Tyrphostins. 3. Structure-Activity Relationship Studies of α-Substituted Benzylidenemalononitrile 5-S-Aryltyrphostins†

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Received March 19, 1993®

In this study we describe an extension of our previous studies on *cis*-benzylidenemalononitrile tyrphostins. We have introduced *S*-aryl substituents in the 5 position (meta vis-a-vis the malononitrile moiety). We find that these compounds are potent blockers of EGFR kinase and its homolog HER-2 kinase. Interestingly, we find that certain *S*-aryltyrphostins discriminate between EGFR and HER-2 kinase in favor of the HER-2 kinase domain by almost 2 orders of magnitude. When examined in intact cells it was found that these selective *S*-aryltyrphostins are equipotent in inhibiting EGF dependent proliferation of NIH 3T3 harboring either the EGF receptor or the chimera EGF/neu (HER1-2). These findings suggest that the antiproliferative activity of these tyrphostins is mainly due to the inhibition of a mitogenic signaling element downstream to the growth receptor kinase.

Introduction

In the past few years we have pursued a program to develop synthetic PTK blockers.[1-5] We have generated numerous families of tyrphostins derived from benzenemalonitrile[2,3] and some bisubstrate inhibitors[6] based on the structure of BMNs. As one of the goals of designing PTKs blockers is to aim for selectivity toward various PTKs, an important focus of our attention has been to design PTK blockers aimed at the HER-2 kinase because of its involvement in malignant forms of breast and ovary cancers.[7-9] We have previously shown that certain BMN tyrphostins discriminate between EGF receptor (HER-1) and the closely related erb-2, neu(HER-2) receptor,[3] in favor of the former. In this article we describe, for the first time, tyrphostins which discriminate in favor of the HER-2 kinase as compared to the HER-1 kinase.

Initial experiments showed that the substitution of BMN with *S*-aryl groups enhance the affinity of the compound to the receptor. We therefore generated a series of compounds which differ in the *S*-aryl substitution (Tables I and II) and measured their inhibitory activity on the phosphorylation of polyGAT and their ability to inhibit the autophosphorylation of the EGF receptor as compared with that of ErbB2/neu. We have also measured the ability of these inhibitors to arrest the EGF-dependent proliferation of DHER cells.

Results and Discussion

Structure-Activity Relationship Studies of 5-Substituted Tyrphostins. We have previously shown[2] that addition of hydroxy groups to the aromatic ring of benzylidenemalononitrile-derived tyrphostins increases the inhibitory activity by about 1 order of magnitude per hydroxy group (e.g., 3,4,5-trihydroxybenzylidenemalononitrile have an IC_{50} (μM) = 3). The trihydroxy compound was unstable, and hence we studied the effect of replacement of hydroxy groups by methoxy and/or methyl groups. Replacement of the 3-(or 5-)hydroxy group by methoxy or methyl groups did not have much effect on the inhibitory activity (e.g., 3-methoxy-4,5-dihydroxybenzylidenemalononitrile IC_{50} (μM) = 6[2] and 3-methyl-4,5-dihydroxybenzylidenemalononitrile (compound **25**, Table II), IC_{50} (μM) = 5.6). Combination of these two substitutions, namely 3-methoxy-, 4-hydroxy-, 5-methylbenzylidenemalononitrile (compound **27**, Table II), abolishes the inhibitory activity (IC_{50} (μM) = 2600). In order to increase the latitude for possible modifications aimed at improving the inhibitory activity and selectivity of hydroxybenzylidenemalononitrile-derived tyrphostins, we have replaced the methyl group of compound **27** by substituted thiomethyl groups in position 5. We studied the structure-activity relationship of these substitutions at the α position of BMN (general formula shown in Table I).

We have also previously shown[3] that substitution of the nitrile group at the α position of benzylidenemalononitrile-derived tyrphostins by unsubstituted or substituted amides, thioamides, or ketones improves the inhibitory activity. Shirashi et al. have shown[10] that replacement of the α-cyano group by an amide and introduction of a (phenylthio)methyl group at position 5 of analog **27** (Table II) gave a highly potent inhibitor (compound **6**, Table I, IC_{50} (μM) = 2.9 compared to IC_{50} (μM) = 2600 of compound **27**).

The first set of compounds studied had a cyano group at the α position. Introduction of a (phenylthio)methyl group at position 5 of analog **27** (Table II), increased the inhibitory activity by a factor of 7 (compound **23**, Table I, IC_{50} (μM) = 370 compared to IC_{50} (μM) = 2600 of compound **27**). Introduction of an 3-carboxy substitution on the phenylthio ring improved the inhibitory activity by a factor of 10 (compound **17**, IC_{50} (μM) = 36 compared to IC_{50} (μM) = 370 of compound **23**). Replacement of the carboxyphenyl ring by carboxymethyl decreased the inhibitory activity (compound **21** IC_{50} (μM) = 96 compared to IC_{50} (μM) = 36 of compound **17**). Replacing the cyano group at the α position of compound **17** by of a carboxamide group did not effect the inhibitory activity (compound **18**, IC_{50} (μM) = 37 compared to IC_{50} (μM) = 36 of compound **17**). On the other hand, replacement of the cyano group

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† Abbreviations: EGF, epidermal growth factor; PTK, protein tyrosine kinase; BMN, benzylidenemalonitrile; PolyGAT, copoly-Glu6Ala3Tyr (random); PDGF, platelet-derived growth factor.

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® Abstract published in *Advance ACS Abstracts*, October 1, 1993.

