NMe_2), 2.60 and 2.63 (2 s, total of 3 H, NCH_3), 2.90 (m, 3 H, CH_2), 3.05 (m, 2 H, CH_2), 5.10 (m, 1 H, CHN), 7.12 (br s, 4 H, ArH), 7.27 (m, 1 H, ArH), 7.51 (m, 2 H, ArH); IR ($CDCl_3$) 1630 (s, CO) cm^{-1}; MS (EI) m/e 376 (M^+). Anal. ($C_{20}H_{22}N_2OCl_2$) C, H, N, Cl.

***trans*-2-(Dimethylamino)-1-(*N*-methyl-3',4'-dichlorobenzamido)-1,2,3,4-tetrahydronaphthalene (6b).** By the same method as described for **5b**, 14 (2.04 g, 10.0 mmol) was converted to 1.30 g (35%) of oily dichlorobenzamide **6b**. Crystals were obtained from ether: mp 134-136 °C; ^1H NMR (200 MHz) (mixture of two conformers, ca 3:2) δ 1.50 (m, 1 H), 1.85 (m, 1 H), 2.03-2.08 (m, 1 H), 2.06 and 2.39 (2 s, total of 6 H, NMe_2), 2.63 and 2.73 (2 s, total of 3 H, NCH_3), 2.80-2.95 (m, 2 H), 4.75 and 6.02 (2 d, J = 10 Hz, total of 1 H, CONCH), 7.15 (m, 4 H, ArH), 7.41 (m, 2 H, ArH), 7.65 (m, 1 H, ArH); IR ($CDCl_3$) 1640 (CO) cm^{-1}; MS (FAB) m/e 377, 379 $(M + H)^+$. Anal. ($C_{20}H_{22}N_2OCl_2$) C, H, N, Cl.

Biological Methods. Analgesic activities were determined in a battery of in vivo tests[1] utilizing female CF-1 mice (18-22 g). The test compounds were dissolved or suspended in 25% aqueous (carboxymethyl)cellulose and administered subcutaneously in a volume dose of 10 mL/kg. Fifteen minutes later the mice were subjected to the radiant heat tail flick, tail pinch, and HCl writhing (abdominal stretch) tests. Animals failing to respond to these nociceptive stimuli were scored as analgesic. Six mice were tested at each dose level and additional doses at 0.3 log intervals were tested if the previous dose resulted in >2/6 analgesic mice. ED_{50}'s were calculated by the method of Spearman and Karber for those compounds that were active at one or more doses.

Acknowledgment. We thank Mr. Solomon Joseph and Mr. R. A. Lewis for laboratory assistance. This research was supported by a grant from the Upjohn Co.

Tyrphostins. 2. Heterocyclic and α-Substituted Benzylidenemalononitrile Tyrphostins as Potent Inhibitors of EGF Receptor and ErbB2/neu Tyrosine Kinases†

Aviv Gazit,‡,§ Nir Osherov,‡ Israel Posner,‡ Pnina Yaish,‡ Enrique Poradosu,‡ Chaim Gilon,§ and Alexander Levitzki*,‡

Department of Biological Chemistry, Institute of Life Sciences, and Department of Organic Chemistry, Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel. *Received October 22, 1990*

We have previously described a novel series of low molecular weight protein tyrosine kinase inhibitors which we named tyrphostins. The characteristic active pharmacophore of these compounds was the hydroxy-*cis*-benzylidenemalononitrile moiety. In this article we describe three novel groups of tyrphostins: (i) one group has the phenolic moiety of the *cis*-benzylidenemalononitrile replaced either with other substituted benzenes or with heteroaromatic rings, (ii) another is a series of conformationally constrained derivatives of hydroxy-*cis*-benzylidenemalononitriles in which the malononitrile moiety is fixed relative to the aromatic ring, and (iii) two groups of compounds in which the position trans to the benzenemalononitrile has been substituted by ketones and amides. Among the novel tyrphostins examined we found inhibitors which discriminate between the highly homologous EGF receptor kinase (HER1) and ErbB2/neu kinase (HER2). These findings may lead to selective tyrosine kinase blockers for the treatment of diseases in which ErbB2/neu is involved.

Introduction

In recent years it has become apparent that protein tyrosine kinases (PTKs) mediate proliferative signals as well as metabolic signals.[1] This realization promoted the hypothesis that PTK blockers (tyrphostins) can in principle become useful antiproliferative agents[2,3] and useful molecular tools to investigate signal transduction of PTKs. We have therefore synthesized low molecular weight PTK blockers[2-4] which we demonstrated to be competitive inhibitors of EGF receptor kinase and insulin receptor kinase[5] and showed them to be effective blockers of EGF-dependent proliferation.[2,3,6] We have developed tyrphostins which discriminate up to a factor of 10^3 in favor of EGF receptor kinase as compared to insulin receptor kinase. Furthermore, tyrphostins which were found to be effective in inhibiting EGF receptor kinase in vitro and EGF-dependent proliferation were indeed shown to inhibit the EGF-induced tyrosine phosphorylation of intracellular proteins including phospholipase C[7] and therefore EGF-induced phosphotidylinositol bisphosphate (PIP_2) breakdown.[8] These findings encouraged us to examine whether EGF receptor selective tyrphostins can inhibit EGF-dependent proliferation of human keratinocytes. Indeed, we can demonstrate strong inhibitory effects of EGF receptor directed tyrphostins on the EGF-induced proliferation of normal human keratinocytes, of SCL-1 carcinoma, and of guinea pig epidermal cells in organ culture.[9] In this report we examine novel tyrphostins which deviate in structure from the classical BMN moiety. We prepared three classes of novel tyrphostins: (i) those in which the aromatic nucleus has been replaced by a heterocyclic ring, (ii) those in which the *cis*-cyano group was conformationally con-

* Address correspondence to Professor Alexander Levitzki.

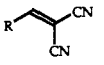
† Abbreviations: EGF, epidermal growth factor; PDGF, platelet-derived growth factor; PolyGAT, copoly-Glu_6Ala_3Tyr (random); BMN, benzylidenemalononitrile; PTK, protein tyrosine kinase.

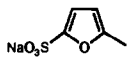
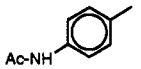
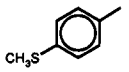
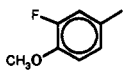
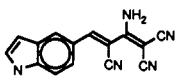
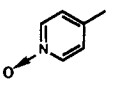
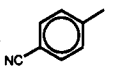
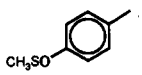
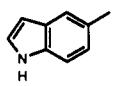
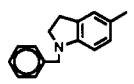
‡ Department of Biological Chemistry.
§ Department of Organic Chemistry.

(1) Yarden, Y.; Ullrich, A., *Ann. Rev. Biochem.* 1987, *56*, 881.
(2) Yaish, P.; Gazit, A.; Gilon, C.; Levitzki, A. *Science* 1988, *242*, 933.
(3) Gazit, A.; Yaish, P.; Gilon, C.; Levitzki, A. *J. Med. Chem.*, 1989, *32*, 2344.
(4) Levitzki, A. *Biochem. Pharmacol.* 1990, *40*, 913-918.
(5) Schechter, Y.; Yaish, P.; Chorev, M.; Gilon, C.; Braun, S.; Levitzki, A. *EMBO* J. 1989, *8*, 1671.
(6) Lyall, R. M.; Zilberstein, A.; Gazit, A.; Gilon, C.; Levitzki, A.; Schlessinger, J. *J. Biol. Chem.* 1989, *264*, 14503.
(7) Margolis, M.; Rhee, S. G.; Felder, S.; Merric, M.; Lyall, R.; Levitzki, A.; Ullrich, A.; Schlessinger, J. *Cell* 1989, *57*, 1101.
(8) Posner, I.; Gazit, A.; Gilon, C.; Levitzki, A. *FEBS Lett.* 1989, 287.
(9) Dvir, A.; Milner, Y.; Chomsky, N.; Gilon, C.; Gazit, A.; Levitzki, A. *J. Cell Biol.*, in press.

0022-2623/91/1834-1896$02.50/0

Table I. Structures, Inhibitory, and Antiproliferative Activities of Aromatic and Heteroaromatic Tyrphostins

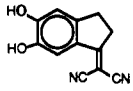
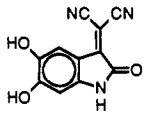
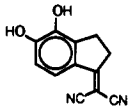
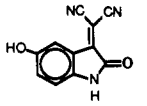
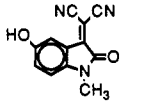
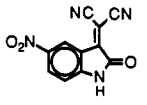
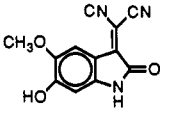
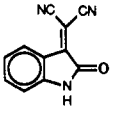
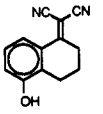


chemical data no.	R[a]	IC_{50}, μM biochemical data:[b] PolyGAT phosphorylation	biological data:[c] EGF-dependent proliferation
1[d]		225	ND[f]
2		>250	ND
3		>325	ND
4		532	ND
5		>625	ND
6		800	ND
7[e]		820	60 (≫100)
8		>1250	ND
9[d]		1300	ND
10		1400	ND
11		1480	ND
12		1800	ND
13[e]		2200	≫100
14		>2500	ND

[a] Residue R of the general formula except no. 7. [b] Compounds are arranged according to descending IC_{50} values for tyrphostins inhibition of PolyGAT phosphorylation. Each IC_{50} value was obtained from inhibition curves with 10–12 experimental points, each point in triplicates. Repeated experiments yield values within 5–7% of each other. [c] Numbers in parentheses refer to the concentration required to inhibit serum-dependent cell growth. [d] These compounds were prepared according to ref 3. [e] These compounds were found to inhibit PDGF receptor Tyrosine kinase activity and PDGF-induced mitogenesis (IC_{50} (μM) of compound 7 = 7, compound 13 = 12)[12]. [f] ND = not determined.

strained so that the *cis*-cyano groups was coplanar with the aromatic ring, and (iii) *cis*-BMN compounds in which the α-position of the BMN moiety has been substituted by ketones and amide derivatives. Potencies of the compounds have been examined as blockers of EGF receptor kinase (HER1) and of ErbB2/neu kinase (HER2)[10,11] in vitro. In addition the potency of these compounds was measured as blockers of EGF-dependent mitogenesis in NIH3T3 cells harboring the EGF receptor.

Table II. Structures and Inhibitory and Antiproliferative Activities of Conformationally Constrained Tyrphostins

chemical data no.	structure	IC_{50}, μM biochemical data:[a] PolyGAT phosphorylation	biological data:[b] EGF dependent proliferation
15		0.5	30 (>150)
16		2.5	≫16 (unstable)
17		7	ND[c]
18		12.5	unstable
19		26	ND
20		40	20
21		60	≥120
22		>250	ND
23		1200	ND

[a] Compounds are arranged according to descending IC_{50} values for tyrphostins inhibition of PolyGAT phosphorylation. Each IC_{50} value was obtained from inhibition curves with 10–12 experimental points, each point in triplicate. Repeated experiments yielded values within 5–7% of each other. [b] Numbers in parentheses refer to the concentration required to inhibit serum-dependent cell growth. [c] ND = not determined.

