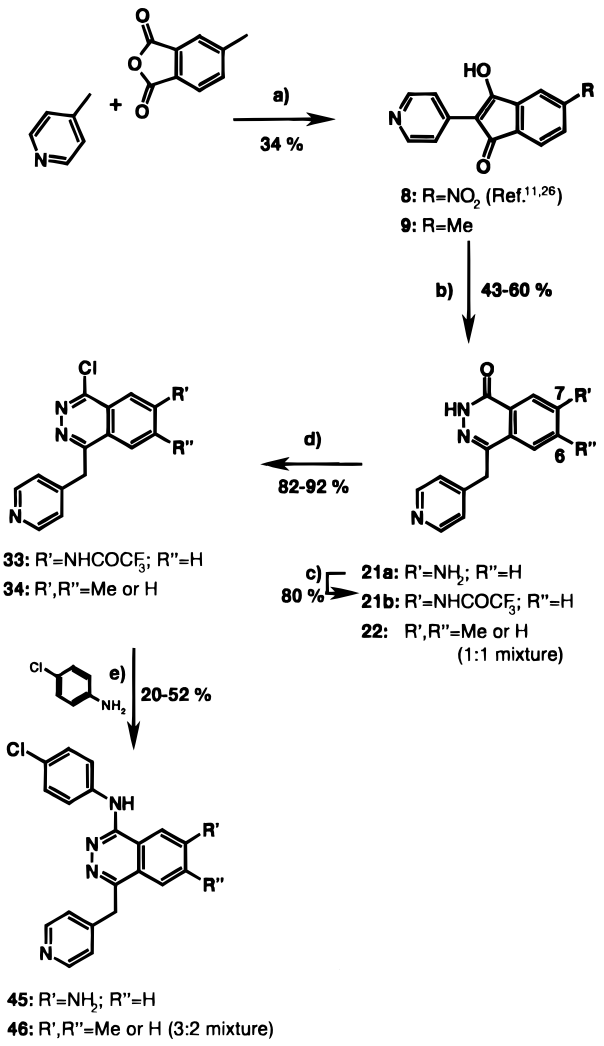
Subsequent condensation of **2**−**7**, **12**, and **13** with hydrazine hydrate led to the phthalazones **15**−**20** and **27** or the tetrahydrophthalazone **28**. Conversion of the unsymmetrical derivatives **8**−**11** (Schemes 3 and 4) gave the corresponding regioisomeric products **21**−**26**, the structures of which were proven by $^1H$ NMR

**Scheme 2.** Synthesis of the Phthalazine Derivatives **41**−**44**[a]




[a] Conditions: (a) melting at 180−210 °C; (b) $H_2N-NH_2 \cdot H_2O$, ↑↓; or $H_2N-NH_2 \cdot H_2O$, EtOH, ↑↓; (c) $POCl_3$, $CH_3CN$, HCl, 50 °C; (d) neat, 110 °C; (e) $P_2O_5$, $Et_3N \cdot HCl$, 170 °C.

**Scheme 3.** Synthesis of the Phthalazine Derivatives **45** and **46**[a]

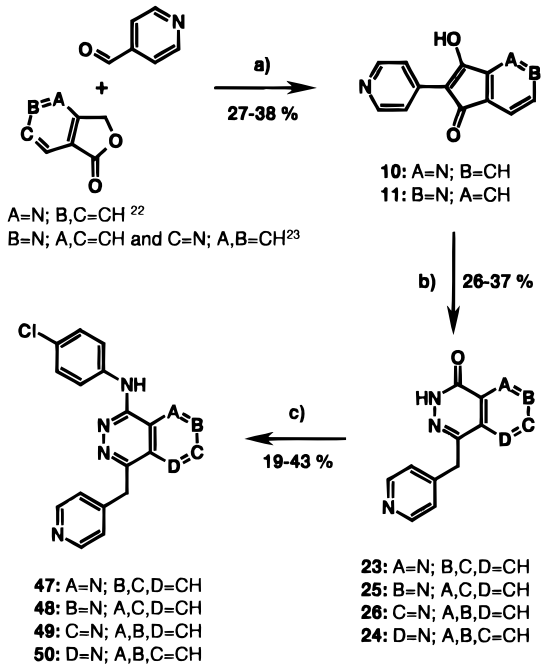



[a] Conditions: (a) melting at 165 °C; (b) $H_2N-NH_2 \cdot H_2O$, ⇅; (c) $(CF_3CO)_2O$; (d) $POCl_3$, $CH_3CN$, HCl, 50 °C; (e) EtOH, HCl, ⇅; or *n*-butanol, 100 °C.

spectroscopy (nuclear Overhauser effects between the $H_2C$ group and the neighboring aromatic HC protons). During the condensation of the nitro derivative **8** with hydrazine, the nitro group was reduced to an amino function as described by Druey and Marxer.[11] Whereas the regioisomeric 6-amino derivative of **21a** was not isolated from the reaction mixture, the methyl derivative **9** yielded **22**, as a ca. 1:1 mixture of regioisomers. Protection of the amino group as a trifluoracetamide gave **21b**. Condensation of the 7-hydroxypyrindin-5-ones **10** and **11** yielded a chromatographically separable mixture of the corresponding isomers **23**−**26**. The monocyclic pyridazine-3-one **29** (Scheme 5) was obtained in modest yield from hydrazine hydrate and **14**, the addition product of 4-picolyllithium to maleic acid anhydride.

Introduction of the 3-anilino group on the pyridazine core was achieved via two methods. In a one-step procedure described by Andersen and Pedersen,[12] a mixture of the pyridazine-3-ones, the respective aniline, phosphorus pentoxide, and triethylamine hydrochloride was melted together to give the anilino derivatives **CGP 79787**, **41**, **43**, **44**, **47**-**50**, **52**, and **53**, directly. Alternatively, the pyridazine-3-ones were activated as the imidoyl chlorides, **30**−**36**, and then reacted with the appropriate aniline to afford the 1-anilinophthalazines **37**-**40**, **42**, **45**, **46**, and **51** and the 3-anilinopyridazine **54**. The best condition for the chlorination of phthalazin-1-ones was stirring at 50 °C in a solution of phosphoryl chloride in slightly acidic acetonitrile. The chloride then could be mildly substituted by anilines in boiling acidic ethanol. This method was preferentially used to convert the chloride **30** into the derivatives **55**−**66** (Table 3).

**Scheme 4.** Synthesis of the Pyridopyridazine Derivatives **47**−**50**[a]

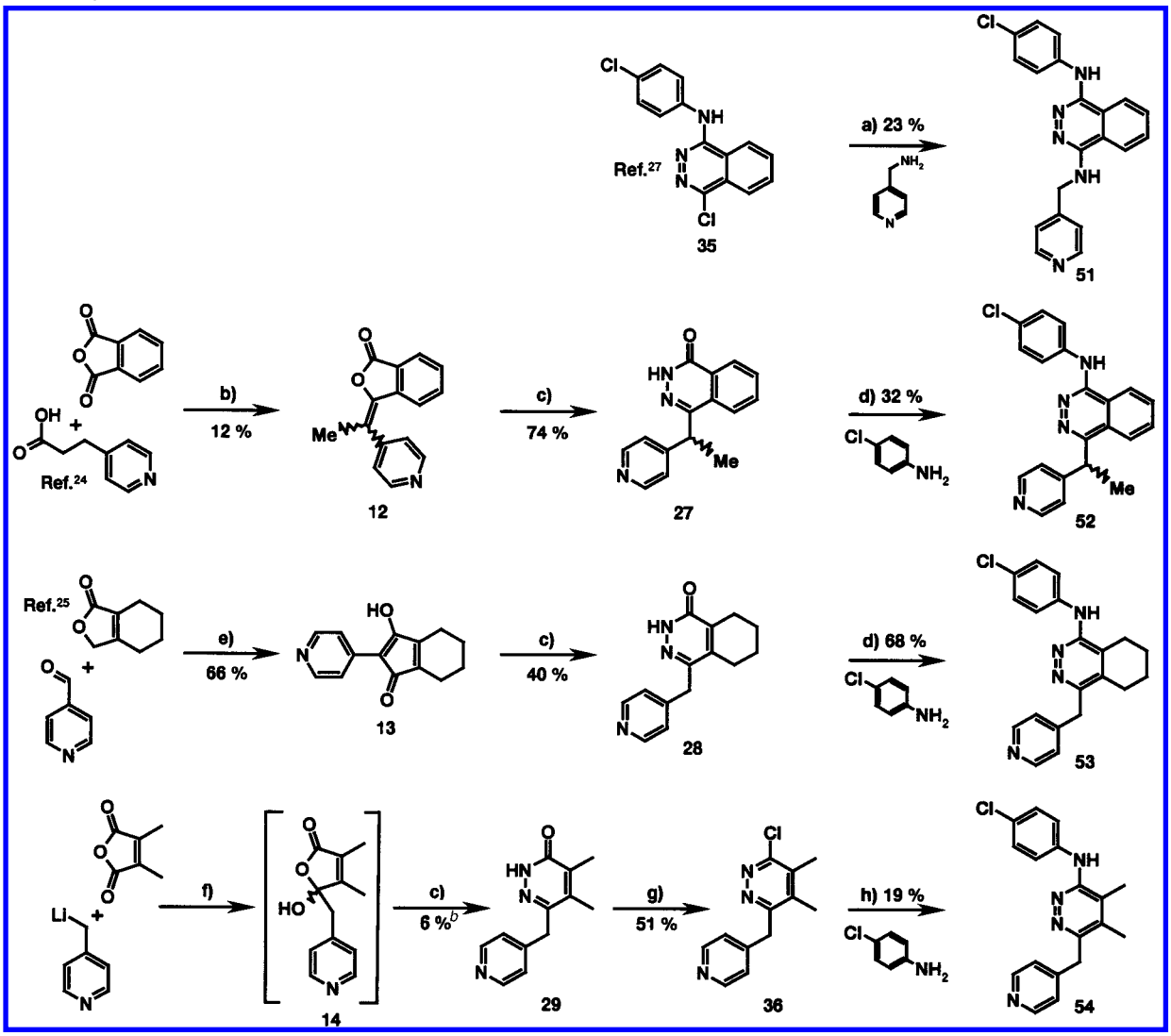



[a] Conditions: (a) MeONa/MeOH, EtCOOEt, 0 °C → ⇅; (b) $H_2N-NH_2 \cdot H_2O$, ⇅; (c) $P_2O_5$, $Et_3N \cdot HCl$, 4-chloroaniline, 170 °C.

## Results and Discussion

To identify lead structures for VEGF receptor tyrosine kinase inhibition, we screened the corporate compound libraries. Kinase inhibition was evaluated by measuring the phosphorylation of the artificial substrate poly-Glu-Tyr by the kinase domain of the Flt-1 VEGF receptor. Via this approach, we identified the anilinophthalazine **1** (see Chart 1) as a potent inhibitor of Flt-1 ($IC_{50} = 0.16$ $\mu$M). Further profiling revealed that **1** is highly selective for the class III protein tyrosine kinase family:[13] **1** inhibits the related VEGF receptor tyrosine kinase[5b] KDR with an $IC_{50}$ of 0.05 $\mu$M and its mouse homologue Flk-1 with an $IC_{50}$ of 0.3 $\mu$M. Its succinate salt, **CGP 79787D** (see Chart 1), blocks the lymphatic endothelial cell receptor tyrosine kinase Flt-4 with an $IC_{50}$ of 0.66 $\mu$M. Additionally, **1** interacts with the PDGF-$\beta$ receptor (platelet derived growth factor-$\beta$; $IC_{50} = 0.7$ $\mu$M) and c-Kit (receptor for stem cell factor; $IC_{50} = 0.7$ $\mu$M). However, the $IC_{50}$ of **1** is higher than 10 $\mu$M against a series of non-class III receptor tyrosine kinases, like the EGF receptor (epithelial growth factor receptor), v-abl (virally transduced Abelson oncogene), c-src (protooncogene), or a serine-threonine kinase like PKC-$\alpha$ (protein kinase C-$\alpha$ ).