0022-2623/93/1836-3556$04.00/0

at the α position of compound **23** by of a carboxamide group dramatically improved the inhibitory activity by a factor of 130 (compound 6, IC_{50} (μM) = 2.9 compared to IC_{50} (μM) = 370 of compound **23**). From these findings we concluded that substitution on the aromatic ring of compound **6** may have an effect on the inhibitory activity as well as the selectivity to the ErbB2/neu tyrosine kinase as compared to that of EGFR tyrosine kinase. We therefore used compound **6** as our parent compound and studied the effect on the inhibitory activity and selectivity of (a) other aromatic and heteroaromatic rings replacing the phenylthio ring at position 5, (b) the distance between the two aromatic rings, (c) the effect of various substituents on the thio aromatic rings, (d) the nature of the group at the α position, and (e) replacement of the methoxy group in position 3 by a hydroxy group. We have also studied the effect of oxidation of the thioether group to sulfone.

(a) The character of the aromatic ring attached to the thiomethyl group at the 5 position has an effect on the inhibitory activity. Replacement of the phenyl ring of compound **5** by various neutral aromatic and heteroaromatic rings caused a decrease of the inhibitory activity. Thus, compound **6** with a phenyl residue has an IC_{50} (μM) of 2.9 compared to 6.6 of compound **10** (with a naphthyl), 12.5 of compound **12** (with 2-benzoxazole), 16.3 of compound **14** (with 2-thiazole), and 18.6 of compound **15** (with 2-benzothiazole).

(b) We have also probed the influence of the distance between the two aromatic rings on the inhibitory activity. Replacement of the (phenylthio)methyl group at position 5 of compound **6** by a (benzylthio)methyl group (compound **3**) has a minor effect on the whole spectrum of biological activities (IC_{50} (μM) of 2.9 for compound **6** compared to 1.95 for **3**).

(c) The influence of diverse substituents at various positions on the aromatic rings at position 5 has been studied. In the case of the phenyl ring (e.g., compound **6**) introduction of a *p*-hydroxy group (e.g., compound **5**) has little influence on the inhibitory activity (IC_{50} (μM) of 2.9 for compound 6 compared to 2.5 for **5**), whereas *p*-methyl decreased the inhibitory activity (IC_{50} (μM) of 8.7 for compound 11). Introduction of 3-carboxy decreased the inhibitory activity by 1 order of magnitude (IC_{50} (μM) of 37 for compound 18 compared to 2.9 for **6**). In the case of a benzyl group at position 5 (e.g., compound **3**), the ortho substituent had little effect on the inhibitory activity (IC_{50} (μM) of 2.5 for compound **4** compared to 1.95 for **3**), whereas the same substituent at the para position decreased the activity 8-fold (IC_{50} (μM) of 14.8 for compound **13** compared to 1.95 for **3**). In the case the benzothiazole residue at position 5, introduction of a chloro substituent on the heteroaromatic benzene moiety improved the inhibitory activity (IC_{50} (μM) of 18.6 for compound **15** compared to 4.4 for compound **7**).

Positively or negatively charged residues at position 5 decreases the inhibitory activity. Introduction of a negative charge on the phenylthio ring at position 5 of compound **6** decreased the activity by a factor of 12 (IC_{50} (μM) of compound 18 = 37 compared to 2.9 for compound **6**). It seems that the decrease in inhibitory activity is due to the charge and not due to the increase in size. Compound **8** with an acetamido group at the ortho position of the phenylthio ring has an IC_{50} (μM) of 5.6 compared to 37 of compound **18**. Introduction of positively charged residues at position 5 also decreased the inhibitory activity as compared to their noncharged analogs. Thus, replacement of the phenyl ring of compound **6** (IC_{50} (μM) of 2.9) by a pyridyl ring decreased the activity by a factor of 9 (IC_{50} (μM) of **16** = 25). A similar phenomenon was observed in the case of heteroaromatic ring at position 5 (e.g., compound **12** with an IC_{50} (μM) of 12.5 compared to 75 for compound **20**).

Replacement of the aromatic ring on the thiomethyl group at position 5 by an ω-alkyl carboxylic acid decreases the activity. Thus, compounds **19**, **21**, and **22** had IC_{50} (μM) of 60, 96, and 125, respectively, as compared to 36 and 37 for compounds **17** and **18**, respectively.

(d) The nature of the group at the α position was studied by replacing the carboxamide group by a nitrile group or substituted ketones. The influence of the group in the α position on the inhibitory activity differed according to the nature of the aromatic ring at position 5. Thus, replacing the α-carboxamido group by a nitrile did not effect the inhibitory activity when the aromatic ring in position 5 is substituted with negatively charged carboxyl group (e.g., compound **18** with (IC_{50} (μM) of 37 and compound **17** with 36). Replacement of the α-carboxamido group by a dihydroxy phenyl keto group of inhibitors with a (benzylthio)methyl group at position 5 improved the inhibitory activity 3-fold (compound 1 with an IC_{50} (μM) of 0.75 compared to that of 1.95 for compound **3**). On the other hand, as was mentioned above, a dramatic loss of activity was noticed when the α-carboxamido group was replaced by a cyano group in phenylthio-substituted inhibitors.

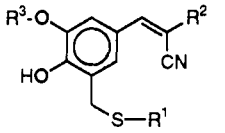
(e) Replacement of the methoxy group in position 3 by a hydroxy group had a profound effect on the inhibitory activity of 5 methyl-substituted benzylidenemalononitriles. Such a replacement improved the inhibitory activity 464-fold (compound **25**, IC_{50} (μM) = 5.6 compared to IC_{50} (μM) = 2600 of compound **27**). Such a modification had a small effect on 5-[(arylthio)methyl]-α-carboxamido inhibitors. Thus, compound **2** with 5-[(benzylthio)-methyl]-3-hydroxy substituents had an IC_{50} (μM) of 0.94 compared to the IC_{50} (μM) of 1.95 of compound **3** with a 3-methoxy substituent. A similar effect was found with 5-[(benzathiazolylthio)methyl]: compound **9** had an IC_{50} (μM) of 6.5 compared to 18.6 of compound **15**.

Because the similarity of the 5-substituted aromatic thiomethyl BMNs' to bisubstrate inhibitors, we have replaced the thioether with sulfone to mimic the phosphate group in the transition state.[6] Indeed, such a modification increased the inhibitory activity by a factor of 4 (IC_{50} (μM) of 85 for compound **26** compared to the IC_{50} (μM) of 370 of compound **23**).