Results and Discussion

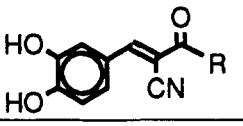
Tables I–III depict the biochemical properties of a series of tyrphostins as inhibitors of EGF receptor kinase. Several of the more potent inhibitors were also examined as blockers of EGF-dependent proliferation of DHER 14 cells which are NIH 3T3 cells that express the EGF receptor introduced by transfection.[6]

Aromatic and Heteroaromatic Tyrphostins (See Table I). We have shown previously[2,3] that tyrphostins derived from hydroxy-BMN's (benzylidenemalononitriles)

(10) Coussens, L.; Yang-Feng, T. L.; Lao, Y.-C.; Chen, E.; Gray, A.; McGrath, J., Seeburg, P. M.; Libermann, T. A.; Schlessinger, J.; Francke, U.; Levinson, A.; Ullrich, A. *Science* **1985**, *230*, 1132.

(11) Lee, J.; Dull, T. J.; Lax, I.; Schlessinger, J.; Ullrich, A. *EMBO J.* **1989**, *8*, 167.

Table III. Structures and Inhibitory and Antiproliferative Activities of α-Keto and α-Amido Tyrphostins[a]



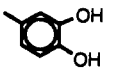
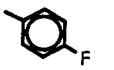
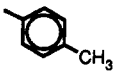
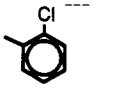
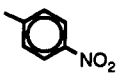
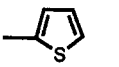
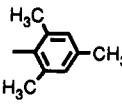
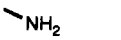
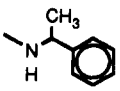
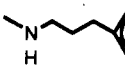
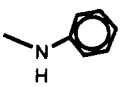
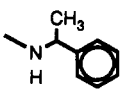
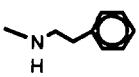
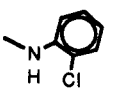
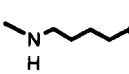
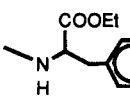
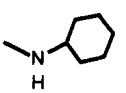
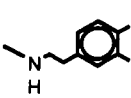
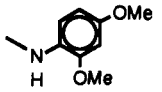
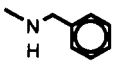
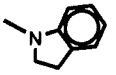
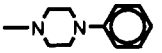
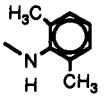
chemical data		IC_{50},[a] μM			
			biological data[d]		
		biochemical data:[b] PolyGAT phosphorylation	autophosphorylation		
			EGFR	ErbB2/neu (HER1-2)	EGF-dependent proliferation
no.[b,c]	structure				
24		0.37	5	18	8 (>50)
25		0.56	5.1	13.6	20 (80)
28		0.88	15	10	7 (100)
29		1.3	14	18	15 (120)
30		1.54	6.6	12	ND[e]
33		2.3	11	3	20 (≪20)
36		3.1	31	35	ND
39		10.7	24	50	ND
40		10	4.5	6.4	15 (60)
44 (−)		0.4	2.5	37	3.5 (10)
46		0.7	0.7	35	2.5 (25)
48		0.7	1.25	42	15 (>50)
50 (+)		0.86	ND	ND	ND
52		0.93	<0.625	23	ND
54		1.1	2.1	57	35 (12)
56		1.1	5	>500	3 (10)
58 (±)		1.53	49	>333	ND
60		1.6	5.9	17	15 (50)
62		1.7	ND	ND	ND

Table III (Continued)

chemical data		IC_{50},[a] μM			
			biological data[d]		
		biochemical data:[b] PolyGAT phosphorylation	autophosphorylation		
			EGFR	ErbB2/neu (HER1-2)	EGF-dependent proliferation
no.[b,c]	structure				
64		1.7	>166	>166	ND
42		2.0	0.1	13.5	3.5 (25)
66		2.1	12	4.9	25 (100)
68		4.3	55	49	ND
70		5.1	10.3	34	5 (>20)
71		>1000	ND	ND	ND

[a] The IC_{50} values for the inhibition of PolyGAT phosphorylation and EGF receptor autophosphorylation were determined at least three times from a full dose–response curve as described by us earlier.[2,3] The IC_{50} values for the inhibition of EGF-dependent HER 14 cell proliferation were determined as described by us earlier.[6] The values in parentheses refer to the IC_{50} value for the inhibition of cell growth stimulated by serum (see discussion). These values are reproducible within less than 15% in repeated experiments. [b] Compounds are arranged according to ascending compound number except compound 42, which is situated between compound 64 and 66. Compounds 24–39 are keto tyrphostins and compounds 40–71 are amide tyrphostins. Signs in parentheses refer to the optical activity of the compounds. [c] Compounds are arranged according to descending IC_{50} values for tyrphostins inhibition of PolyGAT phosphorylation in each group, except compound 40, which is the unsubstituted amide parent compound.[2,3] Each IC_{50} value was obtained from inhibition curves with 10–12 experimental points, each point in triplicate. Repeated experiments yield values within 5–7% of each other. [d] Numbers in parentheses refer to the concentration required to inhibit serum-dependent cell growth in the absence of added EGF as described earlier and in the Experimental Section. [e] ND = not determined.

are potent inhibitors of EGF receptor tyrosine kinase phosphorylation. In order to explore further the SAR of tyrphostins we examined the following parameters: (a) The role of the phenolic ring in the inhibitory activity was studied by its replacement with parasubstituted aromatic rings. These derivatives gave much inferior inhibitors compared to phenolic BMN's, even though some of them have a hydrogen-bond donor at the para position. (b) The phenolic and catechol ring were replaced by a wide range of heterocyclic rings. Most of the heterocyclic BMN were inactive except the indole tyrphostins 7 and 13, which were found to be good inhibitors of PDGF receptor tyrosine kinase and PDGF-induced mitogenesis.[12]

Conformatonally Constrained Tyrphostins (See Table II). In this article we also report on conformationaly constrained tyrphostins. These analogues, which have the dicyanoethylene moiety fixed exo to a bicyclic structure, were prepared in order to determine the conformational requirements of tyrphostins as PTK inhibitors.

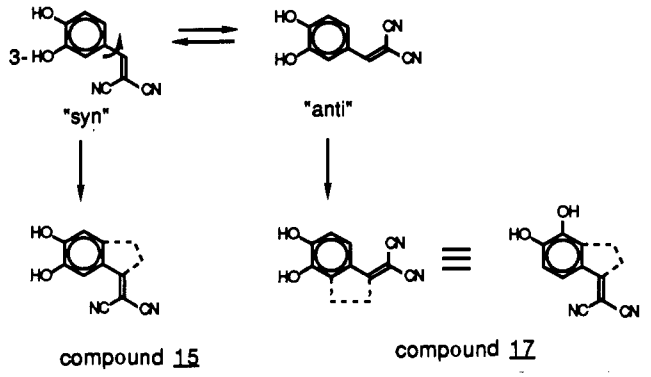
The first group of analogues prepared for this purpose were derivatives of hydroxyisatins. The isatins were prepared by the classical Sandmeyer's route.[13] The 5-hydroxy- and 5,6-dihydroxyisatins were reported in the literature[14] but were poorly characterized and probably impure (black materials[14] compared to our blue compounds). Due to the reactivity of the 3-keto in hydroxyisatins, the condensation with malononitrile can be carried even without base catalysis to give violet, green, and blue isatin-BMN's. The isatin-BMN's were more potent inhibitors than their corresponding "open" tyrphostins: IC_{50} (μM) for compound 18 is 12.5 compared to 375 for 3-OH-BMN,[2] IC_{50} (μM) for compound 21 is 60 compared to 200 for 3-OCH_3-4-OH-BMN, and IC_{50} (μM) is 2.5 for compound 16 compared to 35.0 for 3,4-$(OH)_2$BMN.[2,3] Unfortunately, the isatins exhibit reduced stability and decompose quickly in alkaline aqueous solution and more slowly at pH 7.5. This instability is caused probably by the heterocyclic ring-opening reaction, as is well-known for isatins.[15] The improved inhibitory effect of the hydroxyisatin-BMN's prompted us to prepare their carbocyclic analogues. Thus the rigid five-membered ring derivatives compound 15 and compound 16 have similar IC_{50} (μM) of 0.5 and 2.5, respectively. In contrast compound 23, in which the dicyanoethylene is exo to a more flexible six-membered ring, has an IC_{50} (μM) of 1200 (compared to 375 for 3-OH-BMN[3]).

These results suggest that optimal potency is achieved with tyrphostins that have coplanar catecholic ring and *cis*-cyano group. Even slight deviation from planarity as in compound 23 seems to decrease the inhibitory potency.

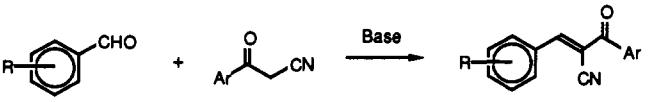
The favorable orientaton of the catechol moiety for inhibitory activity of the constrained BMN-indanone was determined by comparison of the IC_{50}'s (μM) of compound 15 (0.5) and compound 17 (7.0). This indicates that the second hydroxyl group at position 3 (of the open ring analogue) potentiates the inhibitory activity better than in position 5 (Scheme I). An alternative explanation is as follows: the two analogues, compounds 15 and 17, were prepared to probe the relative conformation of the *cis*-cyano group. In the nonconstrained analogue two coplanar conformations are possible "syn" and "anti" (Scheme 1).

(12) Bryckaert, M. C.; Eldor, A.; Gazit, A.; Osherov, N.; Gilon, C.; Fontenay, M.; Levitzki, A.; Tobelem, G. Submitted.
(13) *Organic Syntheses*; Wiley: New York, 1941; Collect Vol. I, p 327.
(14) Giovannini, E. *Helv. Chim. Acta* **1957**, *40*, 249.
(15) Poppl, F. D. *Adv. Heterocycl. Chem.* **1975**, *18*, 1–59.

Scheme I. Conformational Analysis of (Dihydroxyindanylidene)malononitriles 15 and 17



Scheme II. Synthesis of α-Keto-*cis*-cinnamonitriles by the Knoevenagel Condensation of Cyanomethyl Ketones



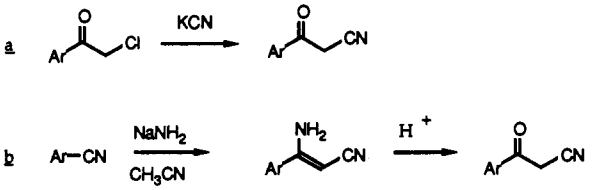
(The conformer "syn" is the one in which the distance between the 3-OH and the *cis*-cyano is shorter.)

These two conformations are fixed in compounds **15** and **17**, respectively. Since the inhibitory activity of compound **15** is 14-fold better than that of compound **17**, we conclude that in the dihydroxy-*cis*-BMN series the active conformation for inhibition is the one where the malononitrile moiety and the catechol ring are coplanar and fixed in the cis-"syn" configuration. Replacement of the cyano group trans (or α) to the catechol ring also yields improved inhibition. These findings are reported below. Incorporation of these substitutions into the rigid indanone-BMN ring is expected to give very potent tyrphostins in the nanomolar range and work in this direction is in progress.