**Scheme 5.** Synthesis of the Derivatives **51**−**54**[a]



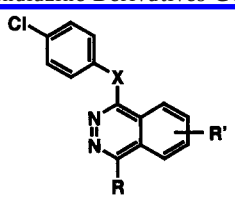

[a] Conditions: (a) 90 °C; (b) NaOAc, $CH_3CONMe_2$, 180 °C; (c) $H_2N-NH_2 \cdot H_2O$, EtOH, ↑↓; or $H_2N-NH_2 \cdot H_2O$, ↑↓; or $H_2N-NH_2 \cdot H_2O$, BuOH, 120 °C; (d) $P_2O_5$, $Et_3N \cdot HCl$, 170 °C; (e) MeONa/MeOH, EtCOOEt, 0 °C → ↑↓; (f) THF, −78 °C → rt; (g) $POCl_3$, 120 °C; (h) BuOH, 130 °C. [b] Yield for 2 steps

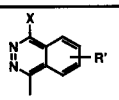
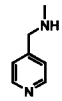
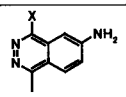
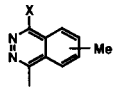
Since a drug must enter the cells in order to inhibit the tyrosine kinase activity of the receptor, the effect of **1** was tested in cellular, VEGF-induced receptor autophosphorylation assays. CHO cells (chinese hamster ovarian cells) ectopically expressing the VEGF receptor KDR were stimulated with VEGF. The degree of receptor autophosphorylation was measured in a sandwich ELISA assay by trapping KDR with an antibody specific for KDR and detecting phosphorylation with an antiphosphotyrosine antibody. Thus, **1** inhibits the cellular response with an $ED_{50}$ of 34 nM. In a similar cell-based receptor autophosphorylation assay using human umbilical vein endothelial cells (HUVEC), which naturally express the KDR receptor, the compound also inhibits the VEGF-induced phosphorylation with an $ED_{50}$ of 17 nM. Additionally, the compound inhibits the autophosphorylation of the PDGF receptor induced by PDGF in BALB/c 3T3 cells ($ED_{50} = 0.6\ \mu M$) as well as the c-Kit autophosphorylation, induced by stem cell factor (SCF) in Mo-7e cells ($ED_{50} \leq 0.1\ \mu M$). Again, **1** does not inhibit the autophosphorylation of other kinases like v-erbB2 (oncogene of avian erythroblastosis virus) or Ins-R (insulin receptor) in cell based assays, thus indicating that it inhibits the tyrosine kinase of growth factor receptors belonging to the same family (class III) with high selectivity compared to other kinases.

Oral application to mice of 50 mg/kg of **1** as a solution in water resulted in peak levels of ≥ 20 $\mu$M drug at ≈30 min after dosage. Even after 4 h, concentrations were still above 3 $\mu$M. This observation proves that **1** is orally well absorbed. Additional salt forms of its free base, **CGP 79787** (see Chart 1), were prepared: Among them, the succinate **CGP 79787D** has the best physicochemical properties, and when formulated in DMSO/Tween 80, it was also well absorbed orally. In vivo studies with **CGP 79787D** which demonstrate that it inhibits angiogenesis, tumor growth, and the occurrence of metastasis are described in a separate publication.[14]

Assuming that **CGP 79787** exerts its inhibitory action by binding to the ATP cleft of the enzyme, a reasonable hypothesis considering that the available X-ray crystal structures of protein kinases indicate that no other binding pockets usually exist in their catalytic domains, we modeled its binding mode. Thus, **CGP 79787** was docked in a model of the ATP binding site of KDR constructed using the available X-ray structures[15] of the

**Table 1.** Inhibition of VEGF-R Tyrosine Kinases by the Phthalazine Derivatives **CGP 79787D**, **37**−**46**, **51**, and **52**[a]

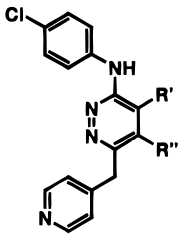


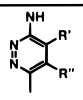
| cpd |  |  | X | formula | enz. inhibition $IC_{50}$ [μM] Flt-1 | KDR |
|---|---|---|---|---|---|---|
| CGP 79787D | | | NH | $C_{20}H_{15}ClN_4 \cdot C_4H_6O_4$ | 0.077 | 0.037 |
| 37 | | | NMe | $C_{21}H_{17}ClN_4$ | 2.3 | 3.5 |
| 38 | | | O | $C_{20}H_{14}ClN_3O$ | 0.06 | 1.6 |
| 39 | | | S | $C_{20}H_{14}ClN_3S$ | 0.07 | 1.3 |
| 40 | |  | NH | $C_{20}H_{15}ClN_4 \cdot HCl$ | 9.8 | >10 |
| 41 | |  | NH | $C_{20}H_{15}ClN_4$ | >10 | >10 |
| 42 | |  | NH | $C_{19}H_{14}ClN_5$ | 0.76 | 0.41 |
| 43 |  |  | NH | $C_{21}H_{17}ClN_4$ | 0.62 | 0.24 |
| 44 | |  | NH | $C_{22}H_{19}ClN_4$ | >10 | >10 |
| 51 | |  | NH | $C_{20}H_{16}ClN_5$ | 2.6 | 4.7 |
| 52 | |  | NH | $C_{21}H_{17}ClN_4$ | 0.42 | 0.21 |
| 45 |  |  | NH | $C_{20}H_{16}ClN_5$ | 0.65 | 1.7 |
| 46 |  | | NH | $C_{21}H_{17}ClN_4 \cdot HCl$[b] | 3.0 | 4.7 |

[a] The data represent averages of at least three determinations. [b] Mixture of 6- and 7-methyl derivatives.

kinase domain of the fibroblast growth factor receptor 1 (FGFR1), another class III growth factor receptor. The putative binding mode of **CGP 79787** in the ATP binding cleft of KDR (shown in Chart 2) results from extensive docking analyses aimed at identifying a model consistent with the structural complementarity between the inhibitor and the cleft as well as the available structure−activity relationships.[16] This homology model for the ATP binding site was recently validated by the published X-ray of an unligated KDR by Agouron.[17] According to our hypothesis, **CGP 79787** does not form direct hydrogen bonds with the peptide backbone of the hinge region as does ATP and many reported kinase inhibitors[18] but rather occupies the hydrophobic regions of the binding site. The anilino moiety is located in the hydrophobic pocket formed by residues Val 914, Val 912, Val 897, Leu 887, Cys 1043, Phe 1045 and the hydrocarbon part of the side chain of Lys 866,[19] while the phthalazine bicycle makes hydrophobic contacts with Leu 1033, Gly 920, and Leu 838. Although no direct hydrogen bonds with the hinge region are possible in this binding orientation, the anilino NH group of the inhibitor is located at distances from the backbone of Glu 915 and Cys 917, allowing water-mediated hydrogen bonds to be formed (Glu 915 and Cys 917 are the residues of the hinge region that should be involved in bidentate hydrogen bonding with the adenine ring of ATP). In addition, the essential pyridyl nitrogen of the inhibitor is assumed to be engaged in a hydrogen bond with the side chain of Lys 1060, a residue belonging to the activation loop of the kinase. Lys 1060 is not conserved outside the tyrosine kinases of the PDGF

**Table 2.** Enzyme Inhibition of the 1-(4-Chloroanilino)-4-(4-pyridylmethyl)pyridazine Derivatives **47**−**50**, **53**, and **54**[a]



| cpd |  | formula | enz. inhibition $IC_{50}$ [μM] Flt-1 | KDR |
|---|---|---|---|---|
| **47** |  | $C_{19}H_{14}ClN_5$ | 1.7 | >10 |
| **48** |  | $C_{19}H_{14}ClN_5$ | 0.47 | 1.4 |
| **49** |  | $C_{19}H_{14}ClN_5$ | 0.23 | 0.61 |
| **50** |  | $C_{19}H_{14}ClN_5$ | 0.24 | 0.13 |
| **53** |  | $C_{20}H_{19}ClN_4$ | 3.6 | >10 |
| **54** |  | $C_{18}H_{17}ClN_4$ | 0.26 | 0.87 |

[a] The data represent averages of at least three determinations.

family (PDGF-R, c-Kit, KDR, Flt-1, Flk-1) and therefore may contribute to the selective recognition of **CGP 79787** by the members of this family. However, on the basis of the model one cannot completely rule out an alternative hydrogen bond with either the proximal Asn 1031 or a residue of the glycine rich loop which may be prone to conformational rearrangement.[15b]

Tables 1−3 summarize results of structure−activity investigations on the anilinophthalazine lead, probing the active site in view of model validation by modifying the bulk or the donor−acceptor properties first on the aniline-NH, then on the pyridyl group, the benzene ring, and finally in the aniline substitution pattern. The aniline-NH is important for good inhibition of KDR (see **37**−**39** in Table 1). Even though the ether derivative **38** and the thioether **39** retain the activity against Flt-1, it appears that for good KDR inhibition a secondary NH is necessary. The position of the pyridine nitrogen is crucial for activity, since both the 3- and 2-pyridyl derivatives (**40**, **41**) are inactive. The 4-pyrimidyl derivative **42**, which retains the 4-pyridine nitrogen, still moderately inhibits the kinases. To hinder interactions between cytochrome $P_{450}$ isozymes and the pyridine-nitrogen, the ortho methyl-derivatives **43** and **44** were prepared. The one methyl group of **43** is tolerated ($^{KDR}IC_{50}$ = 0.24 μM); the dimethyl derivative **44** is inactive at concentrations up to 10 μM, however. Whereas the extension of the pyridyl-methyl side chain by an extra nitrogen atom (**51**) decreases the activity significantly, branching the pyridyl-methyl side chain (**52**) does not result in a tremendous loss in activity ($^{KDR}IC_{50}$ = 0.21 μM; for the racemate). A polar substituent on the phthalazine core (**45**) leads to a weak inhibitor of KDR, whereas phthalazines bearing lipophilic substitutents (e.g. **46**) are weak inhibitors of both VEGF-receptor kinases.

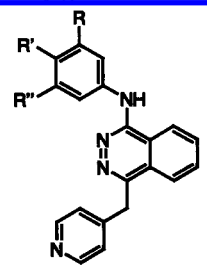
The effects of substitutions in the condensed benzene ring of the phthalazine are shown in Table 2: Introduction of a hydrogen-bond acceptor to possibly pick up additional interactions with the enzyme by the systematic replacements of ring carbons by nitrogens (**47**−**50**) reduced the activity, the only well-tolerated substitution for KDR-inhibition being the 5-aza one (**50**). Increasing the steric demand by hydrogenation of the benzene ring to the tetrahydrophthalazine **53** also reduced activity. However, the simple monocyclic analogue (**54**) retains some activity against Flt-1.