Discrimination between the ErbB2/neu and the EGFR Tyrosine Kinases. We have tested the ability of 5-substituted tyrphostins to inhibit HER1-2/ErbB2 kinase as compared to HER-1/EGFR kinase. The chimera HER1-2 kinase possesses the EGF binding domain of the EGF receptor and the kinase domain of the HER-2/neu/ErbB2 receptor as described previously[3]. Table I shows that one of the BMN *S*-aryltyrphostins discriminates by up to a factor of 54 in favor of HER1-2 kinase.

The nature of the aromatic ring at position 5 had a major effect on the selectivity of the inhibitor as measured by the ratio of inhibition of the ErbB2/neu vs the EGFR kinase autophosphorylation. All the inhibitors (except one) with various aromatic substituents at position 5 could not discriminate between the ErbB2/neu and the EGFR

Table I. Structures and Inhibitory and Antiproliferative Activities of 5-*S*-Aryl-Substituted Tyrphostins



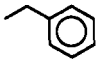
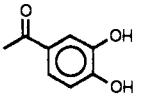
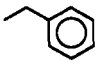
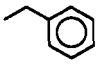
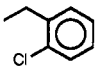
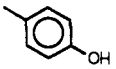
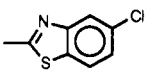
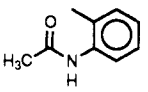
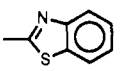
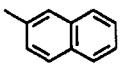
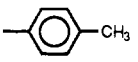
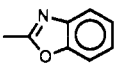
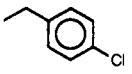
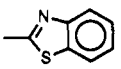
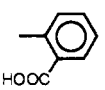
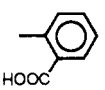
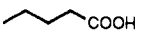
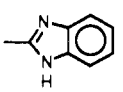
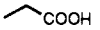
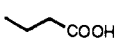
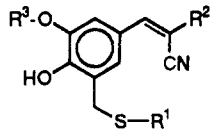
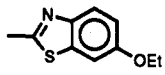
chemical data				IC$_{50}$,[a] μM			
				biochemical data:[c] PolyGAT phosphorylation by EGFR	biological data		
	structure				autophosphorylation		EGF-dependent proliferation of HER-1
no.[b]	R^1	R^2	R^3		EGFR	ErbB2/neu (HER 1-2)	
1			-Me	0.75	4.5	6.2	2
2		$-CONH_2$	-H	0.94	0.64	1.02	3
3		$-CONH_2$	-Me	1.95	0.90	0.20	12.5
4		$-CONH_2$	-Me	2.5	1.00	0.25	10
5		$-CONH_2$	-Me	2.5	2.5	1.6	2
6		$-CONH_2$	-Me	2.9	0.40	0.13	12.5
7		$-CONH_2$	-Me	4.4	>83	15	4.5
8		$-CONH_2$	-Me	5.6	22	3.3	9
9		$-CONH_2$	-H	6.5	3.2	8.1	>50
10		$-CONH_2$	-Me	6.6	1.7	4.6	10
11		$-CONH_2$	-Me	8.7	1.75	1.65	12
12		$-CONH_2$	-Me	12.5	5.3	1.5	6
13		$-CONH_2$	-Me	14.8	16	3	20
14		$-CONH_2$	-Me	16.3	8.8	2.0	>50
15		$-CONH_2$	-Me	18.6	19	0.35	35
16		$-CONH_2$	-Me	25	3.4	0.70	16
17		-CN	-Me	36	8	>8	>50
18		$-CONH_2$	-Me	37	0.71	0.45	>50
19		$-CONH_2$	-Me	60	48	16	6.5
20		$-CONH_2$	-Me	75	14.5	6.1	35
21		-CN	-Me	96	54	91	>50
22		$-CONH_2$	-Me	125	ND	ND	>50

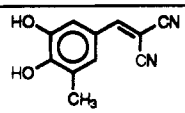
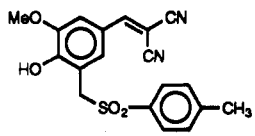
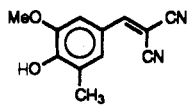
Table I (Continued)



chemical data				IC_{50},[a] μM			
	structure			biochemical data:[c] PolyGAT phosphorylation by EGFR	biological data		
					autophosphorylation		EGF-dependent proliferation of HER-1
no.[b]	R^1	R^2	R^3		EGFR	ErbB2/neu (HER 1-2)	
23		-CN	-Me	370	6.7	2.5	6
24		$-CONH_2$	-Me	>100	>167	ND	ND

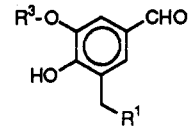
[a] The IC_{50} values for the inhibition of PolyGAT phosphorylation and EGF receptor autophosphorylation were determined at least three times from a full dose–response curve as described by us earlier.[15] Each IC_{50} value was obtained from inhibition curves with 10–12 experimental points, each point in triplicate. Repeated experiments yield values within 10–20% of each other. The IC_{50} values for the inhibition of EGF-dependent HER 14 cell proliferation were determined as described earlier.[15] [b] Compounds are arranged according to ascending compound number. [c] Compounds are arranged according to descending IC_{50} values for tyrphostins inhibition of PolyGAT phosphorylation. ND = not determined.

Table II. Structures and Inhibitory and Antiproliferative Activities of 5-Substituted Tyrphostins

chemical data		IC_{50},[a] μM			
		biochemical data:[c] PolyGAT phosphorylation by EGFR	biological data		
			autophosphorylation		EGF-dependent proliferation of HER-1
no.[b]	structure		EGFR	ErbB2/neu	
25		5.6	62.0	59	13
26		85	23	17.4	15
27		2600	87	112	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.[15] Each IC_{50} value was obtained from inhibition curves with 10–20 experimental points, each point in triplicate. Repeated experiments yield values within 10–20% of each other. The IC_{50} values for the inhibition of EGF-dependent HER 14-cell proliferation were determined as described earlier.[15] [b] Compounds are arranged according to ascending compound number. [c] Compounds are arranged according to descending IC_{50} values for tyrphostins inhibition of PolyGAT phosphorylation.

