[(Alkylamino)methyl]acrylophenones: Potent and Selective Inhibitors of the Epidermal Growth Factor Receptor Protein Tyrosine Kinase

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[(Alkylamino)methyl]acrylophenones and (alkylamino)propiophenones, bearing a spacer moiety such as the benzyloxy or (benzoylsulfonyl)oxy group in the 4-position, represent a novel class of inhibitors of the epidermal growth factor (EGF) receptor protein tyrosine kinase with a high degree of selectivity versus other tyrosine and serine/threonine kinases. The most active compounds inhibited the EGF receptor protein tyrosine kinase from A431 cell membranes with IC_{50} values of <0.5 μM. Derivatives with a benzyloxy substituent in the 4-position of the aromatic ring inhibited both the EGF receptor kinase and the proliferation of an EGF-dependent mouse epidermal keratinocyte cell line (BALB/MK) but were only marginally active in the inhibition of the cellular EGF-dependent tyrosine phosphorylation. Compound **18** inhibited ligand-induced tyrosine phosphorylation and BALB/MK cell proliferation with IC_{50} values of approximately 100 and 1.21 μM, respectively, and showed antitumor activity *in vivo* in a nude mouse model. However, the discrepancy between the IC_{50} values for antiproliferative activity and cellular tyrosine phosphorylation as well as the relatively low tolerability in animals suggests a second site of action of this class of inhibitors. Nevertheless, [(alkylamino)methyl]-acrylophenones and (alkylamino)propiophenones may prove to be interesting tools for studying the action of tyrosine kinases.

Introduction

In recent years, extensive knowledge has been accumulated about the importance of protein tyrosine kinases (PTK's) as mediators of proliferative as well as metabolic signals. The generation of mitogenic signals from abnormally expressed or deregulated PTK's has been associated with a number of proliferative diseases. One of the most promising targets for the therapy of proliferative diseases is the epidermal growth factor receptor (EGF-R) protein tyrosine kinase family of transmembrane growth factor receptors (e.g., c-erbB1, c-erbB2). The EGF-R is a transmembrane glycoprotein that mediates the mitogenic response of cells to the epidermal growth factor (EGF) family of mitogenic polypeptides which include EGF, transforming growth factor α (TGF-α),[1,2] and amphiregulin.[3] The EGF-R and its ligands are involved in epithelial proliferation and have been strongly implicated in malignant tumor growth[4–6] and proliferative skin diseases like psoriasis.[7] The *c-erbB1* gene, which encodes the EGF-R, has been found to undergo gene amplification or overexpression in many human tumors of epithelial or neuroepithelial origin.[5] In addition, the closely related *c-erbB2* gene has been found to be amplified or overexpressed at high frequency in human mammary and ovarian carcinomas.[8]

Enzymatic activity of the intracellular domain of the EGF-R is essential for signal transduction via the EGF-R.[9,10] The activated EGF-R phosphorylates tyrosine residues in its C-terminal tail, as well as intracellular substrate proteins, many of which contain Src-homolgy 2 (SH2) domains.[11] These intracellular phosphorylation events in turn mediate the intracellular signaling cascade which results in activation of nuclear events such as transcription of immediate early genes.[12] The involvement of EGF-R protein tyrosine kinases in epithelial proliferation suggests therefore that enzyme inhibitors could have therapeutic potential in the treatment of malignant and nonmalignant epithelial diseases. Due to the involvement of tyrosine kinases in many signal transduction pathways, it will be important to develop agents with high selectivity at the enzyme level. A number of different classes of compounds have been reported as tyrosine kinase inhibitors (reviewed in refs 13 and 14). Tyrphostins,[15–18] a series of lavendustin analogues,[19,20] 7,8-dihydroxyisoquinoline derivatives,[21] 2-thioindoles,[22] 3-substituted quinoline derivatives,[23] dianilinophthalimides,[24,25] and anilinoquinazolines[26,27] represent interesting classes of potent and selective PTK inhibitors. In the present paper, we describe the synthesis, structure–activity relationships, and biological profile of a novel group of tyrosine kinase inhibitors which are designed to act as multisubstrate complex inhibitors. They preferentially inhibit the EGF-R tyrosine kinase *in vitro* and exhibit weak antitumor activity *in vivo*.

Inhibitor Design

In the course of such a search for PTK inhibitors via the random screening of a pool of CIBA chemicals, we found the [(dimethylamino)methyl]acrylophenone (**1**) and its benzyl derivative **2** to be potent and selective inhibitors of the EGF-R PTK. By further optimization of this lead structure, a series of derivatives thereof with interesting biological properties have been synthesized.

The transition state postulated for the transfer of the γ-phosphate group of ATP to a tyrosine moiety of a

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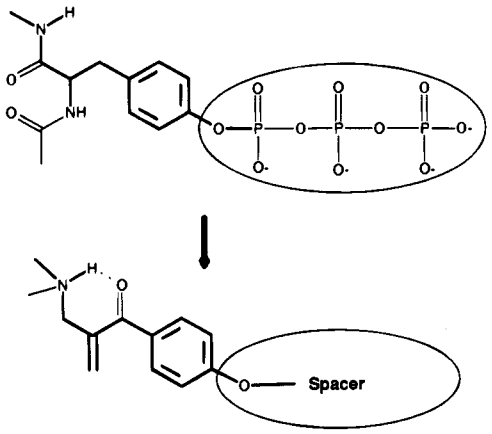


Figure 1. Rational concept: correlation between [(alkylamino)methyl]acrylophenones and tyrosine.

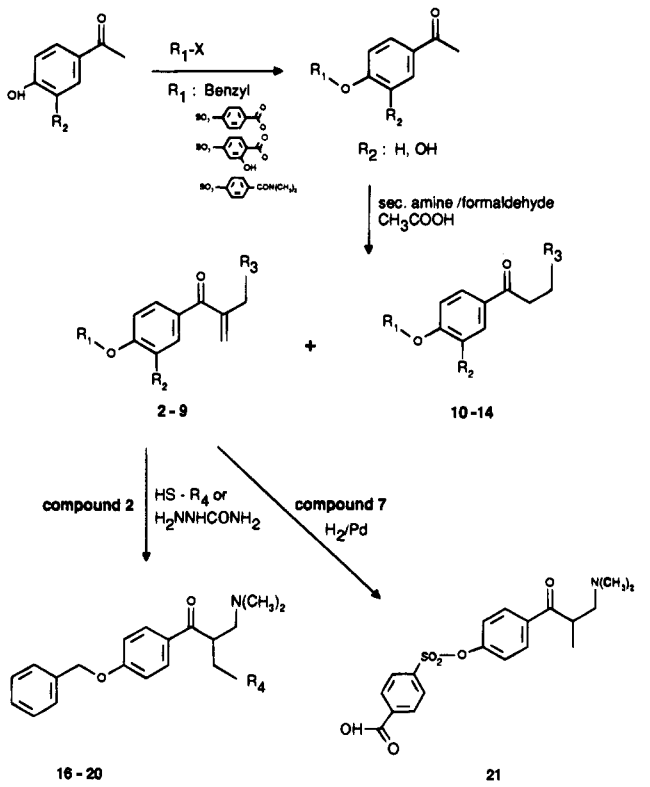
substrate protein suggests the design of bisubstrate-type inhibitors. On the basis of the assumption that the [(dimethylamino)methyl]acrylophenone moiety is a tyrosine mimic (Figure 1) and on the basis of our previous work with (sulfonylbenzoyl)nitrostyrenes,[28] we have approached the rational design of bisubstrate-type PTK inhibitors by combining the [(dimethylamino)methyl]-acrylophenone or [(dimethylamino)methyl]propiophenone moiety with a phosphate mimic/spacer such as a sulfonylbenzoyl or benzyloxy group.