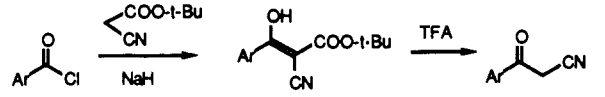
Isatin-based tyrphostins which were found to be effective in in vitro tyrosine kinase assays were found to be only moderately active or inactive in their efficacy to inhibit EGF-dependent cell proliferation. Compounds **18** and **16** are unstable at neutral pH values and therefore their biological activity could not be tested. We are currently attempting to improve the stability of isatin-based tyrphostins in order to be able to reach a stable tyrphostin with enhanced biological activity. Compound **15** has rather less activity on intact cells although its inhibitory activity in vitro was found to be quite good. Since compound **15** is chemically stable, its weak biological activity may be due either to reduced permeability or to its being metabolized rapidly. We are curently investigating these issues.

Keto and Amide Tyrphostins (See Table III). Chemistry of α-Keto Tyrphostins. The method which was used for the synthesis of the α-keto derivatives is a Knoevenagel condensation of aldehydes with cyanomethyl ketones according to Scheme II. Cyanomethyl ketones are usually[16] prepared by two routes describes in Scheme III. The first route (a in Scheme III) involves substitution of aromatic chloro ketones with CN^-. The second method (b in Scheme III) involves condensation of aromatic nitriles and acetonitrile followed by acid-catalyzed hydrolysis of the α-aminocinnamonitrile. The first method is generally inconvenient due to the lachrymatory nature of the chloro ketones and the need to use toxic KCN. The second method was found to be incompatible with base-sensitive

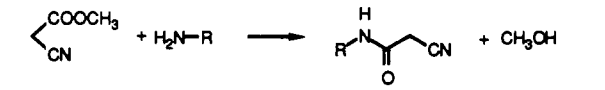
Scheme III. Preparation of Cyanomethyl Ketones



Scheme IV. Novel Preparation of Cyanomethyl Ketones from Aroyl Chloride



Scheme V. Synthesis of Cyanomethyl Amides



functions due to the use of a strong base in the formation of the CH_2CN anion. We have, therefore, developed a simple general method to prepare cyanomethyl ketones, described in Scheme IV. The *tert*-butyl ester activates the cyanomethylene and can subsequently be easily removed with TFA. This method is more efficient and convenient and was used for most of the α-aromatic keto tyrphostins described in this article.[3]

Chemistry of α-Amido Tyrphostins. The substituted α-amido tyrphostins can be formed by condensation of the α-carboxy or the α-acyl halide with amines. However, this method was found to give disappointing yields, even for methylenedioxy derivatives, and inconvenient for catechol derivatives, which have to be protected and then deprotected. We have developed a facile synthesis of cyanomethylamides described in Scheme V.

By simply heating the cyano ester and the amine the desired solid α-cyano amide is obtained directly and due to the activation of cyano and amide can be used for condensation with aldehydes or ketones even with weak bases such as piperidine. Primary amines react readily while anilines react more slowly. The yields were not optimized but can most probably be improved by longer reaction time or higher reaction temperature.

Structure–Activity Relationship Studies of Keto and Amide Tyrphostins. (i) Ketones. We have previously[2,3] shown that a nitrile group cis to the aromatic ring is necessary to achieve maximal potency. In this paper we show that substitution at the α-position with an aromatic keto group improves the affinity of the tyrphostin toward the EGF receptor kinase almost 30-fold (e.g. compound **24**) and improves its antiproliferative activity up to 10-fold as compared to small substituents.[3] The tyrphostins substituted at the α-position with aromatic ketones also show enhanced efficacy toward the ErbB2/neu (HER2) protein tyrosine kinase (e.g. compound **33**). The SAR trend described in Table III demonstrates the essential role of a keto aromatic ring at the trans α-position for improved inhibitory activity. Substitutions on the keto aromatic ring at the α-position have little or no effect on the inhibitory activity [e.g. IC_{50} (μM) of compound **29** = 1.3 compared to that of compound **28** (0.88) or compound **30** (1.54)]. There seems to be a size or conformational demand for the keto aromatic ring at the α-position. Thus compound **39** with the mesitoyl residue has an IC_{50} (μM) of 10.7 compared to 0.37 for compound **24** and 0.88 for compound **28**.

(ii) Amides. The enhanced efficacy of tyrphostins substituted at the α-position with a ring is also observed

(16) For a review of 3-oxoalkanenitriles, see: Elnagdi, M. A.; El-Moghayar, M. R. H.; Elgemeie, G. E. H. *Synthesis* **1984**, 1–26.

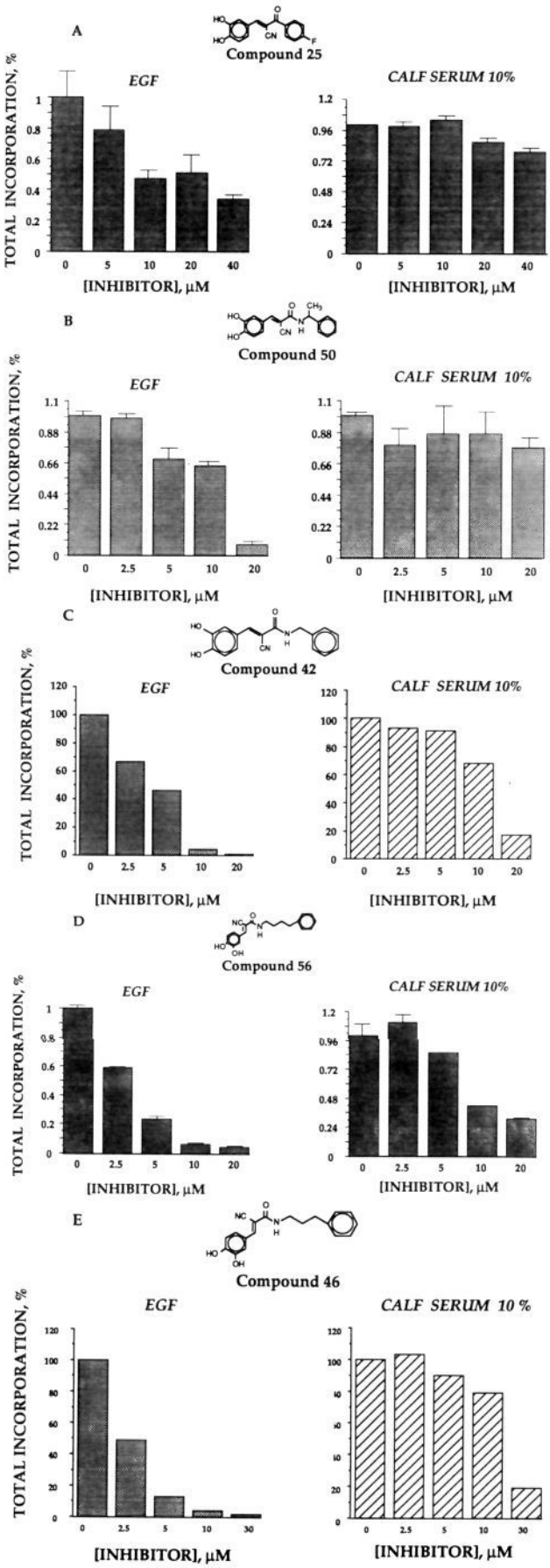


Figure 1. Inhibition of EGF-dependent proliferation of HER 14 cells by ketone and amide tyrphostins. Experimental techniques are described in the Experimental Section. The tyrphostins are arranged according to their increasing potency.

for compounds in which the substituent is an aromatic amide. As in ketones, the compounds are more potent than the parent unsubstituted amide up to 25-fold [e.g. compound **40** IC_{50} (μM) = $10^{2,3}$ compared to that of compound **44** (0.4)].

As, however, can be seen from Table III, the best affinity obtained with the moiety 3,4-dihydroxybenzene-*cis*-malononitrile is in the 0.4 μM range in vitro. In the α-substituted aromatic amide series we have probed the optimal distance of the amido aromatic ring at the α-position. Thus the aromatic ring was removed from the amide nitrogen by gradual addition of methylene groups (compare compounds **46**, **48**, **52**, and **42** and compound **56**). It seems that the inhibitory activity is independent of the distance of the aromatic substituent (e.g. IC_{50} (μM) of compound **48** = 0.7 and compound **46** = 0.68). This led us to the hypothesis that for improved inhibitory activity a substituted amide, but not necessarily of an aromatic nature, is probably needed. We therefore prepared compound **60**, in which the benzene ring of compound **48** was replaced by a cyclohexyl ring. This substitution decreased only by 2-fold the inhibitory activity (IC_{50} (μM) of compound **48** = 0.7, compound **60** = 1.6), in vitro. The potency of the two compounds in intact cells, however, is essentially similar (Table III, see discussion below). The exact location of the ring relative to the amide bond is important as demonstrated by the analogous compounds **60** and **71**. In the latter the amide nitrogen is part of a six-membered ring whereas in the former the cyclohexyl ring is attached to the amide nitrogen. Compound **71** was inactive (IC_{50} (μM) > 1000). Thus we conclude that for optimal activity the ring (be it aromatic or aliphatic) has to be attached to the amide bond at the α-position. The dramatic decrease of activity for compound **71** is not due to the replacement of the secondary amide by a tertiary one or due to conformational restriction of the rings. This is demonstrated by compound **66** (IC_{50} (μM) 2.1). To further explore the structural demands for the N-substituent at the α-amido, we introduced substituents on the benzylic carbon of compound **42**. Introduction of a methyl group increased activity 2.5-fold in the case of the (+)-enantiomer (IC_{50} (μM) of compound **42** = 2.0 and compound **50** = 0.86,) and 5-fold in the case of the (−)-enantiomer (IC_{50} (μM) of compound **44** = 0.4. The difference of only 2-fold in activity between the two enantiomers indicates that the substituents on the α-amido group interact with a minor binding site at the active site. Introduction of a carbethoxy group at the benzylic carbon did not affect activity (IC_{50} (μM) of compound **58** = 1.53).

The best antiproliferative activity obtained is in the range of 3.0–2.5 μM (compound **46** and compound **56**, Table III and Figure 1) 4–20-fold higher than the IC_{50} values observed in in vitro kinase experiments. This relationship between the potency in in vitro kinase activity and the antiproliferative potency has been observed previously.[2,3] This discrepancy is most likely due to two main factors. Firstly, the pharmacokinetic properties of the compounds such as cell permeability, biochemical stability, etc. reduce their potency in intact cells. Secondly, activation of only a fraction of the EGF receptor kinase molecules may be needed for the full mitogenic response. This phenomenon of "spare receptors" is well-documented for many receptor–effector systems.[17] Thus it takes more of a drug to affect the final physiological response than it takes to inhibit the initial kinase response of the receptor. All the amides are more potent antiproliferative agents than the ketones (Table III and Figure 1), although the two groups are similar in their inhibitory effect on

(17) Levitzki, A. *Receptors: A Quantitative Approach*; Benjamin/Cummings Inc. New York, 1984; pp 91–103.

kinase activity. This difference may result from the superior pharmacokinetic properties of the substituted amides.