As previously mentioned, **CGP 79787D** also inhibits other class III tyrosine kinases, like the PDGF receptor kinase and c-Kit (see Table 3). By modification of the aniline part of the 1-anilino-(4-pyridylmethyl)phthalazine we attempted to retain the activity against the VEGF receptor tyrosine kinases and at the same time improve the selectivity with respect to the PDGF receptor and c-Kit: The unsubstituted aniline derivative **55** is a much weaker inhibitor of KDR than the 4-chloro derivative **CGP 79787D**. The 4-chlorine can be replaced by alkyl groups as large as the *tert*-butyl group in either the 3- or 4-position of the aniline phenyl ring (**56**, **57**) or a phenyl (**58**). Whereas the 3-methoxy derivative **59** is a reasonable inhibitor for both VEGF receptor kinases, the 3-hydroxy derivative **60** potently inhibits only Flt-1. Disubstituted aniline derivatives retain good inhibitory activity against KDR tyrosine kinase (**61**−**66**), whereas their activity on the Flt-1 tyrosine kinase is slightly reduced for **64** and **65**. The herein described disubstituted derivatives tend to be also slightly more selective toward c-Kit. Most strikingly, compounds **65** and **66** no longer block c-Kit nor the PDGF receptor tyrosine kinase.

In cellular profiling, the potent KDR receptor tyrosine kinase inhibitors **56**, **57**, **59**, and **61**−**66** are at least as potent as **CGP 79787D**. Among them, **56**, **57**, and **63** turned out to be clearly superior in inhibiting the autophosphorylation process. Preliminary pharmacokinetic studies (50 mg/kg in mice, formulated in DMSO/Tween 80) showed that the described phthalazine derivatives are comparably well-absorbed after oral administration. The low concentrations of parent compound observed for the hydroxyphenyl derivative **60** are the result of an apparently good oral absorption followed by a rapid metabolism. Although the metabolite has not been identified, by retention time it was more polar than the parent, and peak heights of the metabolite exceeded those of the parent by at least 10-fold. The high levels of the metabolite in the circulation indicate that low bioavailability of the parent was the result of metabolic clearance rather than poor absorption.

In direct comparison with **SU 5416**,[8] the phthalazine derivatives summarized in Table 3 are equipotent against KDR on the basis of $IC_{50}$ values. Some derivatives are more selective VEGF receptor tyrosine kinase

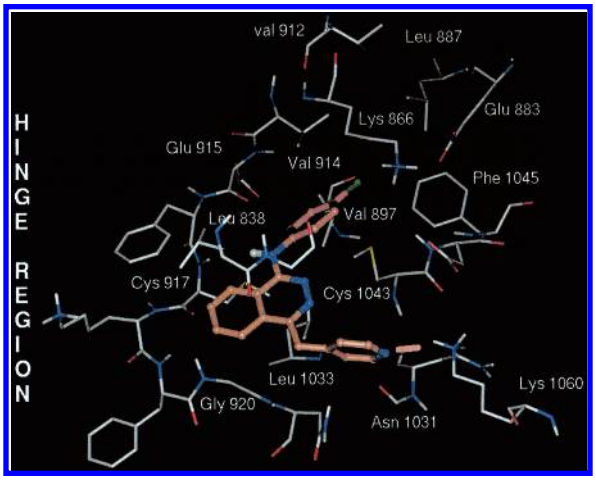
**Table 3.** Enzyme Inhibition of the 1-Anilino-(4-pyridylmethyl)phthalazine Derivatives **CGP 79787D**, **55**−**66**, and **SU 5416**[a]




| cpd | R | R' | R'' | enzymatic inhibition $IC_{50}$ [μM] Flt-1 | KDR | PDGF[b] | c-Kit | Cellular[c] $ED_{50}$ [nM] KDR | Pharmacokin. $C_{max.}$[d] [mM] | $t_{max.}$ [min] |
|---|---|---|---|---|---|---|---|---|---|---|
| **CGP 79787D** | H | Cl | H | 0.077 | 0.037 | 0.6 | 0.7 | 34 | 32 | 15 |
| **55**[e] | H | H | H | 0.06 | 0.95 | 2.8 | >10 | 200 | | |
| **56** | Me | H | H | 0.04 | 0.08 | 1 | 2.5 | 8 | 13 | 30 |
| **57**[e] | H | $^t$Bu | H | 0.24 | 0.21 | 2 | 1 | 9 | 25.6 | 30 |
| **58** | H | Ph | H | 0.23 | 0.20 | 1.4 | 1.4 | 85 | | |
| **59** | OMe | H | H | 0.33 | 0.24 | 0.8 | 3 | 37 | 21 | 30 |
| **60** | OH | H | H | 0.08 | 0.67 | 4 | 7.9 | 250 | 0.5[f] | 30 |
| **61** | Cl | Cl | H | 0.07 | 0.03 | 0.6 | 0.7 | 27 | 9.8 | 30 |
| **62** | OMe | Cl | H | 0.26 | 0.14 | 2 | 3.5 | 24 | 8.0 | 30 |
| **63** | Me | H | Me | 0.15 | 0.04 | 0. 8 | 2.0 | 10 | 10.0 | 30 |
| **64** | $CF_3$ | Cl | H | 0.6 | 0.3 | 4 | 2.7 | 27 | 7.6 | 120 |
| **65** | $CF_3$ | H | Br | 0.6 | 0.38 | >10 | >10 | 39 | 6.0 | 120 |
| **66** | $CF_3$ | H | F | 0.35 | 0.20 | >10 | >10 | 40 | | |
| **SU 5416** | | | | 0.008 | 0.20 | 0.68 | 0.4 | 930 | 0.3 | 30 |

[a] The data represent averages of at least three determinations. [b] PDGF-$\beta$ receptor. [c] Inhibition of VEGF-driven cellular receptor autophosphorylation in CHO cells transfected with the KDR receptor. [d] The pharmacokinetic studies were performed in mice: Drug concentrations in blood samples were analyzed by reversed-phase HPLC 30, 60, 90, and 120 min after oral application of 50 mg/kg in a standardized formulation (DMSO/Tween 80). The value $c_{max}$ represents the highest observed drug concentration at the indicated time point ($t_{max.}$). [e] Dihydrochloride salt. [f] Low concentration of the parent compound but an apparently high concentration (not determined) of an unknown metabolite.

**Chart 2.** Proposed Binding Mode of **CGP 79787** to KDR




inhibitors with respect to other class III tyrosine kinases, however. In our experiments, they are also more potent inhibitors of cellular KDR autophosphorylation than **SU 5416**. Additionally and in contrast to **SU 5416**, the 1-anilino-(4-pyridylmethyl)phthalazine class of compounds is orally well-absorbed, producing drug concentrations in the upper micromolar range upon dosing of 50 mg/kg to mice. This clearly qualifies them for further profiling as antiangiogenic drugs.

## Experimental Section

**Enzyme Assays.** The kinase domains of Flt-1, KDR, c-Kit, and PDGF-$\beta$ were expressed as GST-fusion proteins using the baculovirus system. The in vitro kinase assays were performed in 96-well plates using the recombinant GST-fused kinase domains expressed in baculovirus and purified over glutathione-Sepharose. $^{33}$P-ATP (Amersham) was used as the phosphate donor, and the polyGluTyr (4:1) peptide (Sigma) was used as the acceptor. For PDGF-$\beta$ activity, autophosphorylation was measured.

The buffer conditions were optimized for each kinase: Flt-1 (20 mM Tris-HCl, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 8 $\mu$M ATP, 0.2 $\mu$Ci $^{33}$P-ATP, 3 $\mu$g/mL polyGluTyr); KDR (20 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 1 mM $MnCl_2$, 8 $\mu$M ATP, 0.2 $\mu$Ci $^{33}$P-ATP, 8 $\mu$g/mL polyGluTyr); c-Kit (20 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 2 mM $MnCl_2$, 1 $\mu$M ATP, 0.2 $\mu$Ci $^{33}$P-ATP, 5 $\mu$g/mL polyGluTyr); PDGF-$\beta$ (50 mM Hepes, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 $\mu$M $Na_3VO_4$, 1 mM dithiotreitol, 0.1 $\mu$M ATP, 0.2 $\mu$Ci $^{33}$P-ATP. The reaction was carried out in a

volume of 30 $\mu$l for 10 min at room temperature in the presence of either 1% DMSO or the compound at the required concentration in 1% DMSO. The reaction was stopped by the addition of ethylenediaminetetraacetic acid to a final concentration of 60 mM. The assay mixture was then transferred onto a Immobilon-PVDF membrane (Millipore), which was subsequently washed four times with 0.05% $H_3PO_4$ and once with ethanol. After drying, 10 $\mu$l/well of Microscint cocktail (Packard) was added and scintillation counting was performed (Hewlett-Packard Top Count).

$IC_{50}$ values were calculated by linear regression analysis of the percentage inhibition of each compound in duplicate, at three concentrations (usually, 0.01, 0.1, and 1 $\mu$M or 0.1, 1, and 10 $\mu$M).

**Cellular KDR Kinase Inhibition Assay.** CHO cells, permanently transfected with the VEGF receptor tyrosine kinase KDR were seeded in six-well plates and grown to 80% confluency. Incubation with serial dilutions of compound for 2 h at 37 °C in medium without FCS was followed by addition of VEGF (final concentration 20 ng/mL). After 5 min incubation (37 °C) the cells were washed twice with ice-cold PBS and lysed in 100 $\mu$L per well lysis buffer (50 mM Tris/HCl pH 7.4; 150 mM NaCl; 5 mM EDTA; 1 mM EGTA, 1.5 mM $MgCl_2$; 2 mM Na−vanadate; 1% NP40; 10% glycerol; 1mM PMSF; 80 $\mu$g/mL aprotinin; 50 $\mu$g/mL leupeptin). Nuclei were removed by full speed centrifugation for 10 min at 4 °C using an Eppendorf centrifuge. Protein concentrations of the lysates were determined with the Bio-Rad $D_C$ protein assay kit using BSA as standard. For the second part of the assay a monoclonal antibody to the extracytoplasmic domain of the VEGF receptor was coated to black ELISA plates (OptiPlate HTRF-96; Packard) as capture antibody. After blocking with 1% BSA in PBS, the cell lysates (20 $\mu$g of protein per well) were added in triplicates together with PY-20(AP), an alkaline phosphatase-labeled anti-phosphotyrosine antibody (Transduction Laboratories). After overnight incubation at 4 °C the bound PY-20(AP) was detected with a luminescent alkaline phosphatase substrate (CDP-Star Ready to use with Emerald II; TROPIX). Luminescence was determined using a Packard Top Count microplate scintillation counter. The difference between the signals obtained from unstimulated cells (negative control) and VEGF-stimulated cells (positive control) was considered as 100% VEGF-induced KDR autophosphorylation. The influence of a compound on the VEGF-induced KDR autophosphorylation was expressed as "% inhibition"; the inhibitory activities of different compounds were compared by determination of $ED_{50}$ (50% effective dose) values from dose−response curves.