The α,β-unsaturated ketone moiety in [(alkylamino)-methyl]acrylophenones is known to act as a Michael acceptor and could therefore be associated with the toxic properties of this compound class. In order to improve the therapeutic window of (alkylamino)acrylophenones, a series of masked derivatives has been synthesized by the addition of sulfur nucleophiles like thiophenols or nitrogen nucleophiles like semicarbazine to this Michael acceptor moiety. In addition, by replacement of the dimethylamino moiety by secondary amines and by introduction of an additional hydroxyl group in the meta position of the aromatic ring, structure–activity relationships (SAR) within the class of (alkylamino)acrylophenones and (alkylamino)propiophenones, respectively, have been evaluated.

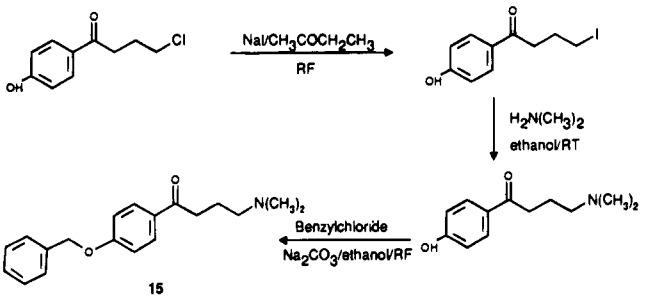
Chemistry

[(Dialkylamino)methyl]acrylophenones or (dialkylamino)propiophenones can easily be prepared in moderate to good yield by Mannich reaction of the corresponding substituted acetophenone with formaldehyde and the corresponding secondary amine according to published procedures (compounds **1**, **2**–**14**).[29,30] Under these reaction conditions, the corresponding [(dialkylamino)-methyl]propiophenones are obtained as byproducts. If necessary, the yield of the (dialkylamino)propiophenone can be improved by replacing acetic acid as solvents by alcohols like ethanol or isoamyl alcohol.[31,32] The spacer groups are introduced by reaction of 4-hydroxyacetophenone with either benzyl chloride, 4-(chlorosulfonyl)-benzoic acid, or 4-(chlorosulfonyl)-2-hydroxybenzoic acid under Schotten–Baumann conditions according to published procedures[28,31,32] (Scheme 1). Starting from 4-(benzyloxy)[(dimethylamino)methyl]acrylophenone (**2**), compound **16** was prepared by the addition of cysteamine and compounds **17**–**19** by the addition of thiophenol, 3,4-dimethylthiophenol, or methyl thiophenol-2-carboxylate, respectively. Compound **20** was obtained by

Scheme 1



Scheme 2



the addition of semicarbazide to compound **2**. Compound **21** was obtained by hydrogenation of compound **7** (Scheme 1).

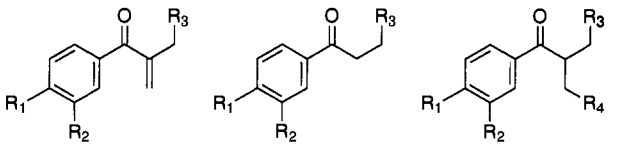
Compound **15** was synthesized in three steps starting from 1-(4-hydroxyphenyl)-4-chlorobutan-1-one (Scheme 2). Unless otherwise indicated, compounds were crystallized as hydrochlorides (Table 1).

Biological Evaluation and Discussion

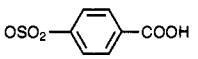
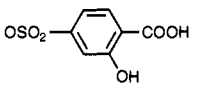
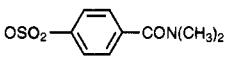
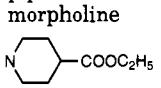
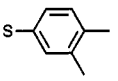
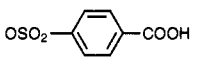
Enzymatic Activity. Compounds were tested against a panel of tyrosine and serine/threonine kinases (Table 2). The most active compounds showed IC_{50} values between 0.18 and 2.5 μM for the inhibition of the EGF-R tyrosine kinase activity from A431 membranes using angiotensin II as phosphate acceptor substrate. When tested for selectivity against the v-abl and c-src tyrosine kinases, no or only marginal inhibition was found for all compounds. In addition, when tested against the serine/threonine protein kinase C (PKC) or cAMP-dependent protein kinase (PKA) (data not shown), no or only marginal inhibition was found (most IC_{50} values > 500 μM).

SAR studies showed that the addition of the benzyloxy group to compound **1** led to a more than 30-fold increase