Table III. Structure of Synthetic Intermediates of Compounds Which Appear in Tables I and II



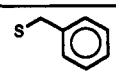
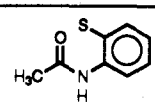
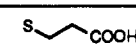
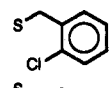
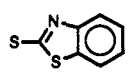
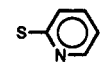
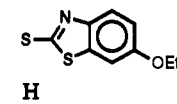
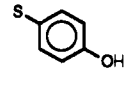
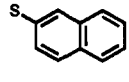
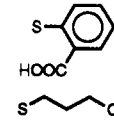
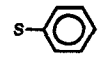
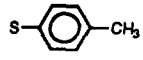
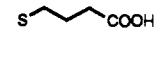
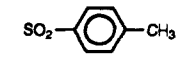
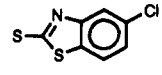
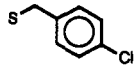
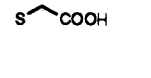
no.	R^1	R^3	no.	R^1	R^3	no.	R^1	R^3	no.	R^1	R^3
28		-Me	33		-Me	38		-Me	43		-Me
29		-Me	34		-Me	39		-Me	44		-Me
30		-Me	35		-Me	40		-Me	45	H	-Me
31		-Me	36		-Me	41		-Me	46		-Me
32		-Me	37		-Me	42		-Me			

Table IV. Synthetic and Structural Data on Compounds and Intermediates Listed in Tables I–III Which Were Prepared According to the General Procedures and the Experimental Section

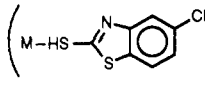
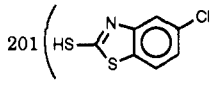
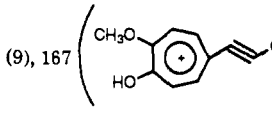
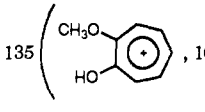
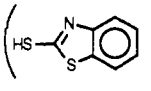
no.	table[a]	method[b]	% yield	mp, °C	solvent[c]	^{1}H NMR, ppm	MS, *m/e*
1	I	B	33	85	A	7.95 (1 H, s, vinyl), 7.83 (1 H, d, J = 1.8 Hz, H_2), 7.60 (1 H, d, J = 1.8 Hz, H_6), 7.40–7.25 (7 H, m, Ph + $H_{2,6}$), 6.98 (1 H, d, J = 7.6 Hz, H_5), 3.94 (3 H, s, OCH_3), 3.78 (2 H, s), 3.75 (2 H, s)	
2	I	C	42	190	A	8.02 (1 H, s, vinyl), 7.63 (1 H, d, J = 2.2 Hz, H_2), 7.38–7.20 (6 H, m), 3.77 (2 H, s), 3.73 (2 H, s)	
3	I	B	50	150	A	8.13 (1 H, s, vinyl), 7.71 (1 H, d, J = 2.0 Hz, H_2), 7.58 (1 H, d, J = 2.0 Hz, H_6), 7.40–7.25 (5 H, m), 3.94 (3 H, s, OCH_3), 3.78 (2 H, s), 3.75 (2 H, s)	354 (M^+, 29), 232 (M – SC_6H_4COOH, 100), 231 (42), 230 (52)
4	I	B	68	135	A	8.13 (1 H, s, vinyl), 7.72 (1 H, d, J = 2.1 Hz, H_2), 7.62 (1 H, d, J = 2.1 Hz, H_6), 7.51–7.27 (4 H, m), 3.94 (3 H, s, OCH_3), 3.89 (2 H, s, CH_2S), 3.82 (2 H, s, CH_2S)	390, 388 (M^+, 8, 18), 232 (38), 231 (23), 230 (32), 160 (12), 158 (20), 125 (100)
5	I	B	47	98	A	8.05 (1 H, s, vinyl), 7.71 (1 H, d, J = 1.9 Hz, H_2), 7.38 (1 H, d, J = 1.9 Hz, H_6), 7.24, 6.77 (4 H, AB q, J = 9.0 Hz), 4.08 (2 H, s, CH_2S), 4.0 (3 H, s, OCH_3)	356 (M^+, 21), 232 (M – SC_6H_4OH, 21), 231 (60), 165 (11), 126 (100)
6	I	B	72	184	A	8.08 (1 H, s, vinyl), 7.73 (1 H, d, J = 2.0 Hz, H_2), 7.57 (1 H, d, J = 2.0 Hz, H_6), 7.4–7.2 (5 H, m), 4.28 (2 H, s, CH_2S), 3.95 (3 H, s, OCH_3)	
7	I	B	60	125	A	8.11–7.30 (6 H, m), 4.74 (2 H, s, CH_2S), 3.93 (3 H, s, OCH_3)	432 (M^+, "transient"), 231 (M–HS–  , 11), 215 (19), 214 (15), 203, 201 (HS–  , 46, 100), 166 (21), 165 (11), 142 (11)
8	I	B	31	112	A	8.12 (1 H, s, vinyl), 7.70–7.10 (6 H, m), 3.97 (2 H, s, CH_2S), 3.88 (3 H, s, OCH_3), 3.37 (3 H, s, NHAc)	397 (M^+, 16), 231 (M – $SC_6H_4NHCOCH_3$, 24), 186 (9), 167 ( , 23), 135 ( , 100), 93 (69)
9	I	C	60	182	A	8.03 (1 H, s, vinyl), 7.70–7.30 (6 H, m), 4.74 (2 H, s, CH_2S)	169 (16), 168 (24), 167 (HS–  , 100), 140 (19), 123 (13), 109 (23), 108 (28), 103 (15)
10	I	B	45	182	A	8.04 (1 H, s, vinyl), 7.87–7.80 (4 H, m), 7.70 (1 H, d, J = 1.8 Hz, H_2), 7.63 (1 H, d, J = 1.8 Hz, H_6), 7.51–7.43 (3 H, m), 4.38 (2 H, s, CH_2S), 3.92 (3 H, s, OCH_3)	390 (M^+, 16), 389 (60), 232 (16), 231 (M – *S*-naphthyl, 87), 186 (10), 160 (HS – naphthyl, 100), 159 (54), 158 (17), 128 (57), 115 (88)
11	I	B	60	168	A	8.96 (br s, NH), 8.05 (1 H, s, vinyl), 7.71 (1 H, d, J = 2.1 Hz, H_2), 7.49 (1 H, d, J = 2.1 Hz, H_6), 7.27–7.10 (4 H, AB q, J_{AB} = 8.5 Hz, H_2), 4.20 (2 H, s, CH_2S), 3.92 (3 H, s, OCH_3), 2.27 (3 H, s, CH_3)	354 (M^+, 60), 231 (M – $SC_6H_4CH_3$, 100), 230 (14), 186 (10), 124 (30), 123 (21)