**Inhibition of PDGF-Stimulated Protein-Tyrosine Phosphorylation.** Inhibition of PDGF-stimulated total cellular tyrosine phosphorylation in Balb/c 3T3 cells was measured using a microtiter ELISA assay.[20] Briefly, Balb/c A31 cells were grown to confluency in collagen IV coated 96-well tissue culture plates. After removal of the medium, cells were treated with 2-fold serial dilutions of compounds in starvation medium (Dulbecco's minimal essential medium, 0.1% bovine serum albumine). Following 2 h incubation at 37 °C, PDGF was added to a final concentration of 50 ng/mL. After 10 more minutes at 37 °C, medium was removed, cells were fixed in cold (−20 °C) methanol, washed with phosphate-buffered saline (PBS), and blocked with PBS containing 0.1% Tween 20 and 3% bovine serum albumine. Subsequently, the cells were incubated with the monoclonal anti-phosphotyrosine antibody 4G10,[21] followed by incubation with an alkaline phosphatase labeled anti-mouse IgG antibody (SIGMA A3688). Bound antibody was detected using *p*-nitrophenyl phosphate (SIGMA) as substrate. Color development was monitored in an ELISA reader at 405 nm.

**Determination of Ligand Induced c-Kit Autophosphorylation.** MO7e cells are a human promegakaryocytic leukemia cell line that requires either granulocyte macrophage colony stimulating factor (GM-CSF), interleukin-3 (IL-3), or SCF for proliferation and were obtained from Grover Bagby, Oregon Health Sciences University. Cells were cultured in RPMI 1640 media supplemented with 10% FCS and 2.5 ng/mL of GM-CSF. Serum-starved MO7e cells (growth in serum-free medium for 16 h) were incubated with drug for 90 min prior to stimulation with SCF (50 ng/mL) for 10 min. Equal amounts of protein of cell lysates were analyzed by Western blotting using 4G10 anti-phosphotyrosine antibodies.[21] Bound antibodies were detected using the ECL Western blotting system from Amersham.

**Bioavailability.** The compounds were dissolved in DMSO to a concentration of 100 mg/mL. The stock solution was diluted 1:20 with an aqueous solution of 1% Tween 80. After brief, low-power sonication, a milky, homogeneous suspension was obtained. Then the formulated compounds were given to female MAG mice that had free access to food and water throughout the experiments. The mice received an average dose of 50 mg compound per kilogram by gavage. At allotted times four mice were sacrificed, heart blood collected into heparinised tubes, and plasma prepared by centrifugation. The plasma was either analyzed immediately or stored frozen at −20 °C. For analysis, the heparinized plasma samples were deproteinated by the addition of an equal volume of acetonitrile. After thorough mixing, the tubes were allowed to stand for 20−30 min at room temperature, and the precipitated protein was removed by centrifugation (10000*g*, 5 min.). The supernatant was collected and analyzed by reversed phase HPLC: 100 $\mu$L of the supernatant was injected onto a Nucleosil C18, 5 $\mu$m analytical column (125 × 4 mm) with a 8 mm guard column; mobile phase, 5% acetonitrile/0.05% trifluoroacetic acid (TFA) in water/0.05% TFA → 80% acetonitrile/0.05% TFA during 15 min + 5 min 100% acetonitrile/0.05% TFA. Compounds were detected by UV absorbance and identified on the chromatograms by retention time and UV spectrum compared to control plasma spiked with compound. Quantitation was by the external standard method using peak heights to quantitate the amounts by reference to a calibration curve. The calibration curve was constructed by the analysis of plasma samples containing known amounts of the compound under evaluation, which had been processed as described above. The limit of quantitation was 0.1−0.2 $\mu$mol/L (compound dependent) under these conditions.

**Chemistry**. All reactions with air- or moisture-sensitive reactants and solvents were carried out under nitrogen atmosphere. In general, reagents and solvents were used as purchased without further purification. THF was freshly distilled from sodium/benzophenone. Column flash chromatography was performed on silica gel 60 (230−400 mesh ASTM, E. Merck) under a positive nitrogen pressure of approximately 0.4 atm. Melting points were determined in an open capillary and are not corrected. $^1$H NMR spectra were collected with Bruker DRX-500 (500 MHz), Bruker AM-360 (360 MHz), Varian Gemini-300 (300 MHz), or Varian Gemini-200 (200 MHz) instruments; chemical shifts of signals are expressed in parts per million (ppm) and are referenced to the deuterated solvents used. MS spectra were collected with an FAB-ZAB, HF (VG Analytical). Elemental analyses were performed by the Ciba Analytical Department and are within ±0.4% of the calculated values.

**Abbreviations:** rt, room temperature; DMPU, 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone.

**3-Hydroxy-2-(pyridin-4-yl)inden-1-one (2).** To an ice-cooled solution of phthalide (201.8 g, 1.50 mol), 4-pyridinecarboxaldehyde (141 mL, 1.50 mol), and ethyl propionate (750 mL) in methanol (1.5 L) was portionwise added sodium methanolate (243 g, 4.5 mol). After 15 min at rt the reaction mixture was heated to 65 °C for 2.5 h and then cooled back to rt. Concentration under reduced pressure and dilution with water (7 L) gave a dark red solution which was extracted with diethyl ether (5 × 2.5 L). Acidification of the aqueous layer with acetic acid (350 mL) and stirring produced a yellowish suspension. Filtration and washing with water (15 L), ethyl acetate (5 L), and finally diethyl ether (3 L) gave 174 g (52%) of **2**: mp 307−308 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.72 (d, 2H), 8.18 (d, 2H), 7.50 (m, 4H); FAB MS $(M + H)^+ = 224$. Anal. ($C_{14}H_9NO_2 \cdot 0.50H_2O$) C, H, N, $H_2O$.

**3-Hydroxy-2-(pyridin-3-yl)-inden-1-one (3).** Preparation from 3-pyridinecarbaldehyde as described for **2** yielded 61.1 g (68%) of **3**: $^1$H NMR (DMSO-$d_6$) $\delta$ 9.75 (s, 1H), 9.45 (d, 1H), 8.23 (d, 1H), 7.80 (dd, 1H), 7.38 (m, 2H), 7.32 (m, 2H); FAB MS $(M + H)^+ = 224$. Anal. ($C_{14}H_9NO_2 \cdot 0.12\ H_2O$) C, H, N.

**3-Hydroxy-2-(pyridin-2-yl)-inden-1-one (4).** A mixture of phthalic anhydride (98.8 g, 0.667 mol) and 2-methylpyridine (130 mL, 1.33 mol) was heated to 140 °C for 15 min and then to 180 °C for 22 h. Recrystallization from ethanol (200 mL) gave 34.4 g (23%) of **4**: mp 288 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.52 (d, 1H), 8.33 (d, 1H), 8.09 (t, 1H), 7.58 (m, 4H), 7.17 (t, 1H); FAB MS $(M + H)^+ = 224$. Anal. ($C_{14}H_9NO_2$) C, H, N.

**3-Hydroxy-2-(pyrimidin-4-yl)-inden-1-one (5).** A mixture of phthalic anhydride (7.87 g, 53.1 mmol) and 4-methylpyrimidine (22 mL, 239 mmol) was heated to 140 °C for 1 h and then to 210 °C for 4 h. At rt the partly crystalline material was stirred in methanol (15 mL). Filtration gave 886 mg of **5**; another 394 mg of product (→1.28 g; 10%) could be isolated from the filtrate by concentration and crystallization from water (30 mL): mp 168−169 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.22 (s, 1H), 8.88 (d, 1H), 8.32 (d, 1H), 8.04 (m, 2H), 7.94 (t, 1H), 7.77 (t, 1H), 6.95 (s, 1H); FAB MS $(M + H)^+ = 225$. Anal. ($C_{13}H_8N_2O_2$) C, H, N.

**3-Hydroxy-2-(2-methylpyridin-4-yl)-inden-1-one (6).** A mixture of phthalic anhydride (27.7 g, 187 mmol) and 2,4-lutidine (21.56 mL, 187 mmol) was heated to 180 °C for 20 h. The black mass was stirred in boiling ethanol (250 mL) and filtered. $SiO_2$ was added to the filtrate, and the mixture was concentrated in vacuo to a powder. Putting this powder on top of a $SiO_2$ chromatography column and eluation with ethyl acetate/methanol 49:1 → 19:1 gave 5.7 g (13%) of **6**: $^1$H NMR (DMSO-$d_6$) $\delta$ 8.61 (dd, 1H), 8.56 (s, 1H), 8.07 (d, 1H), 7.47 (m, 4H), 2.46 (s, 3H); FAB MS $(M + H)^+ = 238$. Anal. ($C_{15}H_{11}NO_2$) C, H, N.

**3-Hydroxy-2-(2,6-dimethylpyridin-4-yl)-indene-1-one (7).** Preparation from 2,4,6-collidine as described for **6** yielded 5.44 g (11%) of **7**: $^1$H NMR (DMSO-$d_6$) $\delta$ 12.9 (sb, 1H), 8.45 (s, 2H), 7.47 (m, 4H), 2.45 (s, 6H); FAB MS $(M + H)^+ = 252$. Anal. ($C_{16}H_{13}NO_2$) C, H, N.

**3-Hydroxy-5-methyl-2-(pyridin-4-yl)-inden-1-one (9).** A mixture of 4-methylphthalic anhydride (47.5 g, 293 mmol) and 4-picoline (28.6 mL, 293 mmol) was heated to 165 °C for 18 h. The resulting material was stirred in boiling ethanol (190 mL), filtered, and washed with ethanol and diethyl ether, yielding 23.7 g (34%) of **9**: $^1$H NMR (DMSO-$d_6$) $\delta$ 8.70 (d, 2H), 8.14 (d, 2H), 7.34 (m, 2H), 7.29 (s, 1H), 2.38 (s, 3H); FAB MS $(M + H)^+ = 238$. Anal. ($C_{15}H_{11}NO_2$) C, H, N, O.

**7-Hydroxy-6-(pyridin-4-yl)-[1]pyrindin-5-one (10).** To an ice-cooled suspension of 7*H*-furo[3,4-*b*]pyridin-5-one[22] (20.27 g, 150 mmol) and 4-pyridinecarbaldehyde (14.1 mL, 150 mmol) in methanol (120 mL) and ethyl propionate (75 mL) was added sodium methanolate (5.4 M in methanol; 27.8 mL, 150 mmol). After 15 min at rt the reaction mixture was heated to 65 °C for 2 h and then cooled to rt again. The reaction mixture was diluted with water (120 mL) and filtered. To the filtrate was added acetic acid (8.57 mL) and the formed precipitate filtered off, yielding 9.2 g (27%) of **10**: FAB MS $(M + H)^+ = 225$. Anal. ($C_{13}H_8N_2O_2 \cdot 0.3H_2O$) C, H, N.

**7-Hydroxy-6-(pyridin-4-yl)-[2]pyrindin-5-one (11).** Prepared from a mixture of 1*H*-furo[3,4-*c*]pyridin-3-one and 3*H*-furo[3,4-*c*]pyridin-1-one[23] and 4-pyridinecarbaldehyde as described for **10** yielded 2.8 g (38%) of **11**: $^1$H NMR (DMSO-$d_6$) $\delta$ 8.75 (d, 1H), 8.68 (d, 2H), 8.59 (s, 1H), 8.30 (d, 2H), 7.39 (d, 1H); FAB MS $(M + H)^+ = 225$.

**3-[1-(Pyridin-4-yl)ethylidene]-3*H*-isobenzofuran-1-one (12).** Phthalic acid anhydride (25.0 g, 169 mmol), 3-pyridin-4-ylpropionic acid[24] (11.8 g, 78 mmol), sodium acetate (1.06 g, 13 mmol), and dimethylacetamide (40 mL) were stirred for 4 h at 180 °C. The reaction mixture was then poured onto a mixture of ice and 0.2 N sodium hydroxide solution (250 mL), stirred, and extracted twice with ethyl acetate. The organic phases were washed with water and brine, dried ($Na_2SO_4$), and concentrated by evaporation. Chromatography (ethyl acetate/methanol 19:1) and crystallization from ethanol yielded 2.28 g (12%) of **12** as a mixture of isomers: FAB MS $(M+H)^+ = 238$. Anal. ($C_{15}H_{11}NO_2$) C, H, N.