Table 1. Physicochemical Properties of Compounds 1–21



1–9 10–15 16–21

compd	R_1	R_2	R_3	R_4	formula	FABMS	anal.
1	H	H	$N(CH_3)_2$	n/a	$C_{11}H_{13}NO\cdot HCl$	190 $(M+H)^+$	C,H,N,Cl
2	$OCH_2C_6H_5$	H	$N(CH_3)_2$	n/a	$C_{19}H_{21}NO_2\cdot HCl$	296 $(M+H)^+$	C,H,N
3	$OCH_2C_6H_5$	OH	$N(CH_3)_2$	n/a	$C_{19}H_{21}NO_3\cdot HCl$	312 $(M+H)^+$	C,H,N,Cl
4	$OCH_2C_6H_5$	H	piperidine	n/a	$C_{12}H_{25}NO_2\cdot HCl$	336 $(M+H)^+$	C,H,N,Cl
5	$OCH_2C_6H_5$	H	morpholine	n/a	$C_{21}H_{23}NO_3\cdot HCl$	338 $(M+H)^+$	*a*
6	$OCH_2C_6H_5$	H		n/a	$C_{22}H_{26}N_2O_2\cdot HCl$	351 $(M+H)^+$	C,H,N,Cl[c]
7		H	$N(CH_3)_2$	n/a	$C_{19}H_{19}NO_6S$	390 $(M+H)^+$	C,H,N,S
8		H	$N(CH_3)_2$	n/a	$C_{19}H_{19}NO_7S$	406 $(M+H)^+$	C,H,N,S[c]
9		H	$N(CH_3)_2$	n/a	$C_{21}H_{24}N_2O_5S\cdot HCl$	417 $(M+H)^+$	*b*
10	$OCH_2C_6H_5$	H	$N(CH_3)_2$	n/a	$C_{18}H_{21}NO_2\cdot HCl$	284 $(M+H)^+$	C,H,N,Cl
11	$OCH_2C_6H_5$	H	piperidine	n/a	$C_{21}H_{25}NO_2\cdot HCl$	324 $(M+H)^+$	C,H,N,Cl
12	$OCH_2C_6H_5$	H	morpholine	n/a	$C_{20}H_{23}NO_3\cdot HCl$	326 $(M+H)^+$	C,H,N,Cl
13	$OCH_2C_6H_5$	H		n/a	$C_{24}H_{29}NO_4\cdot HCl$	396 $(M+H)^+$	*b*
14	$OCH_2C_6H_5$	OH	$N(CH_3)_2$	n/a	$C_{18}H_{21}NO_3\cdot HCl$	300 $(M+H)^+$	C,H,N,Cl
15	$OCH_2C_6H_5$	H	$CH_2N(CH_3)_2$	n/a	$C_{19}H_{23}NO_2\cdot HCl$	298 $(M+H)^+$	*b*
16	$OCH_2C_6H_5$	H	$N(CH_3)_2$	$SCH_2CH_2NH_2$	$C_{21}H_{28}N_2O_2S\cdot 2HCl$	373 $(M+H)^+$	C,H,N,S,Cl[c]
17	$OCH_2C_6H_5$	H	$N(CH_3)_2$	SC_6H_5	$C_{25}H_{27}NO_2S\cdot HCl$	406 $(M+H)^+$	C,H,N,S,Cl
18	$OCH_2C_6H_5$	H	$N(CH_3)_2$		$C_{27}H_{31}NO_2S\cdot HCl$	434 $(M+H)^+$	C,H,N,S,Cl
19	$OCH_2C_6H_5$	H	$N(CH_3)_2$		$C_{27}H_{29}NO_4S\cdot HCl$	464 $(M+H)^+$	C,H,N[c]
20	$OCH_2C_6H_5$	H	$N(CH_3)_2$	$NHNHCONH_2$	$C_{20}H_{26}N_4O_3$	371 $(M+H)^+$	*b*
21		H	$N(CH_3)_2$	H	$C_{19}H_{21}NO_6S$	392 $(M+H)^+$	*b*

[a] Hygroscopic. [b] No elemental analysis (only small amount of compound available). [c] Carbon analysis outside the limits required, probably due to inclusion of hydrates. [c] n/a = not applicable.

in activity against the EGF-R PTK (compound **2**). An additional hydroxyl group in the ortho position to the benzyloxy substituent (compounds **3**, **14**) or replacement of the dimethylamino group by other secondary amino groups had only minor effects on the inhibitor activity (compounds **4**–**6**, **11**–**13**). The amino group in the β-position of the keto group is crucial. Replacement of the dialkylamino group by a hydroxy group (data not shown) or chain extension to (aminoethyl)propiophenones led to an inactive compound, **15**. Addition of a (sulfonylbenzoyl)oxy or (hydroxysulfonylbenzoyl)oxy moiety to compound **1** led to inhibitors in the submicromolar range (compounds **7**–**9**). Interestingly, catalytic reduction of the C–C double bond in compound **7** led to a complete loss of activity (compound **21**).

Addition of S or N nucleophiles to compound **2** did not increase the inhibitory potency. In contrast, a slight decrease of activity was observed (compounds **16**–**19**). Nevertheless, some of these derivatives showed interesting cellular properties. Kinetic analysis of compound **18** showed competitive-type inhibition against ATP and noncompetitive or mixed-type competitive inhibition to the artificial substrate angiotensin II (data not shown).

In contrast to the potent inhibition found against the EGF-R PTK in membranes from A431 cells, [(alkylamino)methyl]acrylophenones and (alkylamino)propiophenones only marginally inhibited the recombinant intracellular domain of the EGF-R (EGF-R-ICD)[33] (data not shown). The reason for this lack of inhibition is not known but may reflect minor folding differences between the recombinant ICD enzyme and the membrane-bound holoreceptor.

Antiproliferative Activity. Compounds were tested for their antiproliferative activity using BALB/MK mouse epidermal keratinocytes, whose proliferation depends on EGF.[36] As shown in Table 2, the 4-(benzyloxy)acrylophenones **2**–**6**, which were potent EGF-R PTK inhibitors at the enzyme level, also showed potent antiproliferative activity. The free acids **7** and **8** of the (benzoylsulfonyl)oxy series were inactive in the cellular assays which might be due to the fact that negatively charged acidic groups are known to be responsible for poor penetration into cells. In fact, the dimethylamine **9** again showed satisfactory antiproliferative activity. As expected, compounds **15** and **21** which did not inhibit the EGF-R kinase *in vitro* were devoid of any antipro-

Table 2. Biological Properties of Compounds 1–21[a]

compd	enzymatic activity (IC_{50}, μM)				cellular activity (IC_{50}, μM)	
	EGF-R	v-abl	c-src	PKC	inhibition of EGF-dependent tyrosine phosphorylation	antiproliferative activity BALB/MK cells
1	5.6	>100	>100	500	>100	9.3
2	0.18	>100	>100	215	>100	6.1
3	0.67	>50	50	86	>100	3.3
4	1.1	>100	>100	140	>100	3.5
5	1.1	>100	>100	110	~100	1.8
6	1.5	>100	>100	310	>100	0.95
7	0.23	50	>100	>500	100	>50
8	0.47	>50	50	140	50	>50
9	0.7	70	>50	180	nt	2.8
10	1.5	>100	>100	>100	>100	46.2
11	1.8	>100	>100	>500	100	25.6
12	1.5	>100	>100	420	>100	33.9
13	0.3	>100	>100	>500	>100	40.2
14	0.36	>100	>50	500	50	9.3
15	>100	>100	nt	nt	>100	>50
16	8.8	>50	>100	>100	25	4.1
17	7.3	>50	>50	>500	100	1.6
18	2.1	>100	>100	>500	~100	1.21
19	8.8	>100	>100	>500	25	1.24
20	3.9	>100	>100	>500	50	2.0
21	100	nt	nt	nt	100	>50
tyrphostin	70	>100	>100	>500	nt	31.1
genistein	1	10	75	15	>100	9.1

[a] IC_{50} values represent the mean and standard deviations of three independent determinations. nt = not tested.

liferative activity. Despite the potent inhibition of the EGF-R PTK, compounds **10–14** of the 4-(benzyloxy)-(dialkylamino)propiophenone series showed only marginal antiproliferative activity, probably due to poor penetration into cells. Compounds **17–20** belong to the most potent inhibitors of this series in this proliferation assay.