(iii) Discrimination between EGFR and ErbB2/neu Kinase by Amide Tyrphostins. Table III shows that most of the tyrphostins examined do not discriminate between the EGF receptor kinase (HER1) and the ErbB2/neu kinase (HER2). However, a few of the tyrphostins mainly from the substituted amide family show some discrimination between the two kinases. It should be noted that the two growth factor receptors are highly homologous in the kinase domain.[10,11] The degree of discrimination increases in the series: compounds **44**, **46**, **48**, **56** > compound **66** > compound **68**. It is interesting to note that any of the substituted ketones tested do not discriminate between EGF receptor kinase and the ErbB2/neu kinase. These findings support biological experiments which demonstrate that the EGF receptor kinase and the ErbB2/neu kinase are able to transmit different mitogenic signals.[18] These findings encourage us to exploit further these differences in order to achieve potent and highly selective inhibitors for the ErbB2/neu tyrosine kinase. The enhanced activity of the ErbB2/neu protein tyrosine kinase is implicated in the malignant forms of breast carcinomas in women[19] and ovarian cancer.[20] It is therefore likely that tyrphostins aimed at this PTK may be of therapeutic value.

(iv) The Effect of Serum-Dependent Growth. Tyrphostins also inhibit serum-supported cell growth but with diminished efficacy. Each tyrphostin shows a different level of selectivity as is also apparent from Table III and Figure 1. The ability of the tyrphostins to inhibit serum-dependent growth most likely reflects their kinase inhibitor potency against growth factor receptors stimulated by growth factors present in serum such as PDGF.

Experimental Section

Materials and Methods. All starting materials were purchased from Aldrich. ^1H and ^{13}C NMR spectra were recorded on a Bruker WP-200 pulsed FT spectrometer. Chemical shifts are in ppm relative to TMS internal standard. Mass spectra were recorded with a MAT 311 instrument. Combustion analyses for all new compounds were within 0.4% of the theoretical value.

Synthetic Methods. The compounds are numbered according to their notation in Tables I–V and are arranged in an ascending order except compounds for which general procedures are described. The general procedures A–D for the synthesis of tyrphostins and the synthesis of unconventional tyrphostins are described in detail in the Experimental Section. The synthesis and characterization of tyrphostins and intermediates which were prepared according to the general procedures A–D in this article and procedures A–I in ref 3 are shown in Table V.

Compound 2, (5-Sulfofurfural)Sodium Salt. To 1 g (5 mmol) of aldehyde and 0.4 g (6 mmol) of malononitrile in 20 mL of H_2O was added 0.3 g of NaAc. The reaction was heated for 0.5 h at 80 °C, cooled, and left at 0 °C overnight. Filtering and washing with ethanol gave a light violet solid (cherry red in solutions): 0.34 g; 27% yield; NMR (DMSO-d_6) δ 8.31 (1 H, s, vinyl), 7.42 (1 H, d, J = 3.5 Hz), 6.81 (1 H, d, J = 3.5 Hz).

Compound 12, [4-(Methylsulfinyl)Benzylidene]Malononitrile. To 0.75 g (5 mmol) of 4-(methylthio)benzaldehyde in 30 mL of H_2O and 30 mL of dioxane was added 3 g of sodium periodate. Stirring at room temperature for 16 hours and extracting with CH_2Cl_2 gave an oil, a mixture of 60:40 sulfoxide–starting material by NMR (0.75 g of aldehyde with 1.1 g of $NaIO_4$, 14 h at room temperature gave 38:62).

Chromatography on silica gel with CH_2Cl_2 gave recovered starting material, followed by 4-(methylsulfinyl)benzaldehyde (eluted with 3% CH_3OH in CH_2Cl_2) (0.5 g, 60% yield) as a viscous oil: NMR ($CDCl_3$) δ 10.09 (1 H, s, CHO), 8.09, 7.85 (4 H, AB q, J_{AB} = 8.5 Hz), 2.81 (3 H, s, CH_3).

The aldehyde (1 g, 6 mmol) was condensed with malononitrile according to procedure A[3] to give 0.2 g (16% yield) of yellow solid: mp 160 °C (from the mother liquid was obtained 0.35 g slightly impure solid); NMR ($CDCl_3$) δ 8.06, 7.82 (4 H, AB q, J_{AB} = 8.5 Hz), 7.84 (1 H, s, vinyl, overlaps with AB), 2.80 (3 H, s, CH_3); MS m/e 217 (M + 1, 7), 216 (M^+, 59), 201 (M – CH_3, 100), 200 (M – O, 21), 173 (M – O – HCN, 9), 170 (M – 46, 10), 153 (M – CH_3SO, 11), 126 (M – CH_3SO – HCN, 21), 114 (13), 100 (126 – CN, 12).

Compound 14, [(*N*-Benzyl-5-indolinyl)methylene]malononitrile. (a) *N*-Benzylindoline. To 6.4 g (53 mmol) of indoline and 7.7 g (61 mmol) of benzyl chloride in 100 mL of ethanol was added 6 g of Na_2CO_3 and the reaction mixture refluxed 2 h. Extraction with CH_2Cl_2 gave 8 g of oil. Chromatography of the oil gave 7 g (63% yield) of a light yellow oil (darker on standing in light). NMR ($CDCl_3$) δ 7.35–7.2 (5 H, m), 7.05 (2 H, m), 6.66 (1 H, t, J = 7.2 Hz), 6.51 (1 H, d, J = 7.7 Hz), 4.25 (1 H, s, $CH_2C_6H_5$), 3.31 (2 H, t, J = 8.3 Hz), 2.96 (2 H, t, J = 8.3 Hz).

Small amount of blue compound was observed in part a probably due to oxidation of ethanol to CH_3CHO and malachite dye type condensation. When the reaction was repeated with 12.7 g of indoline, 15.4 g of benzyl chloride, and 16 g of K_2CO_3 in 100 mL of CH_3OH, with 3 h of reflux, no blue compound was observed and the yield was 90% (17.8 g of oil).

(b) 5-Formyl-*N*-benzylindoline. To Vilsmeyer reagent from 12 mL of $POCl_3$ and 30 mL of DMF in 200 mL of trichloroethylene was added 17 g of *N*-benzylindoline. The reaction was heated for 3 h at 80 °C, decomposed with 50 g of NaAc in water, extracted with CH_2Cl_2, and chromatographed on silica gel. The first fraction gave after trituration with hexane 13 g of light-yellow solid: mp 80 °C; 64% yield; NMR ($CDCl_3$) δ 9.65 (1 H, s, CHO), 7.5–7.3 (7 H, m), 6.46 (1 H, s, J = 6.9 Hz), 4.40 (2 H, s, $CH_2C_6H_5$), 3.55 (2 H, t, J = 8.6 Hz), 3.04 (2 H, t, J = 8.6 Hz).

(c) The aldehyde was reacted according to procedure A[3] to give 0.36 g (75% yield) of bright red solid: mp 187 °C; MS m/e 285 (M^+, 20), 140 (4), 91 (100); NMR ($CDCl_3$) δ 7.80 (1 H, s, vinyl), 7.50–7.20 (7 H, m), 6.44 (1 H, s, J = 8.5 Hz), 4.48 (2 H, s, $CH_2C_6H_5$), 3.67 (2 H, t, J = 8.6 Hz), 3.11 (2 H, t, J = 8.6 Hz).

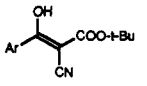
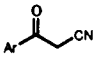
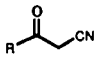
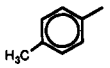
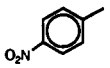
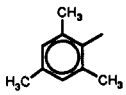
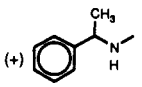
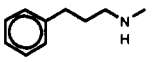
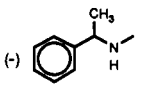
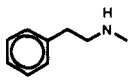
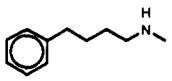
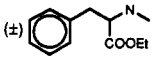
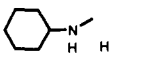
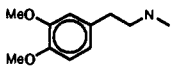
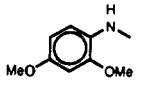
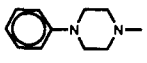
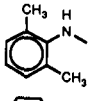
Compound 15, (5,6-Dihdyroxyindanylidene)malononitrile. (a) 5,6-Dihydroxyindanone. Dihydrocaffeic acid (2 g) was stirred 24 h in liquid HF (70 mL) in a KelF system at room temperature. HF was evaporated and the gray solid extracted with ethyl acetate and chromatographed on silica gel to give 0.26 g (14% yield) of a light brown-white solid: NMR (acetone-d_6) δ 7.06 (1 H, s, H_7), 6.99 (1 H, s, H_4), 2.93, 2.54 (4 H, AA'BB' m).

(b) 5,6-Dihydroxyindanone (0.25 g, 1.5 mmol) and malononitrile (0.2 g, 3 mmol) were condensed according to procedure B.[3] The yellow-brown solid obtained after evaporation contained 40% of starting material. Purification on preparative HPLC column (RP-18 25–35μm, 50 g, step gradient from 15:85 CH_3OH–H_2O to 45:55 + 0.1% TFA, UV at 254 nm, F = 9 mL/min) gave 40 mg of yellow solid: mp 245 °C; NMR (acetone-d_6) δ 7.76 (1 H, s, H_7), 7.02 (1 H, s, H_4), 3.20, 3.11 (4 H, AA'BB' m).

Compound 16, (5,6-Dihydroxy-2-oxo-3-indolinidene)malononitrile. To 60 mg (0.3 mmol) of compound 21 in 10 mL of trichloroethylene was added 110 mg of $AlCl_3$ and 7 drops of pyridine, the suspension was refluxed for 1.5 h and filtered, and the solid was added to water–HCl and extracted with EtOAc. Evaporation gave 15 mg (27% yield) of light blue solid (R_f(CH_2Cl_2) – 0.3, green spot): NMR (acetone-d_6) δ 7.47 (1 H, s, H_4), 6.52 (1 H, s, H_7).

(18) Di Fiore, P. P.; Segatto, S.; Taylor, W. G.; Aaronson, S. A.; Pierce, J. M. *Science*, **1990**, *248*, 79.
(19) Slamon, D. J.; Clark, G. M.; Wong, S. G.; Levin, W. L.; Ullrich, A.; McGire, W. L. *Science* **1987**, *235*, 177.
(20) Slamon, D. J.; Godolphin, W.; Jones, L. A.; Holt, J. A.; Wong, S. G.; Keith, D. E.; Levin, W. J.; Stuart, S.; Udove, J.; Ullrich, A.; Press, M. F. *Science* **1989**, *244*, 707.
(21) McCusic, B. C.; Hecth, R. E.; Cairns, T. L.; Coffman, D. P. *J. Am. Chem. Soc.* **1958**, *80*, 2806.
(22) Bibbo, R. W.; Boykin, D. W. *J. Chem. Res.* **1980**, *5*, 332.
(23) Carson, B. B.; Stoughton, R. W. *J. Am. Chem. Soc.* **1928**, *50*, 2825.
(24) Troxler, F.; Harnisch, A.; Bormann, G.; Seemann, F.; Szabo, L. *Helv. Chim. Acta* **1968**, *51*, 1616.
(25) Arai, H.; Igeta, H.; Tsuchiya, T. *J. Chem. Soc. Chem. Commun.* **1973**, 521.