**3-Hydroxy-2-(pyridin-4-yl)-4,5,6,7-tetrahydroinden-1-one (13).** To an ice-cooled solution of 4,5,6,7-tetrahydro-3*H*-isobenzofuran-1-one[25] (1.48 g, 10.7 mmol) and 4-pyridinecarbaldehyde (1.01 mL, 10.7 mmol) in methanol (8.7 mL) and ethyl propionate (5.4 mL) was added sodium methanolate (5.4 M in methanol; 1.98 mL, 10.7 mmol). After 15 min at rt, the mixture was heated to 65 °C for 2 h and then cooled to rt again. The reaction mixture was concentrated under reduced pressure and the residue stirred in water (5 mL) and filtered, giving 0.62 g (25%) of **13**. The filtrate was extracted twice with diethyl ether, the aqueous layer neutralized with acetic acid, and the formed precipitate filtered off, yielding another 1.00 g (totally 66%) of **13**: mp 258−261 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.19 (d, 2H), 7.82 (d, 2H), 2.13 (m, 4H), 1.62 (m, 4H); FAB MS $(M + H)^+ = 228$. Anal. ($C_{14}H_{13}NO_2$) C, H, N.

**4-(Pyridin-4-yl)methyl-2*H*-phthalazin-1-one (15).** Heating of a suspension of **2** (174 g, 0.78 mol) in hydrazine monohydrate (750 mL) to 110 °C gave a dark red solution from which a new precipitate was formed during 8 h. Cooling to 0 °C, filtration, and washing with ethanol (150 mL) and diethyl ether (5 × 150 mL) afforded 153 g (82%) of **15**: mp 212−213 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.48 (d, 2H), 7.27 (m, 1H), 7.88 (m, 3H), 7.32 (d, 2H), 4.35 (s, 2H); FAB MS $(M + H)^+ = 238$.

**4-(Pyridin-3-yl)methyl-2*H*-phthalazin-1-one (16).** Prepared from **3** as described for **15** yielded 50.6 g (79%) of **16**: mp 188−190 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.6 (s, HN), 8.60 (s, 1H), 8.42 (dd, 1H), 8.28 (d, 1H), 8.00 (m, 1H), 7.88 (m, 2H), 7.69 (d, 1H), 7.32 (dd, 1H), 4.35 (s, 2H). Anal. ($C_{14}H_{11}N_3O$) C, H, N.

**4-(Pyridin-2-yl)methyl-2*H*-phthalazin-1-one (17).** Prepared from **4** as described for **15** yielded 22.2 g (61%) of **17**: $^1$H NMR (DMSO-$d_6$) $\delta$ 12.6 (s, HN), 8.47 (m, 1H), 8.28 (m, 1H), 8.0−7.7 (m, 4H), 7.38 (d, 1H), 7.24 (dd, 1H), 4.47 (s, 2H); FAB MS $(M + H)^+ = 238$.

**4-(Pyrimidin-4-yl)methyl-2*H*-phthalazin-1-one (18).** Heating of a suspension of **5** (1.20 g, 5.35 mmol) in hydrazine monohydrate (344.8 $\mu$L, 6.96 mmol) and ethanol (30 mL) for 5 h to 80 °C, cooling to rt, filtration, and washing with ethanol yielded 1.12 g (88%) of **18**: mp 204−206 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.60 (s, HN), 9.05 (s, 1H), 8.70 (d, 1H), 8.26 (m, 1H), 7.85 (m, 3H), 7.53 (d, 1H), 4.48 (s, 2H); FAB MS $(M + H)^+ = 239$.

**4-(2-Methylpyridin-4-yl)methyl-2*H*-phthalazin-1-one (19).** Preparation from **6** as described for **15** yielded 3.2 g (55%) of **19**: mp 183−184 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.6 (s, HN), 8.33 (d, 1H), 8.29 (m, 1H), 7.85 (m, 3H), 7.18 (s, 1H), 7.10 (d, 1H), 4.28 (s, 2H), 2.40 (s, 3H); FAB MS $(M + H)^+ = 252$. Anal. ($C_{15}H_{13}N_3O$) C, H, N.

**4-(2,6-Dimethylpyridin-4-yl)methyl-2*H*-phthalazin-1-one (20).** Preparation from **7** as described for **15** yielded 3.2 g (57%) of **20**: mp 229−230 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.6 (s, HN), 8.27 (d, 1H), 7.85 (m, 3H), 6.95 (s, 2H), 4.23 (s, 2H), 2.35 (s, 6H); FAB MS $(M + H)^+ = 266$. Anal. ($C_{16}H_{15}N_3O$) C, H, N.

**4-(Pyridin-4-yl)methyl-7-amino-2*H*-phthalazin-1-one (21a).**[11] Preparation from 5-nitro-3-hydroxy-2-(pyridin-4-yl)-inden-1-one (**8**)[26] as described for **15** yielded 17.3 g (43%) of **21a**: $^1$H NMR (DMSO-$d_6$) $\delta$ 12.1 (s, HN), 8.43 (d, 2H), 7.56 (d, 1H; NOE on dd at 7.00 ppm and s at 4.16 ppm), 7.26 (m, 3H), 7.00 (dd, 1H), 6.17 (s, $H_2N$), 4.16 (s, 2H; NOE on d at 7.56 ppm and d at 7.26 ppm); FAB MS $(M + H)^+ = 253$. Anal. ($C_{14}H_{12}N_4O$) C, H, N.

**4-(Pyridin-4-yl)methyl-7-trifluoracetamido-2*H*-phthalazin-1-one Trifluoracetate (21b).** A suspension of **21a** (500 mg, 1.98 mmol) in trifluoracetic anhydride (1.65 mL, 11.9 mmol) was stirred for 48 h at rt. The mixture was diluted with water, filtrated, and washed with water, yielding 729 mg (80%) of **21b**: $^1$H NMR (DMSO-$d_6$) $\delta$ 12.7 (s, HN), 11.8 (s, HN), 8.72 (d, 2H), 8.67 (d, 1H), 8.15 (dd, 1H), 8.03 (d, 1H), 7.78 (d, 2H), 4.53 (s, 2H); FAB MS $(M + H)^+ = 349$.

**4-(Pyridin-4-yl)methyl-6-methyl-2*H*-phthalazin-1-one and 4-(Pyridin-4-yl)methyl-7-methyl-2*H*-phthalazin-1-one (22).** Preparation from **9** as described for **15** yielded 13

g (60%) of a ca. 1:1 mixture of the regioisomers **22**: $^1$H NMR (DMSO-$d_6$) $\delta$ 12.5 (sb, HN), 8.45 (d, 2H), 8.14 (d, $HC^{6-Me}$), 8.06 (s, $HC^{7-Me}$), 7.80 (d, $HC^{7-Me}$; NOE on signals at 7.69, 7.29, and 4.30 ppm), 7.74 (s, $HC^{6-Me}$; NOE on signals at 7.31, 4.31, and 2.46 ppm), 7.69 (d, $HC^{7-Me}$), 7.64 (d, $HC^{6-Me}$), 7.31 (d, $2HC^{6-Me}$), 7.29 (d, $2HC^{7-Me}$), 4.31 (s, $H_2C^{6-Me}$), 4.30 (s, $H_2C^{7-Me}$), 2.47 (s, $H_3C^{7-Me}$), 2.46 (s, $H_3C^{6-Me}$); FAB MS $(M + H)^+ = 252$. Anal. ($C_{15}H_{13}N_3O \cdot 0.16H_2O$) C, H, N, O, $H_2O$.

**5-(Pyridin-4-yl)methyl-7*H*-pyrido[2,3-*d*]pyridazine-8-one (23) and 8-(Pyridin-4-yl)methyl-6*H*-pyrido[2,3-*d*]pyridazine-5-one (24).** Preparation from **10** as described for **15** and chromatography (ethyl acetate/methanol 19:1 → 7:3) gave 1.50 g (16%) of **24** followed by 0.92 g (10%) of **23**. **23**: $^1$H NMR (DMSO-$d_6$) $\delta$ 12.83 (s, HN), 9.04 (d, 1H), 8.46 (d, 2H), 8.33 (dd, 1H), 7.86 (dd, 1H), 7.30 (d, 2H), 4.34 (s, 2H; NOE on signals at 8.33 and 7.30 ppm). Anal. ($C_{13}H_{10}N_4O$) C, H, N. **24**: mp 246−248 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.83 (s, HN), 9.13 (dd, 1H), 8.59 (dd, 1H), 8.43 (d, 2H), 7.85 (dd, 1H), 7.29 (d, 2H), 4.38 (s, 2H; NOE on d at 7.29 ppm). Anal. ($C_{13}H_{10}N_4O$) C, H, N.

**1-(Pyridin-4-yl)methyl-3*H*-pyrido[3,4-*d*]pyridazine-4-one (25) and 4-(pyridin-4-yl)methyl-2*H*-pyrido[3,4-*d*]pyridazine-1-one (26).** Preparation from **11** as described for **15**, chromatography (toluene/2-propanol 19:1 → toluene/2-propanol/$NH_3$ concentrated 90:10:0.25 → 90:20:0.5), and crystallization from 2-propanol gave 0.71 g (25%) of **26** and 0.34 g (12%) of **25**. **25**: mp 204−208 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.93 (s, HN), 9.45 (s, 1H), 9.00 (d, 1H), 8.47 (d, 2H), 7.80 (d, 1H), 7.33 (d, 2H), 4.34 (s, 2H; NOE on d at 7.80 ppm); FAB MS $(M + H)^+ = 239$. **26**: mp 236−237 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.90 (s, HN), 9.32 (s, 1H), 8.96 (d, 1H), 8.47 (d, 2H), 8.08 (d, 1H), 7.34 (d, 2H), 4.43 (s, 2H; NOE on s at 9.32 ppm); FAB MS $(M + H)^+ = 239$.

***rac*-4-[1-(4-Pyridyl)ethyl]-2*H*-phthalazin-1-one (27).** In ethanol (50 mL), **12** (2.20 g, 9.27 mmol) and hydrazine hydrate (597 $\mu$L, 12 mmol) were boiled for 4.5 h under reflux. A white solid settled out, which was filtered off and discarded. Concentration of the filtrate and crystallization from acetonitrile gave 1.75 g (74%) of **27**: mp 202−203 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.7 (s, HN), 8.45 (d, 2H), 8.24 (m, 1H), 7.83 (m, 3H), 7.33 (d, 2H), 4.85 (q, 1H), 1.58 (d, 3H); FAB MS $(M + H)^+ = 252$. Anal. ($C_{15}H_{13}N_3O \cdot 0.15\ H_2O$) C, H, N.

**4-(Pyridin-4-yl)methyl-5,6,7,8-tetrahydro-2*H*-phthalazin-1-one (28).** Preparation from **13** as described for **15** yielded 330 mg (40%) of **28**: mp 193−194 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.6 (s, HN), 8.46 (d, 2H), 7.19 (d, 2H), 3.93 (s, 2H), 2.36 (m, 4H), 1.60 (m, 4H); FAB MS $(M + H)^+ = 242$. Anal. ($C_{14}H_{15}N_3O$) C, H, N.