Table IV (Continued)

no.	table[a]	method[b]	% yield	mp, °C	solvent[c]	^{1}H NMR, ppm	MS, *m/e*
12	I	B	83	20	A	8.16 (1 H, s, vinyl), 7.73, 7.69 (2 H, 2d, J = 1.8 Hz, $H_{2,6}$), 7.49–7.25 (4 H, m), 4.59 (2 H, s, CH_2S), 3.94 (3 H, s, OCH_3)	
13	I	B	55	154	A	8.13 (1 H, s, vinyl), 7.69 (1 H, d, J = 1.6 Hz, H_2), 7.58 (1 H, d, J = 1.6 Hz, H_6), 7.32 (4 H, AB q, J = 8.7 Hz), 3.93 (3 H, s, OMe), 3.77 (2 H, s, CH_2S), 3.74 (2 H, CH_2S)	390, 388 (M^+, 6, 15), 232 (56), 231 (23), 172 (18), 125 (100)
14	I	B	60	150	A	8.15 (1 H, s, vinyl), 7.73 (1 H, d, J = 2.0 Hz, H_2), 7.69 (1 H, d, J = 2.0 Hz, H_6), 4.58 (2 H, s, CH_2S), 4.03 (2 H, t, J = 7.6 Hz), 3.93 (3 H, s, OCH_3), 3.60 (2 H, t, J = 7.6 Hz)	276 (M − 73, 72), 230 (100), 201 (14), 200 (11), 185 (12), 159 (16)
15	I	B	20	268	D	8.02–7.31 (7 H, m), 5.66 (2 H, s), 3.89 (3 H, s, OCH_3)	
16	I	B	43	170	A	8.54 (1 H, m), 8.08 (1 H, s, vinyl), 7.74–7.62 (3 H, m), 7.32 (1 H, m), 7.16 (1 H, m), 4.50 (2 H, s, CH_2S), 3.92 (3 H, s, OCH_3)	341 (M^+, 12), 308 (9), 111 (100)
17	I	B	24	156	D	7.74 (1 H, s, vinyl), 7.86 (1 H, d, J = 7.0 Hz), 7.50 (4 H, m), 7.18 (1 H, m), 4.05 (2 H, s, CH_2S), 3.74 (3 H, s, OCH_3)	366 (M^+, 16), 348 (M − H_2O, 37), 214 (16), 213 (M − SC_6H_4COOH, 86), 154 (17), 153 (13), 136 (100), 108 (27)
18	I	B	52	263	D	8.03 (1 H, s, vinyl), 7.91–7.21 (6 H, m), 4.16 (2 H, s, CH_2S), 3.87 (3 H, s, OCH_3)	384 (M^+, 21), 366 (M − H_2O, 25), 241 (M − SC_6H_4COOH, 100), 154 (30)
19	I	B	28	263	A	8.15 (1 H, s, vinyl), 7.72 (1 H, d, J = 2.0 Hz, H_2), 7.61 (1 H, d, J = 2.0 Hz, H_6), 3.94 (3 H, s, OMe), 3.81 (2 H, s, CH_2S), 2.57 (2 H, t, J = 6.5 Hz), 2.41 (2 H, t, J = 6.5 Hz), 1.90 (2 H, quin, J = 6.5 Hz)	233 (M − $HS(CH_2)_3COOH$, 13), 232 (57), 231 (76), 230 (100), 192 (12), 186 (18), 157 (15), 147 (14), 137 (36), 102 (38)
20	I	B	13	215	A	8.14 (1 H, s, vinyl), 7.71–7.67 (2 H, 2d, J = 1.8 Hz, $H_{2,6}$), 7.1 (1 H, d, J = 8.1 Hz, H_7), 7.05 (1 H, br s, H_4), 6.98 (1 H, br d, J = 8.1 Hz, H_6), 4.57 (2 H, s, CH_2S), 3.92 (3 H, s, OCH_3)	394 (M^+, 41), 360 (21), 328 (24), 231 (29), 178 (15), 164 (100)
21	I	B	82	148	A	8.12 (1 H, s, vinyl), 7.74 (1 H, d, J = 2.1 Hz, H_2), 7.60 (1 H, d, J = 2.1 Hz, H_6), 3.95 (2 H, s, CH_2S), 3.93 (3 H, s, OMe), 3.26 (2 H, s, SCH_2COOH)	304 (M^+, 24), 286 (M − H_2O, 53), 245 (M − CH_2COOH, 27), 213 (M − SCH_2COOH, 100), 185 (16), 156 (32)
22	I	B	37	205	A	8.15 (1 H, s, vinyl), 7.74 (1 H, d, J = 2.0 Hz, H_2), 7.60 (1 H, d, J = 2.0 Hz, H_6), 3.96 (3 H, s, OMe), 3.86 (2 H, s, CH_2S), 2.75–2.60 (4 H, m)	336 (M^+, 56), 230 (M − SCH_2COOH, 100), 188 (21), 187 (29), 186 (19), 185 (10), 159 (14), 157 (18)
23	I	B	98	173	C	7.65 (1 H, d, J = 2.0 Hz, H_2), 7.48 (1 H, s, vinyl), 7.29–7.21 (5 H, m), 7.09 (1 H, d, J = 2.0 Hz, H_6), 4.12 (2 H, s, CH_2S), 3.95 (3 H, s, OCH_3)	
24	I	B	46	185	A	8.11 (1 H, s, vinyl), 7.85–7.72 (3 H, m), 7.47 (1 H, d, J = 2.5 Hz, H_2), 7.10 (1 H, d, J = 2.5 Hz, H_6), 4.70 (2 H, s, CH_2S), 4.10 (2 H, q, J = 7.0 Hz), 3.94 (3 H, s, OMe), 1.44 (3 H, t, J = 7.0 Hz)	
25	II	C	16	197	A	7.96 (1 H, s, vinyl), 7.59 (1 H, d, J = 2.0 Hz, H_2), 7.31 (1 H, d, J = 2.0 Hz, H_6), 2.24 (3 H, s, CH_3)	
26	II	B	37	177	A	8.05 (1 H, s, vinyl), 7.81 (1 H, d, J = 2.0 Hz, H_2), 7.59 (2 H, d, J_{AB} = 8.3 Hz), 7.36–7.34 (3 H, m, overlaps of H_6 and right wing AB), 4.54 (2 H, s, CH_2SO_2), 3.88 (3 H, s, OCH_3), 2.41 (3 H, s, CH_3)	