Table IV. Structures of Synthetic Intermediates Which Do Not Appear in Tables I–III[a]

Ar or R	general structures of intermediates		
			
	26	27	
	31	32	
	34	35	
	37	38	
			43
			45
			47
			49
			51
			53
			55
			57
			59
			61
			63
			65
			67
			69
			71

[a] See also Schemes II–V, Table V, and the Experimental Section.

Compound 17, (4,5-Dihydroxyindanylidene)malononitrile. (a) 2,3-Dimethoxyhydrocinnamic Acid. Hydrogenation (EtOH, Pd/C) of 10 g of 2,3-dimethoxycinnamic acid gave 9.2 g (92% yield) of white solid: mp 48 °C; NMR ($CDCl_3$) δ 7.03–6.95 (1 H, m), 6.82–6.76 (2 H, m), 3.86, 3.83 (2 s, 6 H, OCH_3), 2.96, 2.71 (4 H, AA'BB', m).

(b) 4,5-Dimethoxyindanone. To 20 g of polyphosphoric acid was added 9 g of 2,3-dimethoxyhydrocinnamic acid. The viscous oil was stirred for 6 h at 100 °C, water added, and the reaction extracted with CH_2Cl_2. Evaporation gave a red oil which was chromatographed on silica gel to give light red solid (2 g, 24% yield). A sample was triturated with benzene–hexane to give white solid: mp 140 °C (lit.[26] mp 75 °C); MS *m/e* 192 (M^+, 100), 177 (M – CH_3, 39), 149 (M – CO – CH_3, 12), 121 (13), 107 (17), 106 (10); NMR ($CDCl_3$) δ 7.53 (1 H, d, *J* = 8.3 Hz, H_7), 6.98 (1 H, d, *J* = 8.3 Hz, H_6), 3.96 (3 H, s, OCH_3), 3.93 (3 H, s, OCH_3), 3.08, 2.71 (4 H, AA'BB', m).

(c) (4,5-Dimethoxyindanylidene)malononitrile was prepared according to procedure B[3] (16 h of reflux): yellow-green solid; mp 174 °C; 69% yield; NMR ($CDCl_3$) δ 8.16 (1 H, d, *J* = 8.8 Hz, H_7), 7.01 (1 H, d, *J* = 8.8 Hz, H_6), 4.0 (3 H, s, OCH_3), 3.90 (3 H, s, OCH_3), 3.26, 3.20 (4 H, AA'BB', m).

(4,5-Dihydroxyindanylidene)malononitrile. To 0.36 g (1.5 mmol) of 4,5-dimethoxyindanone in 20 mL of CH_2Cl_2, under argon cooled to −10 °C, was added 1 mL (10 mmol) of BBr_3. The yellow brown suspension was stirred for 1.5 h in the cold (TLC shows only starting material). Then it was stirred for 3 h at room temperature and decomposed on ice. Extraction with ethyl acetate gave yellow solid, a mixture of mono- and dihydroxy derivatives. Chromatography on silica gel and elution with 2–7% methanol in CH_2Cl_2 gave (a) 95 mg (30% yield) of yellow solid (mp 275 °C), which is probably the 4-methoxy-5-hydroxy derivative (or the 4-hydroxy-5-methoxy isomer) [NMR (acetone-d_6) δ 7.89 (1 H, d, *J* = 8.6 Hz, H_7), 7.21 (1 H, d, *J* = 8.6 Hz, H_6), 4.0 (3 H, s, OCH_3), 3.28, 3.14 (4 H, AA'BB', m)]; (b) a yellow solid (15 mg, 5% yield, mp 270 °C) the 4,5-dihydroxy derivative [NMR (acetone-d_6) δ 7.80 (1 H, d, *J* = 8.5 Hz, H_7), 7.04 (1 H, d, *J* = 8.5 Hz, H_6), 3.27, 3.15 (4 H, AA'BB', m)].

Compound 18, (5-Hydroxy-2-oxo-3-indolinidene)malononitrile. 5-Hydroxyisatin was prepared by a similar route to compound **21**, from *p*-hydroxyaniline.

5-Hydroxyisatin (0.08 g, 0.5 mmol) and malononitrile (0.05 g, 0.75 mmol) in 30 mL of ethanol were refluxed for 1 h. The solvent was evaporated and the residue chromatographed on silica gel (5:95 methanol–$CHCl_3$). The dark-blue band was collected to give 0.08 g (77% yield) of a violet-black solid: mp 295 °C dec; NMR (acetone-d_6) δ 7.52 (1 H, dd, $J_{4,6}$ = 2.4 Hz, $J_{4,7}$ = 0.4 Hz, H_4), 7.11 (1 H, dd, $J_{6,7}$ = 8.5 Hz (AB), $J_{4,6}$ = 2.4 Hz, H_6), 6.90 (1 H, dd, $J_{6,7}$ = 8.5 Hz, $J_{4,7}$ = 0.4 Hz, H_7). MS *M/e* 211 (M^+, 100), 185 (M – CN, 24), 184 (M – HCN, 43), 149 (42), 125 (23), 113 (21), 111 (47).

Compound 19, (*N*-Methyl-5-hydroxy-2-oxo-3-indolinidene)malononitrile. (a) To cooled (4.4 g, 32.4 mmol) 4-methoxy-N-methylaniline in 100 mL of CH_2Cl_2 was added 5 mL (58 mmol) of oxalyl chloride followed by 5 g (37 mmol) of $AlCl_3$. After 2 h of reflux, H_2O + ice was added. The CH_2Cl_2 layer was seperated, washed with water, dried, and evaporated to give dark red solid. Chromatography on silica gel gave a violet solid (2.3 g, 37% yield), which contained about 15% impurities.

(b) Isatin (1.8 g, 9.4 mM) from part a and 1 g (15 mM) of malononitrile in 50 mL of EtOH were refluxed for 1 h. Cooling and filtering gave 0.95 g (22% yield) of greenish-violet crystals: mp 193 °C; one blue spot on TLC (R_f(CH_2Cl_2) = 0.5); solution in CH_2Cl_2 is blue and in acetone blue-violet; NMR ($CDCl_3$) δ 7.64 (1 H, d, $J_{4,6}$ = 1.5 Hz, H_4), 7.13 (1 H, dd, *J* = 8.7, 1.5 Hz, H_6), 6.76 (1 H, d, $J_{6,7}$ = 8.7 Hz, H_7), 3.83 (3 H, s, OCH_3), 3.20 (3 H, s, NCH_3); MS *m/e* 239 (M^+, 98), 224 (M – CH_3, 100), 196 (M – CH_3 – CO, 28), 141 (42), 114 (28).

(*N*-Methyl-5-hydroxy-2-oxo-3-indolinidene)malononitrile. To 0.21 g of (*N*-methyl-5-methoxy-2-oxo-3-indolindine)malononitrile in 30 mL of CH_2Cl_2 was added 1 mL of BBr_3. After 5 h at room temperature, water was added and the CH_2Cl_2 phase chromatographed on silica gel to give 90 mg (46% yield) of a violet solid: mp 125 °C; NMR ($CDCl_3$) δ 7.42 (1 H, d, *J* = 1.6 Hz, H_4), 7.10 (1 H, dd, *J* = 8.6, 1.6 Hz, H_6), 6.80 (1 H, d, *J* = 8.6 Hz, H_7), 3.24 (3 H, s, NCH_3).

Compound 21, (5-Methoxy-6-hydroxy-2-oxo-3-indolinidene)malononitrile. (a) 3-Hydroxy-4-methoxyisonitrosoacetanilide. To 6.6 g (39 mmol) of chloral hydrate and 30 g of Na_2SO_4 in 10 mL of water was added 51 g (36 mmol) of

(26) Horning, E. C.; Walker, G. N. *J. Am. Chem. Soc.* **1954**, *76*, 1700.

Table V. Synthetic and Structural Data on Compounds and Intermediates That Were Prepared According to the General Procedures in Reference 3 and the Experimental Section

