Benzo[f]quinazoline Inhibitors of Thymidylate Synthase: Methyleneamino-Linked Aroylglutamate Derivatives

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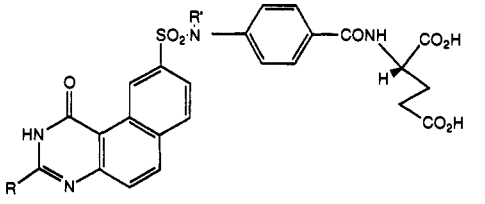
Syntheses of several new inhibitors of thymidylate synthase (TS) structurally related to folic acid are described in which the pterin portion of the folate molecule is replaced by a benzo[f]quinazoline moiety, but which retain the natural methyleneamino link to the benzoylglutamate side chain. The effect on enzyme activity and cytotoxicity of various changes in the structure of the (p-aminobenzoyl)glutamate side chain are reported. Replacement of the benzamide portion of the (p-aminobenzoyl)glutamate moiety with 2-fluorobenzamido, 2-isoindolinyl, 1,2-benzisothiazol-2-yl, and 2-thenamido moieties varied in effect from a 9-fold diminution of TS activity to a 5-fold enhancement, while cytotoxic potency on SW-480 and MCF-7 tumor lines showed increases ranging from 3.6- to 450-fold. The detrimental effect on enzyme activity and cytotoxicity of alkyl substitution on the PABA nitrogen is confirmed for these compounds, in contrast with several series of previously reported quinazoline antifolates (2). Substitution of a C3-methyl substituent for 3-amino had little effect on TS activity but was beneficial in terms of solubility and cytotoxicity. The excellent combination of TS inhibitory activity, FPGS substrate activity, and affinity for the reduced folate transport system in the most potent of these derivatives, **3e**, resulted in IC_{50} values of 0.2–0.8 nM against these tumor lines.

We have previously reported[1] the potent thymidylate synthase (TS) inhibitory activity of a number of benzo[f]quinazoline (p-aminobenzoyl)glutamates of type 1 in which the heterocycle was linked to the benzoylglutamate moiety via a sulfonamide bridge. Although a wide variety of folate analogues[2-8] with fraudulent linkages between the heterocycle and the side chain (e.g. the "reversed bridge" analogues **2a**[2-8] and the sulfur and oxygen isosteres **2b**[2,3,5]) have often shown significant inhibition of thymidylate synthase, particularly as the polyglutamates, the normal folate-like methyleneamino link has been a feature of the most potent TS inhibitors yet reported, namely the quinazoline series exemplified by **2c-g**.[2,9-17] Accordingly, we set out to introduce this structural feature into benzo[f]quinazoline inhibitors, i.e. to synthesize molecules of type **3**. Within this series we examined the effect of several variations in the aromatic residue of the side chain, the alkylation of the bridge nitrogen, and the relative merits of methyl *vs* amino as a C3 substituent in the heterocycle, areas which had produced useful structure/activity information in the sulfonamidobenzoquinazolines[1] and in other folate analogues.[8,11-17]

We report here how this strategy led to some of the most potent thymidylate synthase inhibitors yet described.



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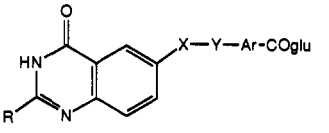
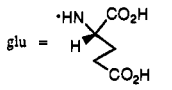


2

2a X = NH; Y = CH_2
2b X = CH_2; Y = O, S
2c-g X = CH_2; Y = NR'
2c R = R' = CH_3; Ar = 2'-F-1,4-phenylene
2d R = R' = CH_3; ArCOglu =
2e R = CH_3; R' = propargyl; ArCOglu =
2f R = R' = CH_3; Ar = 1,4-phenylene
2g R = R' = CH_3; Ar = 2,5-thienylene



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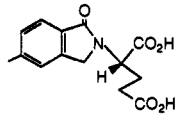

Chemistry

The general route of synthesis of these analogues is shown in Scheme 1.

The starting 3,9-dimethyl- (**4**) and 3-(pivaloylamino)-9-methylbenzo[f]quinazolines (**5**) were prepared in several steps from m-tolylacetic acid essentially as described previously.[18-21] Brominations of the 9-methyl substituents were carried out with N-bromosuccinimide in benzene. In the 3,9-dimethyl derivative, bromination occurred selectively on the 9-substituent. Brominations yielded greater than 80% of the desired bromomethyl intermediates (**6** and **7**) by ^1H NMR, and these were used in the alkylation step without further purification.