**4,5-Dimethyl-6-(4-pyridylmethyl)-2*H*-pyridazine-3-one (29).** To a solution of diisopropylamine (26.1 mL, 184 mmol) in THF (200 mL) was added a 1.6 M solution of butyllithium (124 mL, 198 mmol) in THF at 0 °C. After cooling to −20 to −30 °C, a solution of 4-picoline (19.3 mL, 198 mmol) in THF (200 mL) was added dropwise and the reaction mixture was stirred for 60 min at −30 °C. A solution of maleic acid anhydride (10 g, 102 mmol) in THF (100 mL) was then added dropwise at −78 °C and the mixture was stirred for 1 h at −78 °C and for 2 h at rt. After this time the reaction mixture was diluted with 2 N HCl (500 mL) and washed twice with ethyl acetate. This aqueous layer was concentrated by evaporation and the pH adjusted to alkaline with 2 N NaOH. The solution was washed again with ethyl acetate (2×) and then reacidified with 2 N HCl and evaporated. The resulting orange residue was filtered through silica gel ($CH_2Cl_2$/MeOH 5:1) and the material thus obtained [$^1$H NMR ($CDCl_3$): $\delta$ 3.20 (s, 2H), 1.25 (s, 3H), 1.20 (s, 3H); FAB MS $(M + H)^+ = 220$] was processed without further purification.

A solution of 2 g of the crude product obtained above (**14**) and hydrazine hydrate (1.1 mL, 22 mmol) in *n*-butanol (2 mL) was heated under nitrogen for 2 h to 120 °C. After cooling to rt, the resulting emulsion was concentrated by evaporation and then, after addition of a small amount of water, extracted three times with dichloromethane. The combined organic extracts were filtered through cotton, and the solvent was evaporated. The resulting yellow oil was redissolved in dichloromethane and the product was precipitated with diisopropyl ether to yield 130 mg (6%) of **29** as light-yellow crystals: $^1$H NMR ($CDCl_3$) $\delta$ 12.3 (sb, 1H), 8.55 (dd, 2H), 7.10 (d, 2H), 3.95 (s, 2H), 2.15 (s, 3H), 2.00 (s, 3H); FAB MS $(M + H)^+ = 216$.

**1-Chloro-4-(4-pyridylmethyl)phthalazine (30).** To **15** (29 g, 122 mmol) in acetonitrile (450 mL) and HCl/dioxane (4 N; 61 mL, 244 mmol) was added phosphoryl chloride (28 mL, 306 mmol). After stirring for 27 h at 50 °C, a solution of $NaHCO_3$ (119 g) in water (1.45 L) was added to the ice-cooled white suspension. Stirring and filtration then afforded 28.7 g (92%) of **30**: $^1$H NMR (DMSO-$d_6$) $\delta$ 8.46 (d, 2H), 8.35 (m, 2H), 8.14 (m, 2H), 7.33 (d, 2H), 4.77 (s, 2H); FAB MS $(M + H)^+ = 256$. Anal. ($C_{14}H_{10}N_3Cl$) C, H, N, Cl.

**1-Chloro-4-(3-pyridylmethyl)phthalazine (31).** To **16** (50 g, 0.21 mol) in ice-cooled acetonitrile (250 mL) was added phosphoryl chloride (42.4 mL, 0.46 mol). After stirring for 10 h at 100 °C, the reaction mixture was concentrated in vacuo, and the residue diluted with dichloromethane, ice−water, and a saturated solution of $Na_2CO_3$ (0.4 L). The organic layer was separated, washed with water and brine, dried ($Na_2SO_4$), and concentrated. Stirring of the residue in ethanol and filtration then led to 24.4 g (45%) of **31**: mp 164−166 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.65 (s, 1H), 8.43 (m, 2H), 8.32 (m, 1H), 8.15 (m, 2H), 7.70 (d, 1H), 7.30 (dd, 1H), 4.77 (s, 2H). Anal. ($C_{14}H_{10}N_3$-Cl) C, H, N.

**1-Chloro-4-(4-pyrimidylmethyl)phthalazine (32):** Preparation from **18** as described for **30** yielded 750 mg (82%) of **32**: FAB MS $(M - H)^+ = 255$.

**1-Chloro-4-(4-pyridylmethyl)-7-trifluoracetamidophthalazine (33):** Preparation from **21b** as described for **30** yielded 404 mg (92%) of **33**: FAB MS $(M + H)^+ = 367$.

**1-Chloro-4-(4-pyridylmethyl)-6-methylphthalazine and 1-Chloro-4-(4-pyridylmethyl)-7-methylphthalazine (34):** Preparation from **22** as described for **30** yielded 5.0 g (82%) of **34** as a mixture of regioisomers: $^1$H NMR (DMSO-$d_6$) $\delta$ 8.45 (m, 2H), 8.20, 8.09 and 7.95 (m, s, m, 3H), 7.34 and 7.30 (2d, 2H), 4.72 (s, 2H), 2.60 and 2.59 (2s, 3H); FAB MS $(M + H)^+ = 270$.

**3-Chloro-4,5-dimethyl-6-(4-pyridylmethyl)pyridazine (36).** A solution of **29** (700 mg, 3.26 mmol) in phosphorus oxychloride (7 mL) was heated for 3 h to 120 °C. The reaction mixture was then poured onto ice water and the pH adjusted to alkaline with 2 N NaOH. The aqueous solution was extracted three times with dichloromethane. The combined organic extracts were filtered through cotton, and the solvent was evaporated. The remaining residue was purified by chromatography (dichloromethane/methanol 19:1) to yield 390 mg (51%) of **36** as brown crystals: $^1$H NMR ($CDCl_3$) $\delta$ 8.50 (dd, 2H), 7.10 (d, 2H), 4.35 (s, 2H), 2.35 (s, 3H), 2.15 (s, 3H); FAB MS $(M + H)^+ = 234$.

**1-(4-Chloroanilino)-4-(4-pyridylmethyl)phthalazine (CGP 79787).** A mixture of phosphorus pentoxide (142 g, 1.00 mol), triethylamine hydrochloride (137.6 g, 1.00 mol), and 4-chloroaniline (127.6 g, 1.00 mol) was heated to 170 °C for 40 min. To the melt was added **15** (59.3 g, 0.25 mol) during 5 min and stirring continued at 170 °C for additional 2 h. Then the heating bath was removed. Tetramethylurea (231 mL) was added during 10 min (135 °C), followed by water (830 mL; cooling) and a mixture of water (330 mL) and ammonia (30% in water; 306 mL). At 10 °C, diethyl ether (500 mL) was given to the yellow suspension. Filtration and washing with water (3 L) and diethyl ether (1 L) yielded 70.9 g (82%) of **CGP 79787**: mp 209−212 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.30 (sb, 1 H), 8.60 (d, 1H), 8.46 (d, 2H), 8.0 (m, 5H), 7.40 (d, 2H), 7.33 (d, 2H), 4.59 (s, 2H); FAB MS $(M + H)^+ = 347$. Anal. ($C_{20}H_{15}N_4$-Cl) C, H, N.

**1-(4-Chloroanilino)-4-(4-pyridylmethyl)phthalazine Succinate (CGP 79787D).** A solution of succinic acid (1.77 g, 15 mmol) in ethanol (35 mL) was added to a hot solution of **CGP 79787** (5.0 g, 14.4 mmol) in ethanol (150 mL). As the mixture cooled (scraping) to 0 °C, a crystalline precipitate slowly formed, which was filtered off and washed with ethanol, yielding 5.06 (75%) of **CGP 79787D**: mp 195 °C; $^1$H NMR

(DMSO-$d_6$) $\delta$ 12.2 (sb, 2 $HO_2C$), 9.26 (s, HN), 8.58 (d, 1H), 8.43 (d, 2H), 8.11 (d, 1H), 7.99 (m, 3H), 7.93 (m, 1H), 7.40 (d, 2H), 7.31 (d, 2H), 4.58 (s, 2H), 2.40 (s, 4H). Anal. ($C_{24}H_{21}N_4ClO_4$) C, H, N.

**1-(4-Chloroanilino)-4-(4-pyridylmethyl)phthalazine Dihydrochloride (1).** A solution of **CGP 79787** (115 g, 331 mmol) in methanol (3.25 L) was prepared by heating it up to 50 °C. Then concentrated HCl (37%, 58.05 mL) was added dropwise at 35 °C. After cooling the solution down to 10 °C, diethyl ether (3.35 L) was added. Filtration of the precipitate and washing with diethyl ether (4 L) gave 128 g (84%) of **1**: mp 272−273 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.7 (sb, HN), 9.28 (m, 1H), 8.88 (d, 2H), 8.37 (m, 1H), 8.23 (m, 2H), 8.04 (d, 2H), 7.72 (d, 2H), 7.59 (d, 2H), 5.03 (s, 2H); FAB MS $(M + H)^+$ = 347. Anal. ($C_{20}H_{15}N_4Cl \cdot 2.00\ HCl \cdot 2.17\ H_2O$) C, H, N, Cl, $H_2O$.

**(4-Chlorophenyl)methyl[4-(4-pyridylmethyl)phthalazin-1-yl]amine (37).** A solution of **30** (255 mg, 1.0 mmol) and 4-chloro-*N*-methylaniline (385 $\mu$L, 3 mmol) in DMPU (2 mL) was heated to 100 °C for 15 h. The reaction mixture was diluted with dichloromethane (20 mL) and aqueous ammonia (10%; 10 mL), and the organic layer was separated, washed twice with water (10 mL), dried ($Na_2SO_4$), and concentrated. Chromatography (ethyl acetate/methanol 50:1 → 20:1) and crystallization by adding hexane gave 75 mg (20%) of **37**: mp 128−130 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.48 (d, 2H), 8.18 (d, 1H), 7.86 (t, 1H), 7.74 (t, 1H), 7.55 (d, 1H), 7.36 (d, 2H), 7.29 (d, 2H), 6.97 (d, 2H), 4.68 (s, 2H), 3.54 (s, 3H); HR−FAB MS ($C_{21}H_{17}N_4Cl$) $(M + H)^+$ calcd 361.1220, obsd 361.1222.

**1-(4-Chlorophenoxy)-4-(4-pyridylmethyl)phthalazine (38).** A mixture of **30** (200 mg, 0.78 mmol), $K_2CO_3$ (173 mg, 1.25 mmol), and 4-chlorophenol (120 mg, 0.93 mmol) in DMSO (2 mL) was heated for 2.5 h to 90 °C. The reaction mixture was distributed between water (20 mL) and ethyl acetate (20 mL), and the aqueous phase was separated and extracted with two portions of ethyl acetate. The organic phases were washed with water and brine, dried ($MgSO_4$), and concentrated by evaporation. Chromatography (ethyl acetate/methanol 4:1) gave 96 mg (35%) of **38**: mp 207−208 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.44 (d, 2H), 8.40 (m, 1H), 8.26 (m, 1H), 8.06 (m, 2H), 7.54 (d, 2H), 7.40 (d, 2H), 7.30 (d, 2H), 4.64 (s, 2H); HR-FAB MS ($C_{20}H_{14}N_3ClO$) $(M + H)^+$ calcd 348.0904, obsd 348.0904.

**1-(4-Chlorophenylsulfanyl)-4-(4-pyridylmethyl)phthalazine (39).** Preparation as described for **38** starting from 4-chlorothiophenol gave 83 mg (29%) of **39**: mp 204−206 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.44 (d, 2H), 8.29 (m, 2H), 8.06 (m, 2H), 7.66 (d, 2H), 7.55 (d, 2H), 7.30 (d, 2H), 4.66 (s, 2H); HR-FAB MS ($C_{20}H_{14}N_3ClS$) $(M + H)^+$ calcd 364.0675, obsd 364.0679.