no.[a]	proc[b]	% yield	mp, °C	ref	solvent[c]	^{1}H NMR, ppm	MS, *m/e*
3	[3A]	72	235	21	A	9.75 (br s, NH), 8.18 (1 H, s, vinyl), 8.02, 7.88 (4 H, AB q, J_{AB} = 8.8 Hz), 2.16 (3 H, s, Ac)	
4	[3B]	33	180 dec		A	7.96 (1 H, s, vinyl), 7.57 (2 H, s) [C^{13}: 145.2 ($C_{4,5}$), 141.12 (C_2), 115.7 (CN), 114.0 (C_β), 79.9 (C_α)]	145 (M + 1, 8), 144 (M^+, 100), 118 (M - CN, 11), 117 (M - HCN, 29), 93 (M = 51, 17), 90 (M - 2 HCN, 22)
5	[3A]	87	148		C	7.66 (1 H, s vinyl), 7.82, 7.31 (4 H, AB q, J_{AB} = 8.5 Hz), 2.55 (3 H, s SCH_3)	201 (M + 1, 23), 200 (M^+, 100), 185 (M - CH_3, 15), 167 (M - 33, 14), 158 (M - CH_3 - HCN, 16), 114 (18)
6	[3B]	10	91		C	7.80–7.71 (2 H, m, $H_{2,6}$), 7.65 (1 H, s, vinyl), 7.0 (1 H, t, J = 8.4 Hz, H_5), 4.0 (3 H, s, OCH_3)	
7	[3B]	31	242		A	8.35 (H_4, d, J = 1.6 Hz), 7.96 (H_6, dd, J = 8.7, 1.6 Hz), 7.63 (H_7, d, J = 8.7 Hz), 7.55 (H_3, m), 6.72 (H_2, m)	260 (M + 1, 15), 259 (M^+, 72) 258 (M - 1, 22), 233 (M - CN, 10), 232 (M - HCN, 29), 204 (17), 194 (M - $CH(CN)_2$, 18), 193 (M - $CH_2(CN)_2$, 14), 166 (M - 1 - $CH(NH_2)$=$(CN)_2$, 12), 142 (11), 140 (20)
8		66	70	23	C	7.81 (1 H, d, J = 1.6 Hz, H_2), 7.52 (1 H, s, vinyl), 7.36 (1 H, d, J = 3.7 Hz, H_4), 6.72 (1 H, dd, J = 3.7, 1.6 Hz, H_3)	
10	[3A]	67	157	23	C	7.99, 7.83 (4 H, AB q, J_{AB} = 8.4 Hz), 7.81 (1 H, s, vinyl, overlaps with right wing of AB)	
11	[3B]	25	154	25	A	8.44 (2 H, s), 8.30 (1 H, s)	145 (M + 1, 10), 144 (M^+, 100), 117 (M - HCN, 29), 106 (21), 90 (M - 2 HCN, 13), 81 (21), 79 (M - 65, $C(CN)_2$, 23), 66 (56), 63 (23)
13	[3A]	70	225		A	8.34 (H_4, d, J = 1.8 Hz), 7.93 (H 6, dd, J = 8.6, 1.8 Hz), 7.65 (H_7, d, J = 8.6 Hz), 7.55 (H_3, m), 6.73 (H_2, m)	194 (M + 1, 6), 193 M^+, 23), 149 (11), 111 (12), 97 (24), 85 (26), 83 (29), 71 (54), 57 (100)
20	[3A]	84	235		A	8.88 (1 H, d, $J_{4,6}$ = 1.9 Hz, H_4), 8.51 (1 H, dd, J = 8.9, 1.9 Hz, H_6), 7.33 (1 H, d, $J_{6,7}$ = 8.9 Hz, H_7)	240 (M^+, 100), 210 (M - NO, 75), 207 (42), 195 (M - CO - OH, 39), 166 (M - NO_2 - CO, 36), 139 (M - NO_2 - CO - HCN, 55), 125 (40), 111 (78)
23	[3A]				A	7.69 (1 H, dd, J = 7.9, 0.9 Hz, H_6), 7.24 (1 H, t, J = 7.9 Hz, H_7), 7.13 (1 H, dd, J = 7.9, 1.2 Hz, H_8), 3.04–2.84 (6 H, m)	
26	A	55	98		C	14.42 (1 H, s, enol O), 7.90, 7.29 (4 H, AB q, J_{AB} = 8.2 Hz), 2.43 (3 H, s CH_3), 1.60 (9 H, s, *t*-Bu)	259 (M^+, 4), 203 (M - $(CH_3)_2$=, 65), 186 (M - O - *t*-Bu, 40), 119 CH_3 - $C_6H_4OCO^+$, 100, 91 (tropylium, 24)
27	B	65	95		C	7.82, 7.32 (4 H, AB q, J_{AB} = 8.2 Hz), 4.05 (2 H, s, CH_2CN), 2.44 (3 H, s, CH_3)	159 (M^+, 14), 145 (5), 139 (7), 133 (M - CN, 8), 119 (M - CH_2CN, 100), 91 (92)
28	C	43	172		A	7.95 (1 H, s, vinyl), 7.83 (1 H, d, J = 2.2 Hz, H_2, 7.48 (1 H, dd, J = 2.2, 8.3 Hz, H_6), 7.01 (1 H, d, J = 8.3 Hz, H_5), 7.76, 7.37 (4 H, AB q, J_{AB} = 7.6 Hz), 2.44 (3 H, s, CH_3)	
29[d]	C	82	180		A	7.97 (1 H, s, vinyl), 7.86–7.50 (5 H, m, aromatic), 7.84 (1 H, d, H_2 overlaps Ph), 7.47 (1 H, dd, J = 2.2, 8.4 Hz, H_6), 7.03 (1 H, d, J = 8.4 Hz, H_5)	265 (M^+, 5), 105 ($PhCO^+$, 100), 77 (67)
30[d]	C	18	193		A	7.86 (1 H, s, vinyl), 7.84 (1 H, d, J = 2.0 Hz, H_2), 7.60 (4 H, m, C_6H_4Cl), 7.43 (1 H, dd, J = 2.0, 8.0 Hz, H_6), 7.0 (1 H, d, J = 8.0 Hz, H_5)	
31	A	72	133		C	8.35, 8.15 (4 H, AB q, J_{AB} = 9.0 Hz), 1.62 (9 H, s, *t*-Bu)	290 (M^+, 23), 234 (M - 56, 18), 150 (NO_2 - C_6H_4 - CO^+, 100), 104 (150 - NO_2, 33), 76 (104 - CO, 26)
32	B	88	120	28	C	8.44, 8.14 (4 H, AB q, J = 8.8 Hz, aromatic), 4.16 (2 H, s, CH_2CN)	190 (M^+, 7), 167 (35), 150 ($NO_2C_6H_4CO^+$, 100), 121 (20), 120 (150 - NO, 22), 104 (150 - NO_2, 90), 76 (83)
33	C	50	238		A	8.42, 8.90 (4 H, AB q, J = 8.3 Hz, aromatic), 8.04 (1 H, s, vinyl), 7.86 (1 H, d, J = 2.2 Hz), 7.46 (1 H, dd, J = 2.2, 8.4 Hz), 7.02 (1 H, d, J = 8.4 Hz)	311 (M + 1, 18), 310 (M^+, 100), 263 (M - HNO_2, 10), 150 (NO_2 - $C_6H_4CO^+$, 90), 120 (150 - NO, 15), 104 ($C_6H_4CO^+$, 48), 92 (19)
34	A	31	77		C	8.32 (1 H, 4 line, aromatic), 7.72 (1 H, 4 line, aromatic), 7.19 (1 H, 4 line, aromatic, ABC m), 1.59 (9 H, s, *t*-Bu)	251 (M^+, 12), 195 (M - 56, 100), 178 (M - O - *t*-Bu, 16), 177 (15), 111 (C_4H_3S - CO^+, 87), 83 ($C_4H_3S^+$, 13)
35	B	66	115	28	C	7.80 (2 H, m, aromatic), 7.20 (1 H, 4 line, aromatic), 6.93 (2 H, s, aromatic), 4.01 (2 H, s, (CH_2CN)	151 (M^+, 19), 111 (C_4H_3SCO, 100), 83 (C_4H_3S, 9)
36	C	33	165		A	8.18 (1 H, m, aromatic), 8.04 (1 H, m, aromatic), 7.20 (1 H, m, aromatic), 7.85 (1 H, s, vinyl), 7.86 (1 H, d, J = 2.4 Hz, H_2), 7.55 (1 H, dd, J = 2.4, 8.2 Hz, H_6) 7.01 (1 H, d, J = 8.2 Hz, H_5)	
37	A	10	87		C	6.90 (2 H, s), 2.30 (6 H, s, OCH_3), 2.29 (3 H, s, p-CH_3), 1.60 (9 H, s, *t*-Bu)	287 (M^+, 17), 231 (M - 44, 33), 214 (M - O - *t*-Bu, 19), 213 (35), 185 (M - HCOO - *t*-Bu, 48), 164 (66), 147 ($MesCO^+$, 100), 119 (Mes^+, 44)

Table V (Continued)

no.[a]	proc[b]	% yield	mp, °C	ref	sol-vent[c]	^{1}H NMR, ppm	MS, *m/e*
38	B	81	82		C	6.93 (2 H, s), 3.40 (2 H, s, CH_2CN), 2.30 (6 H, s), 2.24 (3 H, s)	187 (M^+, 17), 164 (23), 147 (M - CH_2CN, 100), 146 (38), 119 (Mes^+, 30), 91 (24), 77 (16)
39	C	37	127		A	7.80 (1 H, d, J = 2.3 Hz, H_2), 7.73 (1 H, s, vinyl), 7.50–6.90 (4 H, m), 2.31 (3 H, s), 2.16 (6 H, s)	307 (M^+, 47), 291 (11), 288 (21), 147 ($MesCO^+$, 100), 119 (Mes^+, 20), 91 (11)
42	C	79	215		A	8.10 (1 H, s, vinyl), 7.68 (1 H, d, J = 2.2 Hz, H_2), 6.97 (1 H, d, J = 8.3 Hz, H_5), 7.93 (1 H, br s, NH), 7.41–7.25 (6 H, m, H_6 + Ph), 4.57 (2 H, d, Bzl-CH_2)	294 (M^+, 48), 293 (28), 277 (28), 174 (25), 164 (40), 151 (17), 105 (100), 99 (88), 77 (26)
43[e]	D	28	143		C	7.33 (5 H, narrow m, aromatic), 6.2 (1 H, br m, NH), 5.10 (1 H, q, J = 7.0 Hz, CHPh), 3.34 (2 H, d, J = 0.5 Hz), 1.54 (3 H, d, J = 7.0 Hz, CH_3)	
44[f]	C	64	135		A	8.04 (1 H, s, vinyl), 7.67 (1 H, d, J = 2.2 Hz, H_2), 7.44 (1 H, dd, J = 2.2, 8.3 Hz, H_6), 6.96 (1 H, d, J = 8.3 Hz, H_5), 7.38–7.10 (5 H, m, aromatic), 5.22 (1 H, quin, m, J = 7.0 Hz, CHPh), 1.58 (3 H, d, J = 7.0 Hz, CH_3)	308 (M^+, 52), 307 (20), 291 (25), 202 (22), 200 (15), 188 (M - $NHCH(CH_3)Ph$, 30), 120 (100, $NHCH(CH_3)Ph$), 106 (58)
45	D	50	46		C	7.33–7.15 (5 H, m, aromatic), 3.31 (2 H, m, $NHCH_2$), 3.30 (2 H, s, CH_2CN), 2.65 (2 H, t, J = 7.3 Hz, CH_2Ph), 1.87 (2 H, q, CH_2), 6.3 (1 H, br s, NH)	
46	C	57	165		A	8.06 (1 H, s, vinyl), 7.67 (1 H, d, J = 2.1 Hz, H_2), 7.38 (1 H, dd, J = 2.1, 8.3 Hz, H_6), 6.98 (1 H, d, J = 8.3 Hz, H_5), 7.6 (1 H, br m, NH), 7.30–7.10 (5 H, m, aromatic), 3.44 (2 H, q, J = 6.2 Hz, $NHCH_2$), 2.70 (2 H, t, J = 6.2 Hz, CH_2Ph), 1.93 (2 H, quin, J = 6.2 Hz, CH_2)	322 (M^+, 74), 203 (21), 201 (100), 200 (33), 188 (51), 136 (23), 91 (57)
47	D	17	192		C	7.50–7.20 (5 H, m, aromatic), 3.56 (2 H, s, CH_2CN)	
48	C	69	258		A	8.12 (1 H, s, vinyl), 7.76 (1 H, d, J = 1.4 Hz, H_2), 7.41 (1 H, dd, J = 1.4, 8.0 Hz, H_6), 6.98 (1 H, d, J = 8.0 Hz, H_5), 9.15 (1 H, br s, NH), 7.70–7.10 (5 H, m, aromatic)	280 (M^+, 42), 202 (18), 188 (M - NHPh, 35), 164 (13), 114 (18), 93 (100), 91 (26)
49[d]	D	54	143		C	identical with that of the (+)-enantiomer	
50[h]	C	52	135		A	identical with that of compound 44	
51	D	36	85		C	7.36–7.17 (5 H, m), 6.5 (br s, NH), 3.53 (2 H, q, J = 7.0 Hz, $NHCH_2$), 3.30 (2 H, s, CH_2CN), 2.85 (2 H, t, J = 7.0 Hz, CH_2Ph)	
52	C	65	185		A	8.05 (1 H, s, vinyl), 7.67 (1 H, d, J = 2.2 Hz, H_2), 7.40 (1 H, dd, J = 2.2, 8.3 Hz, H_6), 6.96 (1 H, d, J = 8.3 Hz, H_5), 7.30 (5 H, narrow m, Ph), 3.60 (2 H, m, $NHCH_2$), 2.92 (2 H, t, J = 7.5 Hz, CH_2Ph)	
53	D	3	117		C	8.26 (1 H, m, aromatic), 7.44–7.10 (3 H, m, aromatic), 3.62 (2 H, s, CH_2CN)	
54	C	60	271		A	8.22 (1 H, s, vinyl), 7.75 (1 H, d, J = 2.2 Hz, H_2), 7.01 (1 H, d, J = 8.3 Hz, H_5), 8.84 (1 H, br s, NH), 8.31 (1 H, m, H_3), 7.55–7.17 (4 H, m, H_6 + $H_{4'-6'}$)	316, 314 (M^+, 11, 32), 279 (M - Cl, 37), 188 (M - NHC_6H_4Cl, 52), 160 (M - $CONHC_6H_4Cl$, 13), 142 (13), 129 (34), 127 (100), 114 (33), 99 (12)
55	D	51	54		C	7.3–7.1 (5 H, m, aromatic), 3.31 (2 H, s, (2 H, s, CH_2CN), 3.27 (2 H, q, J = 7.0 Hz, $NHCH_2$), 2.63 (2 H, t, J = 7.0 Hz, CH_2Ph), 1.60 (4 H, m, $(CH_2)_2$), 6.40 (1 H, br s, NH)	
56	C	60	180		A	8.04 (1 H, s, vinyl), 7.66 (1 H, d, J = 2.1 Hz, H_2), 7.35 (1 H, dd, J = 2.1, 8.3 Hz, H_6), 6.94 (1 H, d, J = 8.3 Hz, H_5), 5.0 (1 H, br m, NH), 7.35–7.20 (5 H, m, aromatic), 3.40 (2 H, m, $NHCH_2$), 2.65 (2 H, t, J = 6.0 Hz, CH_2P), 1.64 (4 H, m, $(CH_2)_2$)	336 (M^+, 95), 245 (42), 203 (42), 188 (89), 132 (36), 91 (100)
57	D	16	75		C	7.32–7.26 (3 H, m, aromatic), 7.14–7.10 (2 H, m, aromatic), 6.50 (1 H, m, NH), 4.85 (1 H, m, NHCH), 4.18 (2 H, q, J = 7.0 Hz, Et), 3.33 (2 H, s, CH_2CN), 3.15 (2 H, m, CH_2Ph), 1.27 (3 H, t, J = 7.0 Hz, Et)	
58	C	70	157		A	8.03 (1 H, s, vinyl), 7.68 (1 H, d, J = 2.2 Hz, H_2), 7.40 (1 H, dd, J = 2.2, 8.2 Hz, H_6), 6.99 (1 H, d, J = 8.2 Hz, H_5), 7.30 (5 H, br s, aromatic), 4.83 (1 H, br q) and 3.25 (2 H, m, ABC $CHCH_2Ph$), 4.22 (2 H, q, J = 7.1 Hz, Et), 1.24 (3 H, t, J = 7.1 Hz, Et)	380 (M^+, 10), 203 (M - $C_6H_5CH_2CH$ - CO_2Et, 23), 188 (M - $NHC_6H_5CH_2$ - CHCOOEt, 100), 177 (85), 120 (36), 91 (40)
59	D	42	131	29	C	3.75 (1 H, m), 3.36 (2 H, s, CH_2CN), 1.87–1.15 (10 H, m)	
60	C	72	243		A	8.03 (1 H, s, vinyl), 7.67 (1 H, d, J = 2.2 Hz, H_2), 7.38 (1 H, dd, J = 2.2, 8.3 Hz, H_6), 6.97 (1 H, d, J = 8.3 Hz, H_5), 2.93 (1 H, br s), 1.95–1.10 (10 H, m)	