Inhibition of EGF-Induced Tyrosine Phosphorylation in Intact Cells. Investigations of the intracellular pathways by which extracellular stimuli activate cell proliferation have shown that ligand binding to receptor-type protein tyrosine kinases (e.g., EGF-R) triggers a cascase of biochemical events. A specific set of intracellular proteins, including the EGF-R itself, are phosphorylated on tyrosine residues in response to EGF.[34,35] Thus, monitoring the modulation of tyrosine phosphorylation as well as receptor autophosphorylation in relation to antiproliferative activity offers a convenient method to study the mode of action and selectivity of protein kinase inhibitors.

Most compounds of this series were tested in a novel cellular ELISA[24] to measure the effects on EGF-stimulated total tyrosine phosphorylation in the A431 human epithelial carcinoma cell line which is known to express high levels of EGF-R.[37,38] Most compounds were inactive in this assay (IC_{50} > 100 μM). Only compounds **5**, **7**, **8**, **11**, **14**, and **16–21** showed low activity with IC_{50} values between 25 and 100 μM (Table 2). No direct correlation between antiproliferative activity and inhibition of tyrosine phosphorylation was observed. Many compounds with potent antiproliferative activity were inactive in this assay (compounds **2–4**, **6**) or showed IC_{50} values between 25 and 50 μM (compounds **14**, **16**, **19**, and **20**).

Compound **5** was further tested for inhibition of ligand-induced EGF-R autophosphorylation assay by Western Blot using anti-phosphotyrosine antibodies.[39] In this assay, EGF stimulation of A431 cells resulted in tyrosine phosphorylation of a protein with a molecular weight of 180 000 (Figure 2, lane 2) which is most

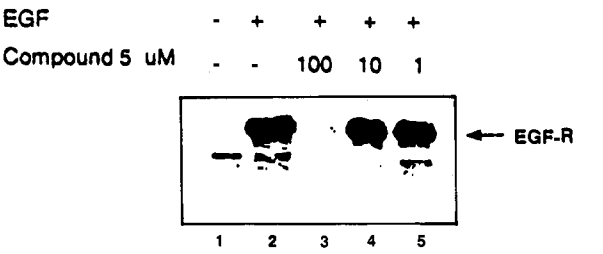


Figure 2. Inhibition of EGF-R autophosphorylation in intact cells. Serum-starved A431 cells were incubated for 90 min with the indicated concentrations of compound **5** (lanes 3–5) prior to stimulation with EGF (100 ng/mL; lanes 2–5) for 10 min, respectively. Equal amounts of protein of cell lysates were analyzed by Western blotting using anti-phosphotyrosine antibodies.[39]

probably the EGF-R itself. Pretreatment of the cells with compound **5** caused a concentration-dependent inhibition of ligand-stimulated EGF-R autophosphorylation. Complete inhibition was observed at 100 μM concentration (lane 3), whereas 10 μM concentration caused no or only marginal inhibition of autophosphorylation (line 4). No inhibition was seen at 1 μM concentration of the drug (lane 5). Overall levels of the EGF-R were not affected by these compounds (data not shown). Although a distinct inhibition of EGF-R autophosphorylation was observed in this assay, there is a discrepancy between the IC_{50} values observed for the inhibition of antiproliferative activity and inhibition of EGF-R autophosphorylation in the Western Blot assay or inhibition of tyrosine phosphorylation in the ELISA assay. This fact may suggest a second site of action for this class of inhibitors, or uptake and accumulation in the cell of compounds may significantly affect their antiproliferative potency.

***In Vivo* Antitumor Activity.** So far, only a few EGF-R PTK inhibitors have been reported to exhibit *in vivo* activity. We have tested compounds **2** and **18** *in vivo* against xenografts of the BT-20 tumor in nude mice. Due to the very low maximal tolerated dose (MTD) of 2 mg/kg compound **2**, only doses of up to 0.2

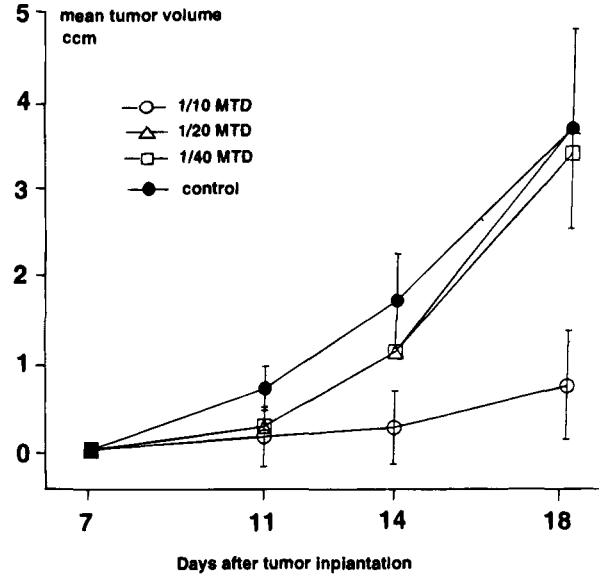


Figure 3. *In vivo* antitumor activity of compound **18**. *In vivo* antitumor activity was tested using xenografts of the human breast carcinoma BT-20. BT-20 pieces of approximately 25 mm^3 were transplanted sc. Drug treatment was started on day 7 after tumor transplantation, when the tumor had reached a diameter of 4–5 mm. Drug ((○) $^1/_{10}$ MTD = 6.3 mg/kg, (△) $^1/_{20}$ MTD = 3.25 mg/kg, (□) $^1/_{40}$ MTD = 1.2 mg/kg, and (●) placebo control) was given ip once daily for 11 consecutive days. Mean tumor volume is given in cm^3 ± standard deviation.

mg/kg could be administered. At these doses, compound **2** showed no or only marginal antitumor effects (data not shown). In contrast, compound **18**, which had an MTD of 62.5 mg/kg after ip application, provoked a pronounced tumor growth inhibition at a dose of 6.3 mg/kg ($^1/_{10}$ of MTD) but no effects at 3.2 mg/kg ($^1/_{20}$ MMTD). No overt cumulative toxicity was observed during treatment (Figure 3).