Table IV (Continued)

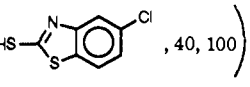
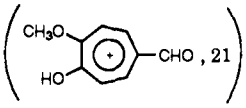
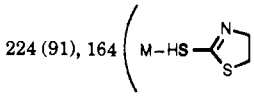
no.	table[a]	method[b]	% yield	mp, °C	solvent[c]	^{1}H NMR, ppm	MS, *m/e*
27	II	B	74	140	A	8.01 (1 H, s, vinyl), 7.63 (1 H, d, J = 2.0 Hz, H_2), 7.44 (1 H, d, J = 2.0 Hz, H_6), 3.92 (3 H, s, OCH_3), 2.24 (3 H, s, CH_3)	
28	III	A	37	48	C	9.79 (1 H, s, CHO), 733 (7 H, br s), 3.96 (3 H, s, OCH_3), 3.73 (2 H, s), 3.71 (2 H, s)	
29	III	A	18	oil	C	9.78 (1 H, s, CHO), 7.41–7.14 (6 H, m), 3.93 (3 H, s, OCH_3), 3.83 (2 H, s, CH_2S), 3.80 (2 H, s, CH_2S)	324, 322 (M^+, 13, 37), 166 (60), 165 (89), 164 (69), 163 (25), 149 (42), 125 (100
30	III	A	49	107	C	9.64 (1 H, s, CHO), 7.28 (1 H, d, J = 1.9 Hz, H_2), 7.13 (1 H, d, J = 1.9 Hz, H_6), 7.20, 6.73 (4 H, AB q, J = 8.3 Hz), 4.04 (2 H, s, CH_2S), 3.89 (3 H, s, OCH_3)	
31	III	A	20	93	C	9.72 (1 H, s, CHO), 7.30 (7 H, m), 4.20 (2 H, s, CH_2S), 3.96 (3 H, s, OCH_3)	
32	III	A	56	130	C	9.81 (1 H, s, CHO), 7.95 (1 H, d, J = 1.9 Hz, $H_{4'}$), 7.64 (1 H, d, J = 8.4 Hz, $H_{7'}$), 7.52 (1 H, d, J = 1.8 Hz, H_2), 7.36 (1 H, d, J = 1.8 Hz, H_6), 7.29 (1 H, dd, J = 8.4, 1.9 Hz, $H_{6'}$), 4.65 (2 H, s, CH_2S), 3.95 (3 H, s, OCH_3)	367, 365 (M^+, 3, 9), 203, 201 ( , 40, 100), 166 (22), 164 (15), 165 ( , 21)
33	III	A	77	oil	C	9.76 (1 H, s, CHO), 7.60–7.10 (6 H, m), 3.92 (2 H, s, CH_2S), 3.83 (3 H, s, OCH_3), 3.38 (3 H s, NHAc)	
34	III	A	50	116	A	9.83 (1 H, s, CHO), 7.95 (2 H, m), 7.50, 7.35 (2 H, m), 7.75 (1 H, d, J = 1.9 Hz, H_2), 7.42 (1 H, d, J = 1.9 Hz, H_6), 4.77 (2 H, s, CH_2S), 3.96 (3 H, s, OCH_3)	
35	III	A	36	145	C	9.70 (1 H, s, CHO), 7.77–7.70 (4 H, m), 7.48–7.44 (1 H, m), 7.38 (1 H, d, J = 1.8 Hz, H_2), 7.33 (1 H, d, J = 1.8 Hz, H_6), 4.32 (2 H, s, CH_2S), 3.97 (3 H, s, OCH_3)	325 (48), 324 (M^+, 98), 166 (18), 165 (97), 161 (30), 160 (HS – naphthyl, 100), 159 (22), 150 (19), 128 (12), 115 (67), 109 (10)
36	III	A	30	60	C	9.71 (1 H, s, CHO), 7.31 (1 H, d, J = 1.8 Hz, H_2), 7.27 (overlap with $CDCl_3$, H_6), 7.24, 7.06 (4 H, AB q, J_{AB} = 8.0 Hz), 4.15 (2 H, s, CH_2S), 3.96 (3 H, s, OCH_3), 2.28 (3 H, s, CH_3)	
37	III	A	25	92	C	9.80 (1 H, s, CHO), 7.36 (1 H, d, J = 1.8 Hz, H_2), 7.32 (1 H, d, J = 1.8 Hz, H_6), 7.35 (4 H, s, C_6H_4Cl), 3.98 (3 H, s, OCH_3), 3.72 (2 H, s, CH_2S), 3.67 (2 H, s, CH_2S)	324, 322 (M^+, 4, 13), 166 (45), 165 (46), 164 (25), 127 (32), 125 (100)
38	III	A	9	195	A	9.81 (1 H, s, CHO), 7.57 (1 H, d, J = 1.8 Hz, H_2), 7.38 (1 H, d, J = 1.8 Hz, H_6), 4.46 (2 H, s, CH_2S), 4.25 (2 H, t, J = 8.0 Hz), 3.93 (3 H, s, OCH_3), 3.52 (2 H, t, J = 8.0 Hz)	283 (M^+, 2), 270 (17), 225 (12), 224 (91), 164 ( , 100), 163 (87), 136 (31), 135 (48), 134 (59), 133 (15), 121 (18), 119 (17)
39	III	A	88	71	C	9.81 (1 H, s, CHO), 8.53 (1 H, m), 7.60 (1 H, m), 7.4–7.3 (3 H, m), 7.14 (1 H, m), 4.39 (2 H, s, CH_2S), 3.96 (3 H, s, OCH_3)	
40	III	A	61	220	A	9.82 (1 H, s, CHO), 8.03 (1 H, dd, J = 7.5, 1.1 Hz, $H_{6'}$), 7.64 (1 H, d, J = 1.8 Hz, H_2), 7.56 (1 H, m), 7.39 (1 H, d, J = 1.8 Hz, H_6), 7.24 (2 H, m), 4.31 (2 H, s, CH_2S), 3.96 (3 H, s, OCH_3)	