**1-(4-Chloroanilino)-4-(3-pyridylmethyl)phthalazine Hydrochloride (40).** A mixture of **31** (1.00 g, 3.9 mmol) and 4-chloroaniline (1.5 g, 11.7 mmol) was heated to 110 °C for 4 h. The reaction mixture was diluted with dichloromethane (10 mL) and the crystallized material filtered off. Recrystallization from methanol yielded 620 mg (41%) of **40**: $^1$H NMR (DMSO-$d_6$) $\delta$ 10.7 (sb, HN), 8.93 (m, 1H), 8.78 (s, 1H), 8.58 (d, 1H), 8.40 (m, 1H), 8.17 (m, 2H), 8.03 (d, 1H), 7.80 (d, 2H), 7.56 (m, 3H), 4.77 (s, 2H); FAB MS $(M + H)^+$ = 347. Anal. ($C_{20}H_{15}N_4Cl \cdot HCl$) C, H, N.

**1-(4-Chloroanilino)-4-(2-pyridylmethyl)phthalazine (41).** Preparation as described for **CGP 79787** starting from **17**, chromatography (ethyl acetate), and crystallization from ethyl acetate gave 309 mg (53%) of **41**: mp 154−156 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.25 (s, 1H), 8.58 (d, 1H), 8.45 (d, 1H), 8.16 (m, 1H), 7.97 (m, 4H), 7.69 (m, 1H), 7.40 (d, 2H), 7.32 (d, 1H), 7.20 (dd, 1H), 4.70 (s, 2H); FAB MS $(M + H)^+$ = 347. Anal. ($C_{20}H_{15}N_4Cl$) C, H, N.

**1-(4-Chloroanilino)-4-(4-pyrimidylmethyl)phthalazine (42).** Preparation as described for **40** starting from **32** and chromatography (ethyl acetate/methanol 19:1) gave 14 mg (10%) of **42**: $^1$H NMR (DMSO-$d_6$) $\delta$ 9.28 (s, 1H), 9.05 (s, 1H), 8.68 (d, 1H), 8.59 (m, 1H), 8.13 (m, 1H), 7.95 (m, 4H), 7.48 (d, 1H), 7.38 (d, 2H), 4.72 (s, 2H); HR-FAB MS ($C_{19}H_{14}N_5Cl$) $(M + H)^+$ calcd 348.1016, obsd 348.1015.

**1-(4-Chloroanilino)-4-[2-methylpyridin-4-ylmethyl]-phthalazine (43).** Preparation as described for **CGP 79787** starting from **19**, chromatography (ethyl acetate/methanol 19:1), and crystallization from acetonitrile gave 193 mg (34%) of **43**: mp 158−159 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.27 (s, 1H), 8.59 (d, 1H), 8.30 (d, 1H), 8.10 (m, 1H), 7.97 (m, 4H), 7.40 (d, 2H), 7.18 (s, 1H), 7.10 (d, 1H), 4.53 (s, 2H), 2.39 (s, 3H); FAB MS $(M + H)^+ = 361$. Anal. ($C_{21}H_{17}N_4Cl$) C, H, N.

**1-(4-Chloroanilino)-4-[2,6-dimethylpyridin-4-ylmethyl]-phthalazine (44).** Preparation as described for **CGP 79787** starting from **20**, chromatography (ethyl acetate/methanol 19:1), and crystallization from acetonitrile gave 983 mg (62%) of **44**: mp 175−176 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.27 (s, 1H), 8.60 (d, 1H), 8.0 (m, 5H), 7.40 (d, 2H), 6.97 (s, 2H), 4.48 (s, 2H), 2.35 (s, 6H); FAB MS $(M + H)^+ = 375$. Anal. ($C_{22}H_{19}N_4Cl$) C, H, N.

**1-(4-Chloroanilino)-4-(4-pyridylmethyl)-7-amino-phthalazine (45).** A mixture of **33** (11 g, 30 mmol), 4-chloroaniline (9.2 g, 72 mmol), and *n*-butanol (90 mL) was heated to 100 °C for 4 h. The reaction mixture was diluted at rt with 5% $Na_2CO_3$ solution (200 mL) and ethyl acetate (250 mL). The aqueous layer was separated off and extracted with ethyl acetate (200 mL). The organic phases were washed with water (4 × 100 mL), dried ($Na_2SO_4$), and concentrated by evaporation. Chromatography (dichloromethane/methanol 9:1) gave 2.2 g (20%) of **45**: mp 277−279 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.86 (s, HN), 8.42 (d, 2H), 7.88 (d, 2H), 7.80 (d, 1H), 7.33 (d, 2H), 7.28 (d, 2H), 7.26 (d, 1H), 7.15 (dd, 1H), 6.15 (s, $H_2N$), 4.40 (s, 2H); FAB MS $(M + H)^+ = 362$. Anal. ($C_{20}H_{16}N_5Cl \cdot 0.2\ H_2O$) C, H, N, Cl.

**1-(4-Chloroanilino)-4-(4-pyridylmethyl)-6-methylphthalazine and 1-(4-Chloroanilino)-4-(4-pyridylmethyl)-7-methylphthalazine Dihydrochloride (46).** A mixture of **34** (500 mg, 1.85 mmol), 4-chloroaniline (248 mg, 1.94 mmol), ethanol (66 mL), and HCl (4 N in dioxane; 0.5 mL) was heated for 3 h to 80 °C. Upon standing at rt, product crystallized slowly from the red solution. Filtration and washing with ethanol and diethyl ether gave 433 mg (52%) of **46** as a ca. 2:3 mixture of the 6-methyl and the 7-methyl derivative: $^1$H NMR (DMSO-$d_6$) $\delta$ 11.2 (sb, HN), 8.96 (d, $HC^{6-Me}$), 8.92 (s, $HC^{7-Me}$), 8.81 (d, $2HC^{6-Me}$), 8.80 (d, $2HC^{7-Me}$), 8.26 (d, $HC^{7-Me}$), 8.20 (s, $HC^{6-Me}$), 8.07 (d, $HC^{6-Me}$), 8.03 (d, $HC^{7-Me}$), 7.93 (d, $2HC^{6-Me}$), 7.90 (d, $2HC^{7-Me}$), 7.70 (d, $2HC^{6-Me}$), 7.68 (d, $2HC^{7-Me}$), 7.57 (d, $2HC^{6-Me}$ + $2HC^{7-Me}$), 4.94 (s, $H_2C^{7-Me}$), 4.91 (s, $H_2C^{6-Me}$), 2.63 (s, $H_3C^{7-Me}$), 2.61 (s, $H_3C^{6-Me}$); FAB MS $(M + H)^+ = 361$. Anal. ($C_{21}H_{17}N_4Cl \cdot 1.94HCl \cdot 0.93H_2O$) C, H, N, Cl.

**8-(4-Chloroanilino)-5-(4-pyridylmethyl)pyrido[2,3-*d*]-pyridazine (47).** Preparation as described for **CGP 79787** starting from **23**, chromatography (ethyl acetate/methanol 50:1 → 25:1), and crystallization from acetonitrile gave 268 mg (43%) of **47**: mp 196−197 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.75 (s, HN), 9.23 (dd, 1H), 8.60 (dd, 1H), 8.46 (d, 2H), 8.25 (d, 2H), 8.00 (dd, 1H), 7.41 (d, 2H), 7.35 (d, 2H), 4.61 (s, 2H); FAB MS $(M + H)^+ = 348$. Anal. ($C_{19}H_{14}N_5Cl$) C, H, N.

**4-(4-Chloroanilino)-1-(4-pyridylmethyl)pyrido[3,4-*d*]-pyridazine (48).** Preparation as described for **CGP 79787** starting from **25**, chromatography (ethyl acetate/methanol 100:1 → 9:1), and crystallization from acetonitrile and methanol gave 90 mg (19%) of **48**: $^1$H NMR (DMSO-$d_6$) $\delta$ 9.96 (s, 1H), 9.63 (s, HN), 9.00 (d, 1H), 8.47 (d, 2H), 8.00 (d, 2H), 7.97 (d, 1H), 7.43 (d, 2H), 7.32 (d, 2H), 4.59 (s, 2H); HR-FAB MS ($C_{19}H_{14}N_5Cl$) $(M + H)^+$ calcd 348.1016, obsd 348.1017.

**1-(4-Chloroanilino)-4-(4-pyridylmethyl)pyrido[3,4-*d*]-pyridazine (49).** Preparation as described for **CGP 79787** starting from **26**, chromatography (ethyl acetate/methanol 50:1 → 19:1), and crystallization from acetonitrile and methanol gave 114 mg (26%) of **49**: mp 227−228 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.59 (s, 1H), 9.47 (s, HN), 9.08 (d, 1H), 8.47 (m, 3H), 8.02 (d, 2H), 7.43 (d, 2H), 7.35 (d, 2H), 4.69 (s, 2H); HR-FAB MS ($C_{19}H_{14}N_5Cl$) $(M + H)^+$ calcd 348.1016, obsd 348.1017.

**5-(4-Chloroanilino)-8-(4-pyridylmethyl)pyrido[2,3-*d*]-pyridazine (50).** Preparation as described for **CGP 79787** starting from **24**, chromatography (ethyl acetate/methanol 50:1 → 25:1), and crystallization from acetonitrile and methanol

gave 288 mg (39%) of **50**: mp 220−222 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.46 (s, HN), 9.24 (d, 1H), 9.03 (d, 1H), 8.43 (d, 2H), 8.0 (m, 3H), 7.40 (d, 2H), 7.32 (d, 2H), 4.64 (s, 2H); FAB MS (M + H)$^+$ = 348. Anal. ($C_{19}H_{14}N_5Cl$) C, H, N.

**1-(4-Chloroanilino)-4-(4-pyridylmethylamino)phthalazine (51).** A mixture of 0.500 g (1.54 mmol) of 1-chloro-4-(4-chloroanilino)phthalazine hydrochloride (**35**)[27] and 2.00 g (18.5 mmol) of 4-aminomethylpyridine was stirred for 36 h at 90 °C. Chromatography (ethyl acetate → ethyl acetate/methanol 19:1) and crystallization from methanol gave 127 mg (23%) of **51**: mp 233−236 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.76 (s, HN), 8.44 (d, 2H), 8.38 (m, 2H), 7.97 (m, 2H), 7.80 (d, 2H), 7.75 (m, HN), 7.36 (d, 2H), 7.28 (d, 2H), 4.75 (d, 2H); FAB MS (M + H)$^+$ = 362. Anal. ($C_{20}H_{16}N_5Cl$) C, H, N.

***rac*-1-(4-Chloroanilino)-4-[1-(4-pyridyl)ethyl]phthalazine (52).** Preparation as described for **CGP 79787** starting from **27**, chromatography (ethyl acetate/methanol 19:1), and crystallization from methanol gave 145 mg (32%) of **52**: mp 132−134 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.28 (s, HN), 8.58 (d, 1H), 8.44 (d, 2H), 8.14 (d, 1H), 8.04 (d, 2H), 7.94 (m, 2H), 7.41 (d, 2H), 7.36 (d, 2H), 5.10 (q, 1H), 1.73 (d, 3H); FAB MS (M + H)$^+$ = 361. Anal. ($C_{21}H_{17}N_4Cl\cdot 0.50MeOH$) C, H, N.