In summary, [(alkylamino)methyl]acrylophenones and (alkylamino)propiophenones represent a novel class of potent inhibitors of the EGF-R protein tyrosine kinase *in vitro* and with high selectivity against other tyrosine and serine/threonine kinases. Compound **18** of this series showed significant antitumor effects *in vivo* on the human breast carcinoma BT-20 in a nude mouse model after ip application. In cells, the compounds showed antiproliferative activity. However, differences between the IC_{50} values of antiproliferative activity and inhibition of EGF-induced total cellular tyrosine phosphorylation suggest a second site of action of this class of inhibitors. In fact, interesting pharmacological activities of [(dialkylamino)methyl]acrylophenones have already been described in the literature. In a SAR study of a series of [(dialkylamino)methyl]acrylophenones, antimicrotubular activity as well as inhibition of platelet aggregation and decrease of serum cholesterol, triglycerides, and phospholipid levels has been reported.[30] Furthermore, evaluation of a series of methoxy-substituted [(dimethylamino)methyl]acrylophenones and related bis-Mannich bases demonstrated potent activity against murine P388 leukemia cells *in vitro* which probably is related to the antimicrotubular effects of this compound class.[40] In addition, anesthetic activity of several (dialkylamino)(benzyloxy)propiophenones has been reported.[31,32] Nevertheless, this class of compounds offers useful tools for studying the action of tyrosine kinases.

Experimental Section

Materials and Methods. For *in vitro* and cellular assays, stock solutions of 10 mM concentration of the compounds were prepared in DMSO and stored at −20 °C. Dilutions for all assays were made up freshly prior to use.

Preparation of Enzymes and Kinase Assays. Determination of EGF-R kinase activity was performed as described using A431 membranes as the enzyme source and angiotensin II as substrate.[41] Purification of protein kinases (v-abl, c-src, PKC) and *in vitro* enzyme assays were performed as previously described.[24,25,28,42–44] Tyrphostin RG-13 022 and genistein served as standard reference inhibitors in all assays.

Western Blot Analysis. Serum-starved A431 cells were incubated for 90 min with the indicated concentrations of drug prior to stimulation with EGF (100 ng/mL) for 10 min.[25] Equal amounts of protein of cell lysates were analyzed by Western blotting using anti-phosphotyrosine antibodies.[39] Bound antibodies were detected using the ECL Western blotting system from Amersham (Amersham, U.K.).

Antiproliferative Assays. Cell growth inhibition was assayed essentially as described previously.[41] Drugs (in a final DMSO concentration of 0.5%) were added 24 h after plating, and growth was monitored after 3–5 days of incubation using methylene blue staining.

***In Vivo* Antitumor Activity.** *In vivo* antitumor activity was tested using xenografts of the BT-20 tumor in nude mice. BT-20 tumor pieces were transplanted sc. The drug was given ip once daily for 11 consecutive days starting on day 7 after tumor transplantation. Stock solutions of 40 mg/mL of test compound were prepared in 100% ethanol containing 0.5% Tween 80 and stirred at 40 °C until a clear solution was obtained. Stock solutions were diluted 1:20 (v/v) with sterile 0.9% NaCl and used for treatment. Solution and dilution were prepared daily prior to application. Tumor growth was followed by measuring perpendicular tumor diameters. Tumor volumes were calculated as previously described[41] using the formula $\pi \times L \times D^2/6$.

Synthesis. Melting points were determined in open capillary tubes and are uncorrected. High-performance liquid chromatography was performed using a Kontron MT 450 apparatus with UV detection at 254 nm. Elemental analyses were within ±0.4% of the theoretical value. UV/vis spectra were obtained with a Perkin-Elmer λ 9 spectrophotometer. Infrared spectra were recorded on a Perkin-Elmer 1310 or 298 spectrophotometer. ^1H and ^{13}C NMR were recorded on a Varian Gemini 200, Varian Gemini 300, or Bruker WM-360 spectrometer. The coupling constants are recorded in hertz (Hz), and the chemical shifts are reported in parts per million (δ, ppm) downfield from tetramethylsilane (TMS). Mass spectra (MS), fast-atom bombardment mass spectra (FABMS), and high-resolution mass spectra (HRMS) were recorded on a VG Manchester apparatus. Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel 60 F-254, Merck), and spots were visualized with UV light, iodine, or phosphoromolybdate. Column chromatography was performed with Merck silica gel 60 (230–400 mesh). Removal of solvents was performed by rotary evaporation under reduced pressure. All reactions involving air- or moisture-sensitive reagents were performed under a positive pressure of argon.

General Method for the Preparation of [(Dialkylamino)methyl]acrylophenones and [(Dialkylamino)methyl]propiophenones. Compounds **2**–**14** were prepared from the corresponding substituted acetophenone by reaction with secondary amines, paraformaldehyde, and acetic acid according to published procedures.[29–32]

1-[4-(Benzyloxy)phenyl]-2-[(dimethylamino)methyl]prop-2-en-1-one (2). In a typical experiment, 11.1 g (50 mmol) of 4-(benzyloxy)acetophenone,[31,32] 3.0 g (170 mmol) of paraformaldehyde, 4.1 g (50 mmol) of dimethylamine hydrochloride, and 10 mL of acetic acid were refluxed for 4 h. Acetic acid was removed by distillation under reduced pressure. The crude product was dissolved in water and the solution acidified with 1 N HCl (pH 2) and extracted with ethyl acetate. Sodium bicarbonate was added to the aqueous phase until pH 8. The aqueous phase was then extracted with methylene chloride

and the organic phase dried with sodium sulfate and evaporated to dryness. The yellow-colored oily residue was dissolved in 30 mL of ethyl ether, and 7.5 mL of 4 N HCl in ethyl ether was added. Crystals of the hydrochloride of compound **2** were filtered off and recrystallized from 2-propanol to yield 6.49 g (40% yield) of pure **2** as colorless crystals of mp 167 °C: MS *m/z* 328 $(M + Na)^+$, 296 $(M + H)^+$, 205 (M − benzyl); ^1H NMR (DMSO-d_6) δ 9 aromatic protons at 7.80 (d, 2 H), 7.40 (m, 5 H), 7.15 (d, 2 H), 2 olefinic protons at 6.59 (s) and 6.10 (s), benzylic-CH_2 group at 5.20 (s), 4.00 (s, 2 H, CH_2 group), 2.75 (s, 6 H, 2 N-CH_3 groups). Anal. ($C_{19}H_{21}NO_2$·HCl) C, H, N, Cl.

1-[4-(Benzyloxy)phenyl]-3-(dimethylamino)propan-1-one (10). From the mother liquor, 450 mg of pure **10** were isolated after chromatography on silica gel and crystallization from 2-propanol or 2-propanol/hexane as colorless crystals of mp 167−169 °C:[31] MS *m/z* 284 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 9 aroimatic protons at 8.00 (d, 2 H), 7.45 (m, 5 H), 7.18 (d, 2 H), benzylic-CH_2 group at 5.25 (s), 3.52 (t, 2 H, CH_2 group), 3.35 (m, 2 H, CH_2 group), 2.80 (s, 6 H, 2 N-CH_3 groups). Anal. ($C_{18}H_{21}NO_2$·HCl) C, H, N, Cl.