Coupling of the side chain moiety to the heterocycle was carried out by heating the appropriate diethyl (p-aminobenzoyl)glutamate analogue with the desired 9-(bromomethyl)benzoquinazoline in dimethylformamide. Diethyl (4-(methylamino)(benzoyl)-L-glutamate (**8b**),[22] diethyl N-(4-amino-2-fluorobenzoyl)-L-glutamate (**8c**),[22] and diethyl (5-amino-2-thenoyl)-L-glutamate (**8d**)[17,23] were prepared by methods previously described.

Diethyl (S)-(5-amino-1-oxo-2-isoindolinyl)glutarate (**8e**) was prepared as outlined in Scheme 2; the presumed (5-amino-3-chloro-1-oxo-2-isoindolinyl)glutarate intermediate and its 1-chloro-3-oxo isomer were not isolated but reduced *in situ* to a 3:1 mixture of **8e** and **13** which was separated by chromatography. The 5-methylamino analogue (**8f**) was made by reductive methylation of **8e** with formaldehyde and sodium cyanoborohydride. Concurrently with these investigations, an alternate route to these intermediates was reported,[2] which avoided the regiochemically ambiguous step of reduction of the phthalimido

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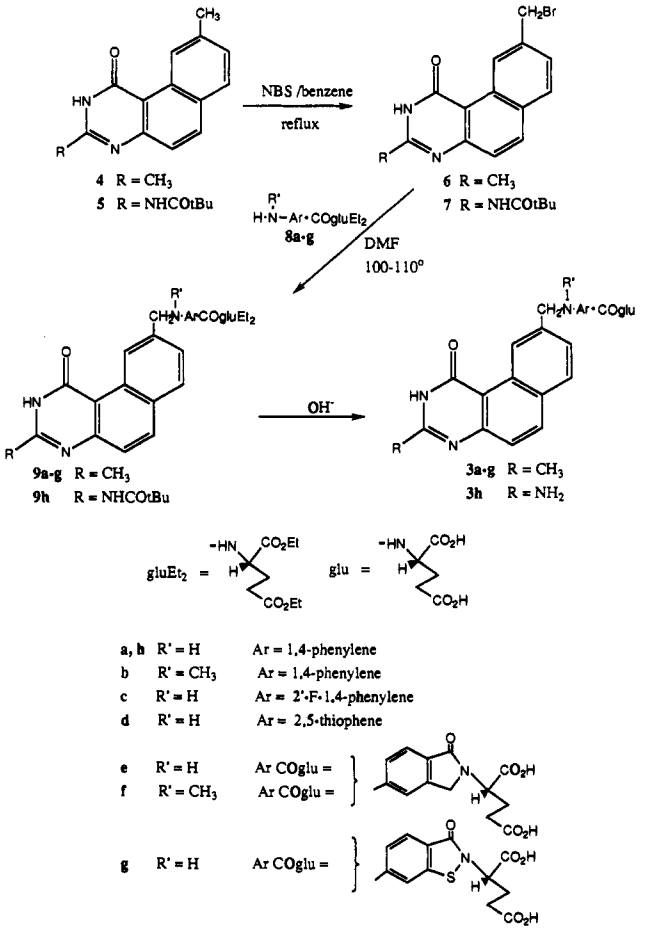
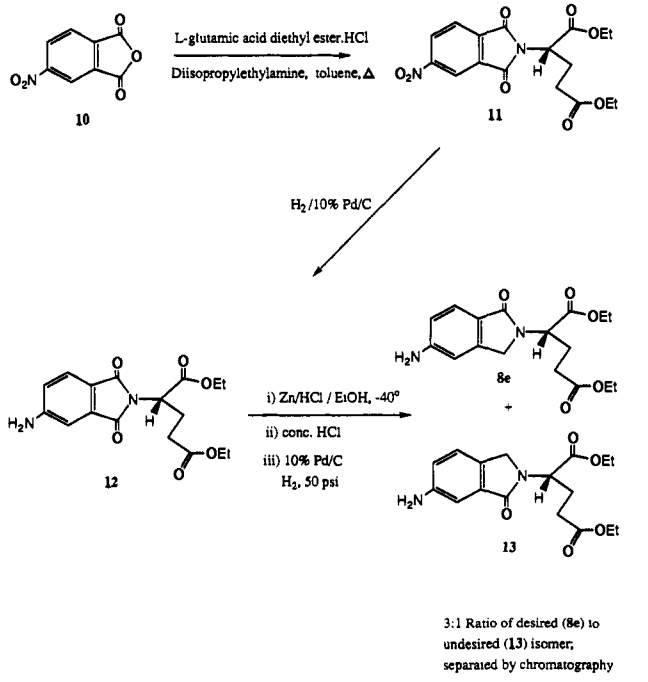
Scheme 1