**1-(4-Chloroanilino)-4-(4-pyridylmethyl)-5,6,7,8-tetrahydrophthalazine (53).** Preparation as described for **CGP 79787** starting from **28**, chromatography (ethyl acetate/methanol 40:1 → 20:1), and crystallization from acetonitrile gave 285 mg (68%) of **53**: mp 181−183 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.46 (d, 2H), 8.00 (s, HN), 7.78 (d, 2H), 7.32 (d, 2H), 7.20 (d, 2H), 4.17 (s, 2H), 2.50 (m, 4H), 1.72 (m, 4H); FAB MS (M + H)$^+$ = 351. Anal. ($C_{20}H_{19}N_4Cl$) C, H, N.

**3-(4-Chloroanilino)-4,5-dimethyl-6-(4-pyridylmethyl)-pyridazine (54).** A solution of **36** (70 mg, 0.3 mmol) and 4-chloroaniline (153 mg, 1.2 mmol) in *n*-butanol (2 mL) was heated in a sealed tube for 20 h to 130 °C. After cooling to rt, the residue was diluted with dichloromethane (100 mL) and the solution washed with saturated aqueous $NaHCO_3$ solution (100 mL). The organic layer was dried ($MgSO_4$) and the solvent removed by evaporation. Purification of the residue by chromatography (dichloromethane/methanol 19:1) gave 18 mg (19%) of **54**: mp 196−199 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.45 (sb, 2H), 7.55 (d, 2H), 7.25 (d, 2H), 7.10 (d, 2H), 6.20 (sb, 1H), 4.25 (s, 2H), 2.15 (s, 3H), 2.10 (s, 3H); HR-FAB MS ($C_{18}H_{17}N_4Cl$) (M + H)$^+$ calcd 325.1220, obsd 325.1222.

**1-Anilino-4-(4-pyridylmethyl)phthalazine Dihydrochloride (55):** A suspension of **30** (30 g, 117 mmol), aniline (11.5 g, 123 mmol), ethanol (390 mL) and HCl (4 N in dioxane; 30 mL) was heated for 2 h to 80 °C. During cooling the solution to rt, the product crystallized. Filtration and washing with ethanol gave 35 g (71%) of **55:** mp 217−220 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.8 (s, HN), 9.23 (m, 1H), 8.86 (d, 2H), 8.34 (m, 1H), 8.22 (m, 2H), 8.00 (d, 2H), 7.57 (m, 4H), 7.40 (m, 1H), 4.97 (s, 2H); FAB MS (M + H)$^+$ = 313. Anal. ($C_{20}H_{16}N_4\cdot 2HCl\cdot 1.85H_2O$) C, H, N, $H_2O$.

**1-(3-Methylanilino)-4-(4-pyridylmethyl)phthalazine (56).** A suspension of **30** (10 g, 39 mmol), *m*-toluidine (4.45 g, 41.5 mmol), ethanol (80 mL), and HCl (4 N in dioxane; 10.3 mL) was heated for 3 h to 80 °C. After cooling to rt, the precipitate was filtered off. The solid was dissolved in water (150 mL). Precipitation by adding concentrated ammonia (25 mL), filtration, and recrystallization from acetonitrile/diethyl ether finally gave 8.25 g (64%) of **56:** mp 144−146 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.08 (s, HN), 8.60 (d, 1H), 8.43 (d, 2H), 8.10 (d, 1H), 7.94 (m, 2H), 7.80 (s, 1H), 7.71 (d, 1H), 7.30 (d, 2H), 7.23 (d, 1H), 6.85 (d, 1H), 4.57 (s, 2H), 2.33 (s, 3H); FAB MS (M + H)$^+$ = 327. Anal. ($C_{21}H_{18}N_4 \cdot 0.2\ H_2O$) C, H, N.

**1-(4-*tert*-Butylanilino)-4-(4-pyridylmethyl)phthalazine Dihydrochloride (57).** Preparation from 4-*tert*-butylaniline as described for **CGP 79787**, chromatography (toluene/acetone 7:3), and crystallization as dihydrochloride salt from methanol/diethyl ether gave 520 mg (27%) of **57**: mp 196−200 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.8 (s, HN), 9.25 (m, 1H), 8.86 (d, 2H), 8.33 (m, 1H), 8.21 (m, 2H), 7.98 (d, 2H), 7.53 (m, 4H), 4.95 (s, 2H), 1.33 (s, 9H); FAB MS (M + H)$^+$ = 369. Anal. ($C_{24}H_{24}N_4\cdot 2HCl\cdot 1.3H_2O$) C, H, N, $H_2O$.

**1-(4-Biphenylamino)-4-(4-pyridylmethyl)phthalazine (58).** Preparation from 4-aminobiphenyl as described for **CGP 79787**, chromatography (dichlormethane/methanol 50:1), and crystallization from acetonitrile gave 396 mg (24%) of **58**: mp 189−191 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.29 (s, HN), 8.64 (d, 1H), 8.46 (d, 2H), 8.09 (m, 3H), 7.97 (m, 2H), 7.70 (d, 4H), 7.46 (t, 2H), 7.33 (m, 3H), 4.60 (s, 2H); FAB MS (M + H)$^+$ = 389. Anal. ($C_{26}H_{20}N_4$) C, H, N.

**1-(3-Methoxyanilino)-4-(4-pyridylmethyl)phthalazine (59).** Preparation from *m*-anisidine as described for **56** and crystallization from acetonitrile gave 10.7 g (80%) of **59**: mp 158−159 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.12 (s, HN), 8.61 (d, 1H), 8.44 (d, 2H), 8.09 (m, 1H), 7.94 (m, 2H), 7.67 (m, 1H), 7.55 (d, 1H), 7.31 (d, 2H), 7.24 (t, 1H), 6.62 (d, 1H), 4.59 (s, 2H), 3.78 (s, 3H); FAB MS (M + H)$^+$ = 343. Anal. ($C_{21}H_{18}N_4O$) C, H, N.

**1-(3-Hydroxyanilino)-4-(4-pyridylmethyl)phthalazine (60).** Preparation from 3-aminophenol and **30** as described for **40**, stirring in a mixture of aqueous $K_2CO_3$ (20%) and ethyl acetate, and washing with boiling methanol gave 260 mg (51%) of **60**: mp 217−219 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.35 (s, 1H), 9.12 (sb, 1H), 8.61 (d, 1H), 8.45 (d, 2H), 8.10 (d, 1H), 7.93 (m, 2H), 7.56 (s, 1H), 7.33 (d, 2H), 7.26 (d, 1H), 7.12 (t, 1H), 6.45 (dd, 1H), 4.57 (s, 2H); FAB MS (M + H)$^+$ = 329. Anal. ($C_{20}H_{16}N_4O \cdot 0.36\ H_2O$) C, H, N, $H_2O$.

**1-(3,4-Dichloroanilino)-4-(4-pyridylmethyl)phthalazine (61).** Preparation from 3,4-dichloroaniline as described for **56** gave 3.2 g (84%) of **61**: mp 254−255 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.44 (s, HN), 8.60 (d, 1H), 8.45 (m, 3H), 8.15 (d, 1H), 7.97 (m, 3H), 7.59 (d, 1H), 7.32 (d, 2H), 4.60 (s, 2H); FAB MS (M + H)$^+$ = 381/383. Anal. ($C_{20}H_{14}N_4Cl_2$) C, H, N.

**1-(3-Methoxy-4-chloroanilino)-4-(4-pyridylmethyl)-phthalazine (62).** Preparation from 3-methoxy-4-chloroaniline as described for **56** and washing in boiling acetonitrile gave 8.1 g (76%) of **62**: mp 243−244 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.27 (s, HN), 8.61 (d, 1H), 8.44 (d, 2H), 8.12 (m, 1H), 7.97 (m, 2H), 7.86 (m, 1H), 7.72 (dd, 1H), 7.37 (d, 1H), 7.32 (d, 2H), 4.60 (s, 2H), 3.88 (s, 3H); FAB MS (M + H)$^+$ = 377. Anal. ($C_{21}H_{17}N_4ClO$) C, H, N.

**1-(3,5-Dimethylanilino)-4-(4-pyridylmethyl)phthalazine (63).** Preparation from 3,5-dimethylaniline as described for **CGP 79787** and crystallization from acetonitrile gave 820 mg (57%) of **63**: mp 174−175 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.02 (s, HN), 8.60 (d, 1H), 8.46 (d, 2H), 8.09 (m, 1H), 7.97 (m, 2H), 7.58 (s, 2H), 7.32 (d, 2H), 6.68 (s, 1H), 4.57 (s, 2H), 2.30 (s, 6H); FAB MS (M + H)$^+$ = 341. Anal. ($C_{22}H_{20}N_4$) C, H, N.

**1-(3-Trifluormethyl-4-chloroanilino)-4-(4-pyridylmethyl)phthalazine (64).** Preparation from 5-amino-2-chlorobenzotrifluoride as described for **56** and washing in boiling methanol gave 11.6 g (70%) of **64**: mp 218−219 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.6 (s, HN), 8.60 (m, 2H), 8.46 (d, 2H), 8.37 (dd, 1H), 8.16 (d, 1H), 8.0 (m, 2H), 7.70 (d, 1H), 7.32 (d, 2H), 4.62 (s, 2H); FAB MS (M + H)$^+$ = 415. Anal. ($C_{21}H_{14}N_4ClF_3$) C, H, N.

**1-(3-Trifluormethyl-5-bromoanilino)-4-(4-pyridylmethyl)phthalazine (65).** 3-Amino-5-bromobenzotrifluoride (10.1 g, 42 mmol) and **30** (10.23 g, 40 mmol) in ethanol (160 mL) and HCl (4 N in dioxane; 10 mL) were heated for 2.5 h to 80 °C. Addition of diethyl ether (100 mL) at rt formed a precipitate which was filtered off at 5 °C and washed with diethyl ether. Water (100 mL), dichloromethane (350 mL), and concentrated ammonia (20 mL) were added to the solid. After 15 min of stirring at 0 °C, the biphasic suspension was filtered, and the crystals were washed with diethyl ether. Recrystallization from dichloromethane and methanol gave 10.7 g (58%) of **65:** mp 225−226 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.62 (s, HN), 8.70 (s, 1H), 8.62 (d, 1H), 8.45 (m, 3H), 8.17 (d, 1H), 8.0 (m, 2H), 7.55 (s, 1H), 7.33 (d, 2H), 4.63 (s, 2H); FAB MS (M + H)$^+$ = 459/461. Anal. ($C_{21}H_{14}N_4BrF_3$) C, H, N.

**1-(3-Trifluormethyl-5-fluoroanilino)-4-(4-pyridylmethyl)phthalazine (66).** Preparation from 3-amino-5-fluorobenzotrifluoride and **30** as described for **40**, distributing in a mixture of aqueous ammonia (10%) and dichloromethane, chromatography (ethyl acetate → ethyl acetate/methanol 9:1),

and crystallization from acetonitrile yielded 116 mg (29%) of **66**: mp 253−255 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.7 (s, HN), 8.62 (d, 1H), 8.46 (d, 2H), 8.37 (d, 1H), 8.26 (s, 1H), 8.17 (d, 1H), 8.01 (m, 2H), 7.33 (d, 2H), 7.25 (d, 1H), 4.63 (s, 2H); FAB MS $(M + H)^+ = 399$. Anal. ($C_{21}H_{14}N_4F_4$) C, H, N.

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