Table V (Continued)

no.[a]	proc[b]	% yield	mp, °C	ref	solvent[c]	^{1}H NMR, ppm	MS, m/e
61	D	60	102		C	6.80 (1 H, d, J = 8.0 Hz, H_5), 6.73 (1 H, dd, J = 8.0, 2.2 Hz, H_6), 6.71 (1 H, d, J = 2.2 Hz, H_2), 3.33 (2 H, s, CH_2CN), 3.88, 3.86 (6 H, 2 s, OCH_3), 3.54 (2 H, q, J = 7.0 Hz, CH_2N), 2.79 (2 H, t, J = 7.0 Hz, $-CH_2-$), 6.16 (1 H, br t, NH)	248 (M^+, 23), 165 (M - $NHCOCH_2CN$, 11), 164 (100), 151 ($C_7H_5(OCH_3)_2$, 97), 149 (10), 107 (10)
62	C	88	226		D	7.90 (1 H, s, vinyl), 7.52 (1 H, d, J = 2.0 Hz, H_2), 7.28 (1 H, dd, J = 2.0, 8.4 Hz, H_6), 6.72 (1 H, d, J = 8.4 Hz, H_5), 8.30 (1 H, t, NH), 6.88–6.70 (3 H, m, aromatic), 3.73, 3.70 (6 H, 2 s, OMe), 3.36 (2 H, q, $NHCH_2$), 2.73 (2 H, t, J = 7.0 Hz, CH_2Ar)	368 (M^+, 11), 164 (100), 151 (38), 137 (11)
63	D	2	130		C	8.13 (1 H, br s, NH), 6.50 (3 H, m, aromatic), 3.88, 3.80 (6 H, 2 s, OCH_3), 3.54 (2 H, s, CH_2CN)	
64	C	65	227		A	8.17 (1 H, s, vinyl), 7.73 (1 H, d, J = 2.2 Hz, H_2), 7.45 (1 H, dd, J = 2.2, 8.3 Hz, H_6), 7.0 (1 H, d, J = 8.3 Hz, H_5), 8.24 (1 H, d, J = 8.8 Hz, $H_{6'}$), 6.68 (1 H, d, J = 2.6 Hz, $H_{3'}$), 6.55 (1 H, dd, J = 2.6, 8.8 Hz, $H_{5'}$), 3.96, 3.80 (6 H, 2 s, OCH_3)	340 (M^+, 100), 188 (M - NHC_6H_3 - OCH_3)$_2$, 25), 153 (77), 152 (44), 138 (51), 114 (19), 109 (22)
65	D	21	175		C	8.16 (1 H, m), 7.20 (2 H, m), 7.10 (1 H, m), 4.10 (2 H, t, J = 8.3 Hz, indoline), 3.59 (2 H, s, CH_2CN), 3.27 (2 H, t, J = 8.3 Hz, indoline)	
66	C	42	215		A	7.79 (1 H, s, vinyl), 7.72 (1 H, d, J = 2.2 Hz, H_2), 7.42 (1 H, dd, J = 2.2, 8.3 Hz, H_6), 6.98 (1 H, d, J = 8.3 Hz, H_5), 7.97 (1 H, m, aromatic), 7.35–7.05 (3 H, m, aromatic), 4.36 (2 H, t, J = 8.3 Hz, indoline), 3.23 (2 H, t, J = 8.3 Hz, indoline)	306 (M^+, 42), 237 (43), 216 (62), 196 (33), 188 (M - indoline, 21), 177 (65), 162 (33), 135 (63), 119 (100), 118 (51)
67	D	35	121		C	7.33–7.29 (2 H, m, aromatic), 6.90–6.10 (3 H, m, aromatic), 3.80 (2 H, m), 3.60 (2 H, m), 3.45 (2 H, s, CH_2CN), 3.22 (4 H, m)	
68	C	65	185		A	7.59 (1 H, s, vinyl), 7.66 (1 H, d, J = 2.2 Hz, H_2), 7.38 (1 H, dd, J = 2.2, 8.3 Hz, H_6), 6.98 (1 H, d, J = 8.3 Hz, H_5), 7.4–6.8 (5 H, m, aromatic), 3.86, 3.32 (8 H, AA'BB' m, piperazine)	349 (M^+, 9), 299 (12), 138 (50), 137 (100), 109 (21)
69	D	3	180		C	7.50–7.10 (3 H, m, aromatic), 3.57 (2 H, s, CH_2CN), 2.25 (6 H, s, CH_3)	
70	C	38	175		A	8.17 (1 H, s, vinyl), 7.76 (1 H, d, J = 2.1 Hz, H_2), 7.46 (1 H, dd, J = 2.1, 8.3 Hz, H_6), 7.0 (1 H, d, J = 8.3 Hz, H_5), 8.81 (1 H, br s, NH), 7.4–7.10 (3 H, m, aromatic), 2.25 (6 H, s, CH_3)	308 (M^+, 65), 188 (M - $NHC_6H_3(CH_3)_2$, 47), 138 (21), 121 (100), 120 (48), 114 (17)
71	C	10	177		A	7.48 (1 H, s, vinyl), 7.64 (1 H, d, J = 2.2 Hz, H_2), 7.32 (1 H, dd, J = 2.2, 8.3 Hz, H_6), 6.96 (1 H, d, J = 8.3 Hz, H_5), 5.0 (1 H, br m, NH), 3.58 (4 H, m, piperidine), 1.64 (6 H, m, piperidine)	272 (M^+, 26), 256 (M - O, 16), 205 (23), 203 (100), 201 (43), 200 (77), 199 (57), 198 (33), 138 (18), 97 (21), 84 (46)

[a] The structures of the compounds are shown in Tables I–IV. [b] Procedures in brackets refer to procedures in ref 3. Procedures in capital letters refer to general procedures in the Experimental Section. [c] Deuterated solvents used for NMR measurements: A = acetone-d_6; C = $CDCl_3$; D = DMSO-d_6. [d] Aryloylacetonitrile used to prepare compounds **29** and **30** was purchased from Aldrich and Lancaster. [e] $[\alpha]^{20}_D$ = +143.0° (c = 1.238, CH_3OH). [f] $[\alpha]^{20}_D$ = -90.5° (c = 0.534, CH_3OH). [g] $[\alpha]^{20}_D$ = -143.0° (c = 1.238, CH_3OH). [h] $[\alpha]^{20}_D$ = +90.7° (c = 0.538, CH_3OH).

3-hydroxy-4-methoxyaniline in 20 mL of H_2O and 3 mL of concentrated HCl, followed by 7.2 g of NH_2OH·HCl. The reaction was heated for $^1/_4$ h, cooled with ice, and filtered to give 1.5 g (20% yield) of a black solid: NMR (acetone-d_6) δ 7.51 (1 H, s, CHNOH), 7.36 (1 H, d, $J_{2,6}$ = 2.5 Hz, H_2), 7.17 (1 H, dd, J = 8.7, 2.5 Hz, H_6), 6.88 (1 H, d, $J_{5,6}$ = 8.7 Hz, H_5), 3.81 (3 H, s, OCH_3).

(b) 5-Methoxy-6-hydroxyisatin. The product from part a (1.5 g) in 8 mL of concentrated H_2SO_4 + 2 mL of H_2O was heated for 10 min, with an internal temperature of 80 °C. The mixture was decomposed on crushed ice and extracted with EtOAc to give 80 mg of red-brown solid: 6% yield; mp 265 °C; NMR (acetone-d_6) δ 7.09 (1 H, s, H_4), 6.50 (1 H, s, H_7), 3.87 (3 H, s, OCH_3).