Table IV (Continued)

no.	table[a]	method[b]	% yield	mp, °C	solvent[c]	^{1}H NMR, ppm	MS, m/e
40					D	9.76 (1 H, s, CHO), 7.90 (1 H, m), 7.57 (1 H, d, J = 1.8 Hz, H_2), 7.37 (1 H, d, J = 1.8 Hz, H_6), 7.20 (2 H, m), 4.19 (2 H, s, CH_2S), 3.96 (3 H, s, OCH_3)	
41	III	A	40	oil	C	9.82 (1 H, s, CHO), 7.45 (1 H, d, J = 1.7 Hz, H_2), 7.33 (1 H, d, J = 1.7 Hz, H_6), 3.95 (3 H, s, OMe), 3.81 (2 H, s, CH_2S), 2.53 (2 H, t, J = 6.7 Hz), 2.42 (2 H, t, J = 6.7 Hz), 1.95 (2 H, quin J = 6.7 Hz), irradiation at 2.53 ppm gave triplet at 1.95 ppm	
42	III	A	7	130	A	9.84 (1 H, s, CHO), 7.54 (1 H, d, J = 1.9 Hz, H_2), 7.40 (1 H, d, J = 1.9 Hz, H_6), 3.97 (2 H, s, CH_2S), 3.95 (3 H, s, OMe), 3.27 (2 H, s, SCH_2COOH)	
43	III	A	31	115	A	9.84 (1 H, s, CHO), 7.55 (1 H, d, J = 1.8 Hz, H_2), 7.37 (1 H, d, J = 1.8 Hz, H_6), 3.95 (3 H, s, OMe), 3.89 (2 H, s, CH_2S), 2.8–2.6 (4 H, m)	
44	III	A	30	97	A	9.82 (1 H, s, CHO), 7.80 (1 H, d, J = 8.7 Hz, $H_{4'}$), 7.73 (1 H, d, J = 1.8 Hz, H_6), 7.48 (1 H, d, J = 2.6 Hz, $H_{7'}$), 7.41 (1 H, d, J = 1.8 Hz, H_2), 7.08 (1 H, dd, J = 8.7, 2.6 Hz, $H_{5'}$), 4.72 (2 H, s, CH_2S), 3.96 (3 H, s, OMe), 1.41 (3 H, t, J = 7.0 Hz)	
45	III		75	99	C	9.80 (1 H, s, CHO), 7.30 (2 H, dd), 3.96 (3 H, s, OCH_3), 2.32 (3 H, s, CH_3)	
46	III		29	136	C	9.77 (1 H, s, CHO), 7.61, 7.25 (4 H, AB q, J = 8.2 Hz, $SO_2C_6H_4$), 7.38 (1 H, d, J = 1.7 Hz, H_2), 7.34 (1 H, d, J = 1.7 Hz, H_6), 4.08 (2 H, s, CH_2SO_2), 3.93 (3 H, s, OCH_3), 2.43 (3 H, s, CH_3)	

[a] Table that shows structural and biological data. [b] See Experimental Section [c] A: acetone-d_6. C: chloroform-d. D: (methyl sulfoxide)-d_6.

PTK's. The only exception is compound **15**. This compound inhibited the autophosphorylation of the ErbB2/neu PTK with an IC_{50} (μM) of 0.375 compared to an IC_{50} (μM) of 19 for the inhibition of the EGFR PTK. We attempted to increase the inhibitory activity of compound **15** while maintaining its selectivity by the following modifications: (a) replacement of the 5-methoxy group by 5-hydroxy (e.g., compound **9**), (b) changing a hetero atom in the hetero aromatic ring at position 5 (e.g., compounds **12** and **20**), and (c) substitutions on the aromatic ring at position 5 (e.g., compounds **7** and **24**). Despite the fact that some modifications increased the inhibitory activity (e.g., compound **7** with an IC_{50} (μM) of 4.4 compared to 18.6 for compound **15**), the selectivity in all these compounds was reduced. Compound **7** was found to be more effective in inhibiting EGFR-catalyzed phosphorylation of polyGAT than EGFR autophosphorylation. The opposite holds true for compound **18** (Table I). EGFR-catalyzed phosphorylation of polyGAT represents the activity of the fully activated receptor toward exogenous substrate whereas autophosphorylation reflects the initial step of receptor activation. This initial step involves the EGF-induced formation of receptor dimer followed by transphorylation. It seems that compound **18** binds well to the EGF receptor active site prior to its activation but poorly to the fully activated conformation of the active site of the activated receptor. Tyrphostin **7** on the other hand, behaves in a opposite manner: it better inhibits the fully formed active site as compared with the site prior to its activation.