The same procedure as for the preparation of **2** and **10** was used for the preparation of compounds **3**−**12** and **14**.

1-[4-(Benzyloxy)-3-hydroxyphenyl]-2-[(dimethylamino)-methyl]prop-2-en-1-one (3): prepared from 1.1 g (4.5 mmol) of 4-(benzyloxy)-3-hydroxyacetophenone,[31] 0.3 g (9 mmol) of paraformaldehyde, and 0.37 g (4.5 mmol) of dimethylamine hydrochloride in 10 mL of acetic acid; colorless crystals (HCl salt) of mp 175−178 °C; MS *m/z* 334 $(M + Na)^+$, 312 $(M + H)^+$, 221 (M − benzyl); ^1H NMR (DMSO-d_6) δ phenolic proton at 9.70 (s), 8 aromatic protons at 7.50 (d, 1 H), 7.48 (s, 1 H), 7.40 (tr, 2 H), 7.32 (m, 3 H), 7.15 (d, 1 H), 2 olefinic protons at 6.55 (s) and 6.11 (s), benzylic-CH_2 group at 5.22 (s), 4.05 (s, 2 H, CH_2 group), 2.75 (s, 6 H, 2 N-CH_3 groups). Anal. ($C_{19}H_{21}NO_3$·HCl) C, H, N, Cl.

1-[4-(Benzyloxy)-3-hydroxyphenyl]-3-[(dimethylamino)-methyl]propan-1-one (14): prepared from 0.8 g (3.30 mmol) of 4-(benzyloxy)-3-hydroxyacetophenone, 133 mg (4.4 mmol) of paraformaldehyde, 269 mg (3.30 mmol) of dimethylamine hydrochloride, and 0.03 mL of concentrated HCl in 10 mL of 2-propanol according to a procedure described in the literature;[31,32] colorless crystals (HCl salt) of mp 169−171 °C; MS *m/z* 300 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ phenolic proton at 9.60 (s), 8 aromatic protons at 7.3−7.6 (m, 7 H), 7.15 (d, 1 H), benzylic-CH_2 group at 5.22 (s), 3.3−3.6 (m, 4 H, 2 CH_2 groups), 2.78 (s, 6 H, 2 N-CH_3 groups). Anal. ($C_{18}H_{21}NO_3$·HCl) C, H, N, Cl.

1-[4-(Benzyloxy)phenyl]-2-(piperidinomethyl)prop-2-en-1-one (4): prepared from 5.65 g (25 mmol) of 4-(benzyloxy)-acetophenone,[31] 1.5 g (45 mmol) of paraformaldehyde, and 2.47 mL (45 mmol) of piperidine in 10 mL of acetic acid; colorless crystals (HCl salt) of mp 166−168 °C; MS *m/z* 336 $(M + H)^+$; ^1H NMR (CH_3OH-d_3) δ 9 aromatic protons at 7.85 (d, 2 H), 7.45 (d, 2 H), 7.35 (m, 3 H), 7.15 (d, 2 H), 2 olefinic protons at 6.50 (s) and 6.25 (s), benzylic-CH_2 group at 5.20 (s), 4.08 (s, 2 H, CH_2 group), 3.5 (m, 4 H, piperidine), 1.75 (m, 6 H, piperidine). Anal. ($C_{22}H_{25}NO_2$·HCl) C, H, N, Cl.

1-[4-(Benzyloxy)phenyl]-3-piperidinopropan-1-one (11): colorless crystals (HCl salt) of mp 162−165 °C;[31] MS *m/z* 324 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 9 aromatic protons at 7.98 (d, 2 H), 7.45−7.30 (m, 5 H), 7.02 (d, 2 H), benzylic-CH_2 group at 5.15 (s), 3.75 (m, 2 H), 3.44 (m, 2 H), 10 piperidino protons at 3.50 (m, 2 H), 2.70 (m, 2 H), 2.30 (m, 2 H), 1.88 (m, 3 H), and 1.45 (m, 1H). Anal. ($C_{21}H_{25}NO_2$·HCl) C, H, N, Cl.

1-[4-(Benzyloxy)phenyl]-2-(morpholinomethyl)prop-2-en-1-one (5): amorphous HCl salt MS *m/z* 338 $(M + H)^+$, corresponding to $C_{21}H_{23}NO_3$; ^1H NMR (DMSO-d_6) δ 9 aromatic protons at 7.85 (d, 2 H), 7.45 (d, 2 H), 7.5−7.3 (m, 5 H), 7.18 (d, 2 H), 2 olefinic protons at 6.62 (s) and 6.15 (s), benzylic-CH_2 group at 5.22 (s), 4.12 (s, 2 H, CH_2 group), 3.95−3.6 (m, 8 H, morpholine).

1-[4-(Benzyloxy)phenyl]-3-morpholinopropan-1-one (12): colorless crystals (HCl salt) of mp 195−197 °C;[26] MS *m/z* 326 $(M + H)^+$; ^1H NMR (CH_3OH-d_3) δ 9 aromatic protons at 8.05 (d, 2 H), 7.5−7.3 (m, 5 H), 7.22 (d, 2 H), benzylic-CH_2 group at 5.18 (s), 8 morpholino protons at 4.08 (m, 2 H), 3.85 (m, 2 H), 3.55 (m, 2 H), and 3.22 (m, 2 H), 3.55 (m, 4 H). Anal. ($C_{20}H_{23}NO_3$·HCl) C, H, N, Cl.

1-[4-(Benzyloxy)phenyl]-2-[(4-methylpiperazino)methyl]prop-2-en-1-one (6): colorless crystals (HCl salt) of mp 161−162 °C; MS *m/z* 351 $(M + H)^+$, 259 (M − benzyl); ^1H NMR (DMSO-d_6) δ 9 aromatic protons at 7.80 (d, 1 H), 7.5−7.3 (m, 5 H), 7.13 (d, 1 H), 2 olefinic protons at 5.96 (s) and 5.60 (s), benzylic-CH_2 group at 5.20 (s), 8 piperazinyl protons at 3.25 (m, 4 H) and 2.95 (m, 4 H), 2.65 (s, 3 H, N-methyl). Anal. ($C_{22}H_{26}N_2O_2$·HCl) C, H, N, Cl.