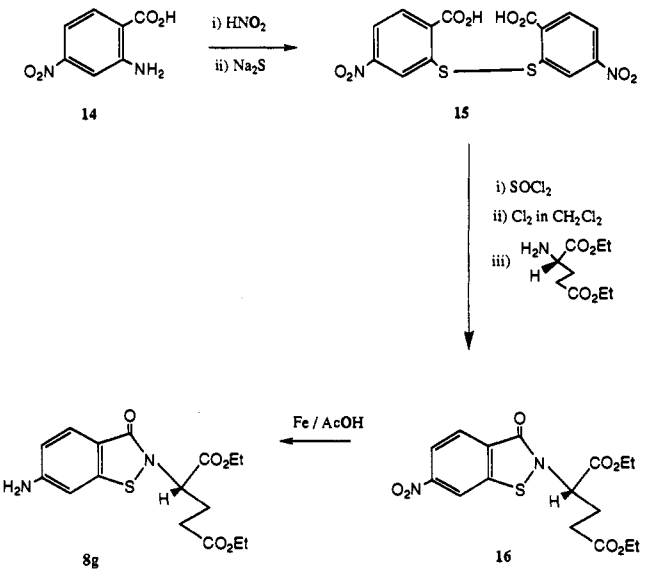
Scheme 2



function; the route reported here, however, is considerably shorter from commercially available starting materials.

Diethyl (*S*)-2-(6-amino-2,3-dihydro-3-oxo-1,2-benzisothiazol-2-yl)glutarate (**8g**) was prepared as shown in Scheme 3. 4-Nitroanthranilic acid (**14**) was diazotized and treated with sodium sulfide to yield the dithiobisbenzoic acid **15**. In a one-pot sequence **15** was converted to the acid chloride with thionyl chloride and treated with chlorine to yield

Scheme 3



the presumed sulfenyl chloride intermediate, which was aminated with diethyl glutamate to give the cyclic (*p*-aminobenzoyl)glutamate analogue **8g**.

Biological Testing

The target diacids **3a-h** were tested as inhibitors of purified human thymidylate synthase (TS) isolated from an *Escherichia coli* harboring a plasmid with *thy* A gene cloned from SV40 transformed human fibroblast cells.[24] The enzyme was assayed and extent of inhibition of the enzyme by the various compounds was determined by the tritium release assay of Roberts[25] as modified by Dev *et al.*[26] Values of the inhibition constant (K_i) were estimated by the method of Henderson for tight-binding noncompetitive inhibitors.[27]

Inhibition of the growth of tumor cells (SW480 colon adenocarcinoma and MCF-7 breast adenocarcinoma) was determined as described previously.[28]

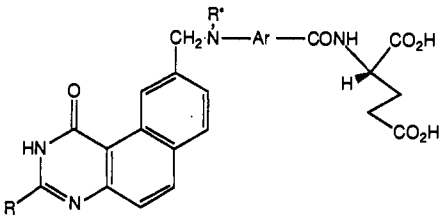
The capacity of the compounds **3a-h** to enter tumor cells by the reduced folate transport system was assessed by measurement of their inhibition of the uptake by MOLT-4 human T-cell leukemia cells of [^{3}H]-methotrexate, itself a substrate for this transport system.[29] In a previous study of the transport of molecules of this type, the uptake of tritium-labeled derivatives where available was shown to parallel the ability of the molecules to bind the transporter.[30] The compounds were also examined for their ability to function as substrates of partially purified hog liver folylpolyglutamate synthetase[31] to provide an estimate of their relative capacity for intracellular glutamylation.[32]

Results and Discussion

In vitro activities of the benzoquinazoline thymidylate synthase inhibitors **3a-h** are shown in Table 1. The introduction of the methyleneamino link to the benzoylglutamic acid side chain, as for example in **3a**, resulted in a marked increase in thymidylate synthase activity (12.5-fold) and cytotoxic potency (7-fold and 4-fold for SW480 and MCF-7, respectively) compared with the corresponding sulfonamide-linked derivative **1a**.[1]