(5-Methoxy-6-hydroxy-2-oxo-5-indolinidene)malononitrile. To 80 mg (0.4 mmol) of the isatin from part b was added 100 mg (1.5 mmol) of malononitrile and the reaction mixture refluxed 6 h, until TLC showed the reaction was finished. Evaporation gave a violet solid, which was purified on silica gel (5:95 CH_3OH–$CHCl_3$). The dark-green band was collected to give 80 mg (80% yield) of a violet solid, mp >300 °C. It is stable on standing but in basic water (green solution) it decomposes in 5 min. On standing several hours on a TLC plate its color changed (Rf (CH_2Cl_2) = 0.5, green-gray spot): NMR (acetone-d_6) δ 7.51 (1 H, s, H_4), 6.54 (1 H, s, H_7), 3.87 (3 H, s, OCH_3); MS m/e 241 (M^+, 66), 227 (12), 226 (M - CH_3, 100), 198 (M - CONH, 28).

Compound 22, (2-Oxo-3-indolinidene)malononitrile. Isatin (1 g, 6.8 mmol) and malononitrile, (0.53 g, 8 mmol) were refluxed for 1 h. Water was added and the suspension filtered and dried to give 1.1 g (83% yield) of a red solid: mp 235 °C (lit.[27] mp 238 °C); NMR (acetone-d_6) δ 8.06 (1 H, d, J = 7.7 Hz), 7.63, (1 H, dt, J = 8.4, 0.8 Hz), 7.20 (1 H, dt, J = 7.8, 1.0 Hz), 7.10 (1 H, dd, J = 7.4, 0.9 Hz); MS m/e 195 (M^+, 100), 169 (M - CN, 39), 168 (M - HCN, 55), 167 (M - CO, 22), 140 (M - CO - HCN, 28), 111 (27), 97 (40).

General Procedures for the Preparation of Cyanomethyl Ketones (See Scheme V). Compound 25, 3,4-Dihydroxy-α-(*p*-fluorobenzoyl)-*cis*-cinnamonitrile. α-*tert*-Butylcarboxy-β-hydroxy-*p*-fluorocinnamonitrile (Procedure A). NaH (50% in oil, 1.3 g, 26 mmol) was washed with hexane. The hexane was decanted and 150 mL of dry ether added. Then 3.7 mL (26 mmol) of *tert*-butyl cyanoacetate was added slowly. After 20 min of stirring, 3.2 g (20 mmol) of *p*-fluorobenzoyl chloride was added to the white suspension. The solution was stirred for 3 h at room temperature and decomposed on ice water. HCl (20 mL) was added and the reaction extracted with 200 mL of CH_2Cl_2. Evaporation gave an oil, which crystallized on standing to white

(27) Capuano, L.; Diehl, V.; Ebner, W. *Chem. Ber.* **1972**, *105*, 3407.

cubic crystals. Trituration with hexane and filtering gave 2 g of a white solid; mp 85 °C; 37% yield; it gives a red color with ethanolic $FeCl_3$; MS *m/e* 263 (M^+, 6), 208 (M − 56, 55), 190 (M − O^+, 20), 123 (F − C_6H_4) − CO^+, 100), 95 (F − $C_6H_4^+$, 40), 75 (13); NMR ($CDCl_3$) δ 14.10 (enol OH, s), 8.05 (2 H, dd, *J* = 9.1, 5.2 Hz), 7.18 (2 H, t, *J* = 9.1 Hz), 1.61 (9 H, ester, s).

(*p*-Fluorobenzoyl)acetonitrile (Procedure B). To 1.35 g of α-(*tert*-butylcarboxy)-β-hydroxy-*p*-fluorocinnamonitrile in 20 mL of CH_2Cl_2 was added 5 mL of TFA. After stirring for 3.5 h at room temperature water was added and the CH_2Cl_2 phase evaporated to give 0.55 g (83% yield) of a white solid: mp 82 °C (lit.[28] mp 79 °C). NMR ($CDCl_3$) δ 7.96 (2 H, dd, $H_{2,6}$), 7.20 (5, $H_{3,4}$), 4.07 (2 H, s, CH_2CN).

3,4-Dihydroxy-(*p*-fluorobenzoyl)-*cis*-cinnamonitrile (Procedure C). 3,4-Dihydroxybenzaldehyde was condensed with (*p*-fluorobenzoyl)acetonitrile according to procedure A:[3] yield 65%; mp 172 °C; MS *m/e* 283 (M^+, 45), 138 (9), 123 ($FC_6H_4CO^+$, 100), 95 ($FC_6H_4^+$, 35); NMR (acetone-d_6) 8 7.98 (1 H, s, vinyl), 7.84 (H_2, d, *J* = 2.2 Hz), 7.48 (H_6, dd, *J* = 2.2, 8.3 Hz), 7.01 (H_5, d, *J* = 8.3 Hz), 7.96 (aromatic, dd, *J* = 8.8, 5.4 Hz), 7.34 (aromatic, t, *J* = 8.8 Hz).

Compound 24, 3,4-Dihdroxy-α-(3,4-dihydroxybenzoyl)-*cis*-cinnamonitrile 2-Cyano-3′,4′-dihdyroxyacetophenone. To 2-chloro-3′,4′-dihydroxyacetophenone (Aldrich), 6 g, 30 mmol) in 30 mL of DMSO was added solid KCN (3 g, 46 mmol). The reaction mixture was stirred at 100 °C for 2.5 h. After cooling, HCl (15 mL) in 100 mL of H_2O was added, and the reaction mixture was extracted with EtOAc. Drying and evaporation gave a red oily solid which was purified by chromatography on silica gel. The first fractions eluted with 3% CH_3OH in CH_2Cl_2 were evaporated and triturated with CH_2Cl_2. Filtering gave 1 g (18% yield) of a light yellow solid: mp 217 °C; NMR (acetone-d_6) δ 7.51 (2 H, m, $H_{2,6}$), 6.97 (1 H, d, *J* = 8.0 Hz, H_5), 4.43 (2 H, s, CH_2CN).

3,4-Dihydroxy-α-(3,4-Dihydroxybenzoyl)-*cis*-cinnamonitrile was prepared according to procedure C from α-cyano-3,4-dihydroxyacetophenone: yield 42%; mp 223 °C; MS *m/e* 297 (M^+, 12), 137 ($(HO)_2C_6H_4CO^+$, 100), 110 (10), 109 (12), 81 (9); NMR (acetone-d_6) δ 7.90 (1 H, s, vinyl), 7.81 (1 H, d, *J* = 2.1 Hz, H_2), 7.47 (1 H, dd, *J* = 2.1, 8.2 Hz, H_6), 7.0 (1 H, d, *J* = 8.2 Hz, H_5), 7.4–7.30 (2 H, m, aromatic), 7.0–6.95 (1 H, m, aromatic).

General Procedure for the Preparation of Cyanomethylamides (See Scheme V). Compound 41, *N*-(Cyanoacetyl)benzylamide (General Procedure D). To benzylamine (15 mL, 0.14 mol) was added methyl cyanoacetate (13 mL, 0.14 mol). The reaction was heated for 16 h at 100 °C without a condensor to allow evaporation of the methanol formed. Cooling gave a dark red solid which was triturated with ethanol, filtered, and recrystallized from 30 mL of ethanol to give 9.4 g (38% yield) of a white solid: mp 113 °C; MS *m/e* 174 (M^+, 63), 147 (M − HCN, 8), 120 (18), 118 (13), 106 (M − $COCH_2CN$, 27), 91 (100), 79 (17), 77 (18); NMR ($CDCl_3$) δ 7.5–7.2 (5 H, m, aromatic), 6.51 (1 H, br s, NH), 4.47 (2 H, d, *J* = 6.0 Hz, Bzl-CH_2), 3.38 (2 H, s, CH_2CN).

(28) Ridge, D. N.; et al. *J. Med. Chem.* **1979**, *22*, 1385.
(29) Osdene, T. S.; Santill, A. A.; McCardle, L. E.; Rosenthale, M. E. *J. Med. Chem.* **1967**, *10*, 165.

Biochemical Methods. Experimental Errors. Biochemical experiments were all conducted in triplicate, and the standard error was computed. The values quoted are within less than 7–10% in repeated experiments.

(i) **General Procedures.** The potencies of the tyrphostins synthesized were tested as inhibitors of EGF receptor catalyzed tyrosine phosphorylation as previously described.[2,3] The antiproliferative potency of the tyrphostins synthesized was examined on NIH3T3 cells (HER 14) which overexpress stably the EGF receptors as previously described.[6]

The effect of some of the tyrphostins was examined as inhibitors of PDGF-dependent phosphorylation and PDGF-dependent proliferation of rabbit vascular smooth muscle cells grown in culture as described elsewhere.[12] Since the ligand for HER2 (ErbB2/neu) has not been identified, we have examined the effect of tyrphostins on the PTK activity of HER2/neu on the chimera HER1-2(11). In this chimeric protein the growth factor binding domain of ErbB2/neu has been replaced by the EGF binding domain of the EGF receptor. Thus one is able to activate the kinase activity of ErbB2/neu with EGF. EGF-dependent phosphorylation of the cimera HER1-2 was determined as described in ref 10 (for details see below).

(ii) **Procedures for Receptor Autophosphorylation.** Tyrphostins were dissolved in DMSO 10%–H_2O–ethanol 45%. Crude membrane extracts (0.125 μg/mL) were preactivated with EGF (20 nM) in 50mM HEPES buffer, pH 7.6, and 125 mM NaCl, for 15 min at 4 °C, as described earlier.[2,3]

Autophosphorylation activity of HER1 kinase (EGF receptor) or HER1-2 kinase was assayed at 4 °C for 30 s in V-shaped 96-well plates. Membrane extracts (8 μL) were added to each well containing reaction mixture (12 μL, 50mM, HEPES, pH 7.4, 125 mM NaCl, 12 mM M_8Ac_2, 2 mM $MnCl_2$, 1 mM $NaVO_3$, 1 μm ATP, and 1 μC_i[γ-^{32}P]ATP, final concentrations) and the given tyrphostin (4 μL). After termination by addition of hot sample buffer, the samples were run on a 6% SDS-polyacrylamide gel electrophoresis minigel, the gels dried, and autoradiography performed during the linear exposure time period. The receptor bands were scanned densitrometrically, and the results analyzed by the Ez-Fit program. All measurements were carried out in triplicate. Repetitive experiments were reproducible within 5–8% or better.

(iii) **Procedure for the Phosphorylation of GAT.** The experimental protocol for GAT phosphorylation catalyzed by EGFR (HER1) was described in detail elsewhere[2,3] IC_{50} values from repeated experiments were within less than 10%.

(iv) **Inhibition of EGF and Serum-Dependent Cell Proliferation.** The HER 14 cell line which overexpresses EGF receptor (HER1) was used. Tyrphostins were added at least 6–8 h prior to stimulation by EGF or serum to serum-starved cells as described by us earlier.[6] The medium containing tyrphostins was replaced every 24 h. For each concentration of tyrphostins three parallel wells of cells were used. As seen in Figure 1 the standard error of the mean was between 5 and 10% in these experiments.

Acknowledgment. This study was supported by Rhone Poulenc Rorer pharmaceuticals, King of Prussia, PA. We thank Drs. J. Schlessinger and A. Ullrich for the cell lines used in this study.