4-[[4-[2-[(Dimethylamino)methyl]acryloyl]phenoxy]-sulfonyl]benzoic acid (7): prepared from 1 g (3.1 mmol) of 4-[4-carboxy(sulfonyloxy)phenyl]acetophenone (prepared from 4-hydroxyacetophenone and benzoic acid 4-sulfochloride according to ref 31), 0.19 g (6.3 mmol) of paraformaldehyde, 0.25 g (3.1 mmol) of dimethylamine hydrochloride, and 5 mL of acetic acid; colorless crystals of mp 173−175 °C; MS *m/z* 390 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 8 aromatic protons at 8.20 (d, 2 H), 8.05 (d, 2 H), 7.83 (d, 2 H), 7.25 (d, 2 H), 2 olefinic protons at 6.68 (s) and 6.15 (s), 4.05 (s, CH_2 group), 2.76 (s, 6 H, 2 N-CH_3 groups). Anal. ($C_{19}H_{19}NO_6S$) C, H, N, S.

4-[[4-[2-[(Dimethylamino)methyl]acryloyl]phenoxy]-sulfonyl]-2-hydroxybenzoic acid (8): prepared from 2 g (6 mmol) of 4-[4-carboxy-3-hydroxy(sulfonyloxy)phenyl]acetophenone, 0.36 g (2 mmol) of paraformaldehyde, 0.49 g (6 mmol) of dimethylamine hydrochloride, and 10 mL of acetic acid; colorless crystals of mp 220−230 °C dec; MS *m/z 406* $(M + H)^+$; ^1H NMR (DMSO-d_6) δ phenolic proton at 10.35, 7 aromatic protons at 8.02 (d, 1 H), 7.83 (d, 2 H), 7.39 (d, 2 H), 7.29 (d, 2 H), 2 olefinic protons at 6.69 (s) and 6.17 (s), 4.08 (s, CH_2 group), 2.80 (s, 6 H, 2 N-CH_3 groups). Anal. ($C_{19}H_{19}NO_7S$) C, H, N, S.

4-[[4-[2-[(Dimethylamino)methyl]acryloyl]phenoxy]-sulfonyl]benzoic acid dimethylamide (9): prepared from 0.79 g (2.27 mmol) of the dimethylamide of 4-[4-carboxy-3-hydroxy(sulfonyloxy)phenyl]acetophenone, 0.14 g (4.54 mmol) of paraformaldehyde, and 0.16 g (2.27 mmol) of dimethylamine hydrochloride in 5 mL of acetic acid; the yellow oil of **9** was converted into its hydrochloride acid salt (hygroscopic); MS *m/z* 417 $(M + H)^+$, corresponding to $C_{21}H_{24}N_2O_5S$; ^1H NMR (DMSO-d_6) δ 8 aromatic protons at 7.90 (d, 2 H), 7.78 (d, 2 H), 7.58 (d, 2 H), 7.09 (d, 2 H), 2 olefinic protons at 6.04 (s) and 5.70 (s), 3.32 (s, CH_2 group), 3.12 (s, 3 H), 2.93 (s, 3 H), 2.28 (s, 6 H).

1-[4-(Benzyloxy)phenyl]-3-(4-carbethoxypiperdino)-propan-1-one (13): prepared from 4.84 g (25 mmol) of 4-(benzyloxy)acetophenone, 5.65 g (25 mmol) of 4-ethylpiperidinecarboxylate hydrochloride, and 2 g (11.5 mmol) of paraformaldehyde in 50 mL of ethanol and 0.25 mL of 1 N HCl according to ref 31; colorless crystals of mp 173−175 °C; MS *m/z* 396 $(M + H)^+$, corresponding to $C_{24}H_{29}NO_4$; ^1H NMR (DMSO-d_6) δ 11.0 (s, 1 H), 9 aromatic protons at 8.0 (d, 2 H), 7.46 (d, 2 H), 7.37 (m, 3 H), 7.17 (d, 2 H), 5.23 (s, 2 H, benzylic-CH_2), 4.1 (q, 2 H), 3.63 (t, 2 H), 3.56 (d, 2 H), 3.37 (m, 2 H), 3.0 (m, 2 H), 2.62 (m, 1 H), 2.04 (m, 4 H), 1.20 (t, 3 H).

1-[4-(Benzyloxy)phenyl]-4-(dimethylamino)butan-1-one (15): prepared in three steps starting from 1-(4-hydroxyphenyl)-4-chlorobutan-1-one.

1-(Hydroxyphenyl)-4-iodobutan-1-one. A 5.20 g (26.2 mmol) sample of 1-(4-hydroxyphenyl)-4-chlorobutan-1-one (Aldrich), dissolved in 40 mL of ethyl methyl ketone, was stirred at 90 °C with 4.50 g (30 mmol) of sodium iodide. The dark-brown suspension was filtered and the filtrate evaporated to dryness. The residue was suspended in 50 mL of ethyl ether, filtered, and evaporated. Crystallization from boiling hexane gave colorless prisms of 1-(4-hydroxyphenyl)-4-iodobutan-1-one of mp 96−97 °C: MS *m/z* 290 (M^+).

1-(4-Hydroxyphenyl)-4-(dimethylamino)butan-1-one. A 7.55 g (26 mmol) sample of 1-(4-hydroxyphenyl)-4-iodobutan-1-one and 100 mL of a 1.12 M solution of diethylamine (104 mmol) in diethyl ether were stirred at room temperature for 24 h. Ether was removed and the residue suspended in 200 mL of buffer at pH 9.5. The aqueous solution was extracted several times with methylene chloride. The organic phase was dried, filtered, and evaporated to dryness. The crude product was suspended in 300 mL of 2-propanol and filtered; 6 mL

4.1 N HCl in ethyl ether was added to the filtrate and the filtrate stored at 0 °C. The product was filtered off and recrystallized twice from 2-propanol. Colorless crystals were obtained of the hydrochloride of 1-(4-hydroxyphenyl)-4-(dimethylamino)butan-1-one of mp 232–235 °C: MS *m/z* 208 $(M + H)^+$.

1-[4-(Benzyloxy)phenyl]-4-(dimethylamino)butan-1-one (15). A 600 mL (5.15 mmol) portion of benzyl chloride and 1.4 g (9.85 mmol) of potassium carbonate were added to a solution of 1.0 g (4.1 mmol) of the hydrochloric salt of 1-(4-hydroxyphenyl)-4-(dimethylamino)butan-1-one in 10 mL of ethanol, and the solution was refluxed for 14 h. The solution was filtered and the filtrate evaporated to dryness. The residue was suspended in water and extracted several times with methylene chloride at pH 10. The organic phase was dried, filtered, and evaporated. The crude product was dissolved in methylene chloride. After dropwise addition of 4 N HCl in ethyl ether, the hydrochloric salt of 1-[4-(benzyloxy)-phenyl]-4-(dimethylamino)butan-1-one was precipitated. Recrystallization from 2-propanol gave colorless crystals of **15** of mp 176 °C: MS *m/z* 298 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 9 aromatic protons at 7.98 (d, 2 H), 7.3–7.6 (m, 5 H), 7.14 (d, 2 H), benzylic-CH_2 group at 5.22 (s), 3.1–3.5 (m, 6 H, 3 CH_2 groups), 3.35 (6 H, 2 N-CH_3 groups). Anal. ($C_{19}H_{23}NO_2$·HCl) C, H, N, Cl.