In contrast with quinazoline TS inhibitors such as **2c-g**, in which alkyl substitution on the methyleneamino nitrogen gave significant increases in TS enzyme activity and cytotoxic potency compared with the NH com-

Table 1. *In Vitro* Activities of Benzoquinazolines **3a–h**



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				K_i(TS)[a]	cell growth inhibn[b] (nM)		MTXupt K_i[c]	FPGS[d]		
		R′	Ar	(nM)	SW480	MCF-7	(μM)	V_m rel%	K_m μM	V/K
3a	CH_3	H	1,4-C_6H_4	0.44	365	18	1.8	126	160	0.79
3b	CH_3	Me	1,4-C_6H_4	22	1500	18	0.5	117	20	5.8
3c	CH_3	H	2′-F-1,4-C_6H_4	0.09	20	0.7	0.85	60	45	1.33
3d	CH_3	H	2,5-thiophene	1.1	100	nd[e]	3.4	113	19	5.9
3e	CH_3	H	1,4-C_6H_4 (GluN-2′CH_2)	0.09	0.8	0.2	0.3	101	0.4	249
3f	CH_3	Me	1,4-C_6H_4 (GluN-2′CH_2)	2.5	500	nd	0.2	131	7.3	18
3g	CH_3	H	1,4-C_6H_4 (GluN-2′S)	4.0	15	nd	0.75	nd	nd	
3h	NH_2	H	1,4-C_6H_4	1.2	600	32	13	66	24.3	2.7
for comparison										
1a	CH_3	H	1,4-C_6H_4 (9-SO_2NH)	5.5	2400	70	4.9	184	2	92

[a] Inhibition constant *vs* purified recombinant human TS. [b] Concentration for 50% reduction in the growth rate upon continuous drug exposure for 72 h (SW480) or 96 h (MCF-7). [c] Inhibition constant *vs* the transport of [^{3}H]MTX by MOLT-4 cells. [d] Substrate activity for hog liver folylpolyglutamate synthetase. V_m (rel %) is velocity compared to a control 50 μM aminopterin run on each test. [e] Not determined.

pounds,[9,10,13,14] similar substitution in the present series of benzoquinazolines resulted in substantial losses of activity. This is also in line with previous observations in the sulfonamide-linked benzoquinazolines **1**.[1] Comparison of **3a** with **3b** and of **3e** with **3f** shows a decrease in TS activity of 50- and 28-fold and a loss of cytotoxicity of 4- and 600-fold, respectively upon methylation of the bridge nitrogen. The loss of cytotoxicity was less dramatic in the former case (**3a** to **3b**) possibly due to improvements in cell membrane transport and FPGS substrate activity, but any positive effects on uptake were more than offset by a reduction in enzyme inhibitory activity.

An increase in TS inhibitory activity was noted on comparison of the "parent" compound **3a** with its 2′-fluoro-substituted derivative **3c** (about 5-fold). The greater enhancement of cytotoxic activity (18-fold for SW480, 26-fold for MCF-7) may be explained in terms of better transport of **3c** into the cells (MTXupt, 2.5-fold) and enhanced intracellular glutamation (FPGS V/K, 1.7-fold). The rotational freedom of the glutamate moiety appeared to be restricted in the 2′-fluoro derivative by hydrogen bonding of the F-substituent with the amide NH group, as evidenced by a strong fluorine NH coupling seen in the NMR. To explore whether this effect might be related to TS activity, transport, or intracellular glutamylation, we synthesized the isoindolinyl derivative **3e**, in which the amide nitrogen was anchored to the 2′-position of the benzene ring by a methylene group. Enhancements of TS inhibition (5-fold), transport (6-fold), and FPGS substrate activity (300-fold) relative to **3a** combined to increase cytotoxic potency by 450- and 90-fold on SW480 and MCF-7 cells, respectively. Similar NMR observations on the 2′-fluoroquinazoline **2c** have been reported,[15,16] and the derivatives **2d** and **2e** made independently, using a different synthetic route to the side-chain moiety.[2] In contrast to the benzoquinazolines these derivatives were reported to show a 2–4-fold diminution in TS activity and a 30-fold reduction in cytotoxic potency (L1210 cells) relative to the conformationally unrestricted analogue **2f**.[2]

When the rigidifying methylene moiety of **3e** was exchanged for a S atom as in **3g**, a 44-fold loss of TS activity and a 19-fold drop in cytotoxic potency was observed compared with the former compound, though the thiazolyl derivative was still considerably more cytotoxic (24-fold) than the parent **3a** despite a 9-fold reduction in TS inhibition.