Antiproliferative Activity of the *S*-Aryltyrphostins. A few of the *S*-aryltyrphostins were also found to be potent antiproliferative agents. Interestingly, the tyrphostins which discriminate between the HER-1 (EGFR) kinase domain and the HER-2 kinase domain *in vitro* do not exhibit differential antiproliferative activity. Thus, when examining the potency of the tyrphostins to block EGF-dependent DNA synthesis in NIH3T3 cells which express either HER-1 or HER1-2, one does not observe the difference characteristic of the *in vitro* kinase experiments (Table I). IC_{50} values for the inhibition of EGF-dependent proliferation of HER1-2 cells by compounds **5**, **8**, **15**, and **18** were within the standard deviation of the results obtained for HER1 cells (not shown). A number of possible explanations may account for this apparent discrepancy. Since the compounds compete with ATP as well as with the substrate,[11,12] the blockers must overcome high intracellular ATP concentrations which mask their differential activity. Alternatively, or even additionally, the tyrphostins may act not only at the level of HER-1 and HER1-2 kinases but also at a signal transduction element downstream to these receptor kinases. We are currently investigating whether the blockers inhibit different signal transduction elements which may be downstream to the

receptor tyrosine kinases, such as the *src*-type PTKs. We have previously shown that activation of bone marrow fibroblasts by PDGF stimulates the activation of $pp60^{c\text{-}src}$ and that tyrphostins block this activation.[13] Similarly, tyrphostins block the activation of *src*-type tyrosine kinases in platelets activated by thrombin.[14] In both cases these cellular PTKs mediate signals emanating from receptors at the plasma membrane. These findings, therefore, have prompted us to search for the downstream elements for HER-1 and HER-2 kinases among the *src*-type PTKs, a study under way.

Experimental Section

Chemistry. 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.

Typical procedures for each class of compounds are given with one detailed example. Tables I and II show the structures of the tyrphostins synthesized in this work. Table III shows the structures of the corresponding intermediary aldehydes. All compounds shown in Tables I-III were fully characterized spectroscopically (^1H NMR, MS, elemental analysis, and ^{13}C NMR for several compounds). Structural and synthetic data are given in Table IV for the tyrphostins and their intermediary aldehydes.

5-Chlorovanillin was prepared according to ref **16** and was reacted with the appropriate thiols (or with *p*-tolylsulfinic acid for compound **46**) as described in method A below to give the 5-substituted aldehydes. Reaction with cyano acetamide as described in method B (or with malononitrile for compounds **17**, **21**, **23**, **26**, and **27** and with α-cyano-3,4-dihydroxyacetophenone[3] for compound **1**) gave the tyrphostins. The intermediary aldehydes for compounds **12** and **20** were not isolated and used directly. Compound **45** was prepared according to ref 16. Compounds **2**, **9**, and **25** were prepared according to method C from the corresponding 3-methoxy compounds **3**, **15**, and **27**.

Compound 31, 5-[(Phenylthio)methyl]vanilline (Tables III and IV) (Method A). To 1 g (5 mM) of 5-(chloromethyl)-vanillin[16] and 0.6 mL (5.2 mM) of thiophenol in 50 mL of dichloromethane was added 0.7 mL (0.7 mM) of Et_3N. After 3 h of stirring at room temperature the solution was concentrated in vacuo and chromatographed directly on silica gel to give 0.27 g, 20% yield, of white solid, mp 91 °C.[10]

Compound 6, 3-Methoxy-4-hydroxy-5-[(phenylthio)-methyl]-α-carboxamidocinnamonitrile (Tables I and IV) (Method B). One hundred milligrams (0.4 mM) of compound **31** and 50 mg (0.6 mM) of cyanoacetamide in 5 mL of ethanol and 2 drops of piperidine were refluxed for 3 h. After cooling, the mixture was added to 30 mL of water and extracted three times with 25 mL of dichloromethane. After drying on $MgSO_4$, the dichloromethane was evaporated to dryness to give 90 mg of yellow solid, 72% yield, mp 184 °C.

Compound 2, 3,4-Dihydroxy-5-[(benzylthio)methyl]-α-carboxamidocinnamonitrile (Tables I and IV) (Method C). To 100 mg (0.3 mM) of compound **3** in 20 mL of CH_2Cl_2 under Ar was added 0.6 mL (6 mM) of BBr_3. The orange solution was stirred 2.5 h at room temperature, water and HCl were added, and the reaction mixture was extracted with EtOAc. Drying on $MgSO_4$, evaporation, and trituration with EtOAc–benzene gave dark yellow solid, 40 mg, 42% yield, mp 190 °C.

Biochemical Methods. The biochemical protocols to examine the kinase inhibitory potency of tyrphostins on EGF receptor/HER 1 and on the HER 1-2 chimera were described by us previously[3,15] and were used with no modification. Similarly, the antiproliferative activity was determined by measuring the potency of the tyrphostins to block ^3H-thymidine uptake.[3,15]

Acknowledgment. This study was partially supported by Rhone Poulenc Rorer pharmaceuticals, King of Prussia, PA, and partially by the Israel Association Against Cancer. We thank Drs. J. Schlessinger (NYU Medical School) and A. Ulrich (Max Planck Institute, Martinsried) for the cell lines used in this study.

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