3-[(2-Aminoethyl)thio]-1-[4-(benzyloxy)phenyl]-2-[(dimethylamino)methyl]propan-1-one (16). A 670 mg (2 mmol) sample of compound **2** and 240 mg (2.1 mmol) of cysteamine hydrochloride were stirred at room temperature in 20 mL of methylene chloride for 24 h; 10 mL of 1 N HCl was added and the solution extracted several times with methylene chloride. The combined organic extracts were dried over sodium sulfate and evaporated to dryness. The crude residue was chromatographed on silica gel with methylene chloride and ethyl acetate as eluents. Pure **16** was obtained as colorless crystals of mp 89–91 °C which were dissolved in ethanol and converted to the hydrochloric salt of mp 90 °C by adding 3 N HCl in ethanol: MS *m/z* 373 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 9.85 (s, 1 H), 9.2 (s, 1 H), 8.75 (s, 1 H), 9 aromatic protons at 7.98 (d, 2 H), 7.4 (m, 5 H), 7.17 (d, 2 H), benzylic-CH_2 group at 5.22 (s), 4.32 (m, 1 H), 3.53 (m, 8 H, 2 N-CH_3 groups and CH_2 group), 3.33 (m, 2 H, CH_2 group), 3.0 (m, 2 H, CH_2 group), 2.82 (m, 2 H, CH_2 group). Anal. ($C_{21}H_{28}N_2O_2S$·2HCl) C, H, N, S, Cl.

The same procedure as for the preparation of **16** was used for the preparation of compounds **17–20**.

1-[4-(Benzyloxy)phenyl]-2-[(dimethylamino)methyl]-3-(phenylthio)propan-1-one (17): colorless crystals (HCl salt) of mp 105–108 °C from methylene chloride/ethanol; MS *m/z* 406 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 14 aromatic protons at 7.75 (d, 2 H), 7.5–7.3 (m, 10 H), 6.93 (d, 2 H), benzylic-CH_2 group at 5.10 (s), 4.51 (m, 1 H), 3.85 (m, 1 H), 3.45 (d, 1 H), 3.25 (dd, 1 H), 2.93 (dd, 1 H), 2.82 (s, 3 H, N-CH_3 group), 2.57 (s, 3 H, N-CH_3 group). Anal. ($C_{25}H_{27}NO_2S$·HCl) C, H, N, S, Cl.

1-[4-(Benzyloxy)phenyl]-2-[(dimethylamino)methyl]-3-[(3,4-dimethylphenyl)thio]propan-1-one (18): colorless crystals (HCl salt) of mp 94–95 °C from 2-propanol/ethyl ether; MS *m/z* 434 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 12 aromatic protons at 7.75 (d, 2 H), 7.42 (m, 5 H), 7.22 (d, 2 H), 7.08 (d, 1 H), 6.92 (d, 2 H), benzylic-CH_2 group at 5.13 (s), 4.42 (m, 1 H), 3.87 (q, 1 H), 3.37 (d, 1 H), 3.23 (dd, 1 H), 2.6–2.8 (m, 8 H, 2 N-CH_3 group and S-CH_2 group), 2.25 (d, 6 H, 2 aromatic CH_3 groups). Anal. ($C_{27}H_{31}NO_2S$·HCl) C, H, N, S, Cl.

2-[[3-[4-(Benzyloxy)phenyl]-2-[(dimethylamino)methyl]-3-oxopropyl]thio]benzoic acid methyl ester (19): colorless crystals (HCl salt) of mp 146 °C from 2-propanol/ethyl ether; MS *m/z* 464 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 13 aromatic protons at 7.9 (m, 3 H), 7.4 (m, 7 H), 7.25 (t, 1 H), 6.95 (d, 2 H), benzylic-CH_2 group at 5.13 (s), 4.75 (m, 1 H), 3.80 (m, 4 H), 3.35 (m, 2 H), 3.15 (dd, 1 H), 2.6–2.8 (m, 6 H, 2 N-CH_3 groups). Anal. ($C_{27}H_{29}NO_4S$·HCl) C, H, N, S, Cl.

1-[4-(Benzyloxy)phenyl]-2-[(dimethylamino)methyl]-3-(*N'*-carbamoylhydrazino)propan-1-one (20). A hot solution of 0.86 g (7.7 mmol) of semicarbazide hydrochloride and 0.54 g (6.6 mmol) of sodium acetate in 15 mL of ethanol was added to a solution of 1.83 g (5.5 mmol) of compound **2** in 25 mL of ethanol. The solution was stirred at room temperature for 90 min and then evaporated to dryness. The residue was chromatographed on a cooled silica gel column with methylene chloride/methanol (4:1) as eluent; 1.32 g of beige crystals of the semicarbazone **20** of mp 160–164 °C was obtained: MS *m/z* 371 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 9 aromatic protons at 8.05 (d, 2 H), 7.6 (s, 1 H), 7.4 (m, 5 H), 7.15 (d, 2 H), 6.0 (s, 2 H, NH_2), benzylic-CH_2 group at 5.25 (s), 4.1 (m, 1 H), 3.4 (m, 12 H), 3.15 (m, 2 H), 2.75 (s, 6 H, 2 N-CH_3 groups), 2.65 (m, 1 H). Anal. ($C_{20}H_{26}N_4O_3$) C, H, N.

1-[4-(Benzyloxy)phenyl]-1-methyl-3-(dimethylamino)-propan-1-one (21). A 200 mg (0.514 mmol) sample of compound **7** in 40 mL of ethanol was hydrogenated at 1 atm over 50 mg of Pd–C at room temperature for 5 h. The catalyst was filtered off and the crude product dissolved in hot 2-propanol. Amorphous **22** (115 mg) was obtained by precipitation with ethyl ether: MS *m/z* 392 $(M + H)^+$, corresponding to $C_{19}H_{21}NO_6S$; ^1H NMR (DMSO-d_6) δ 8 aromatic protons at 8.15 (d, 2 H), 8.05 (d, 2 H), 8.02 (d, 2 H), 7.27 (d, 2 H), 4.1 (m, 1 H), 3.3 (m, 2 H), 2.72 (s, 6 H, 2 N-CH_3 groups), 1.17 (d, 3 H, CH_3 group).

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