Replacement of the benzene ring of the PABA moiety of **3a** by a 2,5-thiophene moiety (**3d**) had little effect on TS activity or cytotoxic potency in the benzoquinazoline series, though the balance of methotrexate uptake inhibition (down 2-fold) and FPGS substrate activity (up 7.5-fold) was different from that in **3a**. In contrast, the thenoylglutamate analogue **2g** in the quinazoline series, though not the most active TS inhibitor (I_{50} = 0.67, 2.7-fold lower than the parent benzene derivative **2f**), had potent tumor cell growth inhibitory activity (IC_{50} = 7 nM *vs* L1210).[17] The compound **2g** is currently a clinical candidate (ICI No. D1694).

Several 2-methyl derivatives in the quinazoline series (**2c-g**, R = CH_3) had been shown to be more cytotoxic than the corresponding 2-amino derivatives despite lower activity against TS.[14] We had observed a similar effect in an earlier series of sulfonamide-linked benzoquinazoline derivatives **1**[1] and had attributed this to observed enhancements of uptake and of FPGS substrate activity. In the methyleneamino-linked benzoquinazolines, replacement of the the 3-amino group of **3h** by 3-methyl to give **3a** resulted in a 3-fold *increase* in TS activity accompanied by a 2-fold increase in cytotoxicity.

Conclusions

Substantial increases in TS inhibitory activity and tumor cell growth inhibition were observed upon replacing the sulfonamido-linking moiety of benzoquinazolines of type **1**[1] with an methyleneamino group as in the derivatives of type **3**. Although a relatively small increase in potency was realized on going from a 3-amino to a 3-methyl substituent in this series, compared with the earlier

reported quinazolines,[14,15] large increases in TS inhibition and cytotoxic activity were gained by rigidifying the glutamate moiety of the side chain. Derivatives of type 3 with a free NH in the methyleneamino bridge were more active than the corresponding N-methylated compounds, paralleling the behavior of the sulfonamide-linked compounds 1[1] and contrasting with the beneficial effect of bridge alkylation in the quinazoline series.[10]

A combination of these structural features made compound 3e (BW 1843U89) a promising candidate for preclinical development as an antitumor agent. We have recently discussed[30] the biochemical and cellular pharmacology of 3e; further studies on the mechanisms of cytotoxicity, biovailability determinations, toxicology, and *in vivo* antitumor efficacy data on this compound will be reported elsewhere.

Experimental Section

^{1}H NMR spectra were recorded on Varian XL-200 and XL-300 spectrometers; chemical shifts are in parts per million downfield from tetramethylsilane, and coupling constants (J) are measured in hertz (Hz). Mass spectra were determined by Oneida Research Services, Whitesboro, NY, on a Finnegan 4500 instrument. Methane was used as the reagent gas in determinations of desorption chemical ionization (CI) mass spectra. Samples were dissolved in methanol prior to deposition on the wire for CI. Analytical samples of intermediates moved as single spots on TLC and were run on Whatman MK6F silica gel plates. The diesters 9 were rigorously purified prior to hydrolysis in dilute sodium hydroxide, allowing the free acids 3 to be isolated without further purification. Column chromatography was carried out on silica gel 60 (E. Merck, Darmstadt, Germany). The benzoquinazolines generally did not have sharp melting points but decomposed gradually above 220 °C. They were also very tenacious of water of crystallization, and in cases where the elemental analysis indicated the presence of water, the ^{1}H NMR spectrum in rigorously-dried DMSO-d_6 reflected this. Analyses were performed by Atlantic Microlab, Inc. All values were within 0.4% of theory.

Diethyl *N*-(4-(((1,2-Dihydro-3-methyl-1-oxobenzo[*f*]quinazolin-9-yl)methyl)amino)-2-fluorobenzoyl)-L-glutamate (9c). To a hot solution of 3,9-dimethylbenzo[*f*]quinazolin-1(2*H*)-one (4) (2.0 g, 8.9 mmol) in benzene (1000 mL) under nitrogen was added *N*-bromosuccinimide (2.0 g, 11 mmol). The solution was stirred at reflux for 1 h and then concentrated *in vacuo* to give crude 9-(bromomethyl)-3-methylbenzo[*f*]quinazolin-1(2*H*)-one (6). The solid was suspended with diethyl *N*-(4-amino-2-fluorobenzoyl)-L-glutamate[23] (6.0 g, 18 mmol) in DMF (20 mL) and stirred under nitrogen at 100 °C for 30 min. The reaction mixture was allowed to cool, *N*-methylmorpholine (1.0 mL, 9.1 mmol) (Aldrich) was added, and the solution was concentrated under vacuum. The residue was purified with silica gel chromatography eluting with methylene chloride–THF (5:1). Fractions containing product were concentrated *in vacuo* to a thick paste, and the solid was suspended in a small volume of diethyl ether, filtered under nitrogen, and dried under vacuum to give 9c as a white solid (2.3 g, 46%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.15 (t, J = 7 Hz, 3H, ester CH_3), 1.18 (t, J = 7 Hz, 3H, ester CH_3), 1.87–2.13 (m, 2H, Glu CH_2), 2.38 (t, J = 7 Hz, 2H, Glu CH_2), 2.43 (s, 3H, C^3-CH_3), 4.02 (q, J = 7 Hz, 2H, ester CH_2), 4.09 (q, J = 7 Hz, 2H, ester CH_2), 4.34–4.44 (m, 1H, Glu CH), 4.57 (d, J = 6 Hz, 2H, C^9-CH_2), 6.39 (dd, J = 15, 2 Hz, 1H, Ar), 6.53 (dd, J = 9, 2 Hz, 1H, Ar), 7.30 (t, J = 6 Hz, 1H, ArNH), 7.44 (dd, J = 9, 9 Hz, 1H, Ar), 7.60 (d, J = 9 Hz, 1H, Ar), 7.61 (dd, J = 8, 2 Hz, 1H, Ar), 7.88 (dd, J = 7, 5 Hz, 1H, Glu NH), 8.01 (d, J = 8 Hz, 1H, Ar), 8.22 (d, J = 9 Hz, 1H, Ar), 9.85 (s, 1H, Ar), 12.53 (s, 1H, N^2H); mass spectrum (CI-CH_4) m/z 563 ((M + 1)$^+$, 100). Anal. ($C_{30}H_{31}FN_4O_6$) C, H, N.

***N*-(4-(((1,2-Dihydro-3-methyl-1-oxobenzo[*f*]quinazolin-9-yl)methyl)amino)-2-fluorobenzoyl)-L-glutamic Acid (3c).** A solution of 9c (2.3 g, 4.1 mmol) in ethanol (25 mL) and 0.2 N NaOH (100 mL) was stirred under nitrogen at room temperature for 3 h. The solution was adjusted to pH 7 with 1 N HCl and reduced in volume under vacuum to remove the ethanol. The product was precipitated by acidifying the solution with 1 N HCl to pH 3 with stirring under nitrogen. The suspension was stirred 15 min, filtered under nitrogen, washed with water, pressed with a sheet of latex to remove excess water, and dried under vacuum to give 3c as a white solid (2.1 g, 93%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.82–2.12 (m, 2H, Glu CH_2), 2.29 (t, J = 7 Hz, 2H, Glu CH_2), 2.43 (s, 3H, C^3-CH_3), 4.32–4.42 (m, 1H, Glu CH), 4.57 (d, J = 6 Hz, 2H, C^9-CH_2), 6.39 (dd, J = 15, 2 Hz, 1H, Ar), 6.53 (dd, J = 9, 2 Hz, 1H, Ar), 7.30 (t, J = 6 Hz, 1H, ArNH), 7.47 (dd, J = 9, 9 Hz, 1H, Ar), 7.59 (d, J = 9 Hz, 1H, Ar), 7.61 (dd, J = 8, 2 Hz, 1H, Ar), 7.73 (t, J = 7 Hz, 1H, Glu NH), 8.01 (d, J = 8 Hz, 1H, Ar), 8.22 (d, J = 9 Hz, 1H, Ar), 9.85 (s, 1H, Ar), 12.42 (br s, 2H, CO_2H'), 12.53 (s, 1H, N^2H). Anal. ($C_{26}H_{23}FN_4O_6$·1.5H_2O·0.33NaCl) C, H, N, Cl, Na.

An essentially similar sequence of reactions with diethyl (4-aminobenzoyl)-L-glutamate (2.8 g, 8.7 mmol) (Aldrich) and diethyl (4-(methylamino)(benzoyl)-L-glutamate[22] (1.4 g, 4.2 mmol) gave, upon coupling of each with 3,9-dimethylbenzo[*f*]quinazolin-1(2*H*)-one (0.5 g, 2.2 mmol) via the bromomethyl derivative and subsequent hydrolysis of the diesters, ***N*-(4-(((1,2-dihydro-3-methyl-1-oxobenzo[*f*]quinazolin-9-yl)methyl)amino)benzoyl)-L-glutamic acid (3a) and *N*-(4-(((1,2-dihydro-3-methyl-1-oxobenzo[*f*] quinazolin-9-yl)methyl)methylamino)benzoyl)-L-glutamic acid (3b)**; data on these compounds and their diester intermediates are given below.

Diethyl *N*-(4-(((1,2-dihydro-3-methyl-1-oxobenzo[*f*]-quinazolin-9-yl)methyl)amino)benzoyl)-L-glutamate (9a) (0.57 g, 47%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.15 (t, J = 7 Hz, 3H, ester CH_3), 1.17 (t, J = 7 Hz, 3H, ester CH_3), 1.87–2.13 (m, 2H, Glu CH_2), 2.39 (t, J = 7 Hz, 2H, Glu CH_2), 2.43 (s, 3H, C^3-CH_3), 4.03 (q, J = 7 Hz, 2H, ester CH_2), 4.07 (q, J = 7 Hz, 2H, ester CH_2), 4.31–4.41 (m, 1H, Glu CH), 4.57 (d, J = 6 Hz, 2H, C^9-CH_2), 6.64 (d, J = 9 Hz, 2H, Ar), 7.02 (t, J = 6 Hz, 1H, ArNH), 7.57–7.67 (m, 4H, Ar), 8.00 (d, J = 8 Hz, 1H, Ar), 8.21 (overlapping d, J = 8 Hz, 2H, Ar, Glu NH), 9.85 (s, 1H, Ar), 12.53 (s, 1H, N^2H); mass spectrum (CI-CH_4) m/z 545 ((M + 1)$^+$, 94.8), 342 (100). Anal. ($C_{30}H_{32}N_4O_6$·0.1H_2O) C, H, N.

***N*-(4-(((1,2-Dihydro-3-methyl-1-oxobenzo[*f*]quinazolin-9-yl)methyl)amino)benzoyl)-L-glutamic acid (3a)** (0.48 g, 93% from diester (0.56 g, 1.02 mmol): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.82–2.12 (m, 2H, Glu CH_2), 2.31 (t, J = 7 Hz, 2H, Glu CH_2), 2.43 (s, 3H, CH_3), 4.28–4.38 (m, 1H, Glu CH), 4.57 (d, J = 6 Hz, 2H, C^9-CH_2), 6.64 (d, J = 9 Hz, 2H, Ar), 7.00 (t, J = 6 Hz, 1H, ArNH), 7.59 (d, J = 9 Hz, 1H Ar), 7.63 (dd, J = 8, 2 Hz, 1H, Ar), 7.63 (d, J = 9 Hz, 2H, Ar), 8.00 (d, J = 8 Hz, 1H, Ar), 8.09 (d, J = 8 Hz, 1H, Glu NH), 8.21 (d, J = 9 Hz, 1H, Ar), 9.85 (s, 1H, Ar), 12.33 (br s, 2H, CO_2H's), 12.53 (s, 1H, N^2H). Anal. ($C_{26}H_{24}N_4O_6$·H_2O) C, H, N.

Diethyl *N*-(4-(((1,2-dihydro-3-methyl-1-oxobenzo[*f*]-quinazolin-9-yl)methyl)methylamino)benzoyl)-L-glutamate (9b) (0.66 g, 53%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.15 (t, J = 7 Hz, 3H, ester CH_3), 1.17 (t, J = 7 Hz, 3H, ester CH_3), 1.88–2.13 (m, 2H, Glu CH_2), 2.40 (t, J = 8 Hz, 2H, Glu CH_2), 2.42 (s, 3H, C^3-CH_3), 3.19 (s, 3H, NCH_3), 4.03 (q, J = 7 Hz, 2H, ester CH_2), 4.08 (q, J = 7 Hz, 2H, ester CH_2), 4.33–4.43 (m, 1H, Glu CH), 4.91 (s, 2H, C^9-CH_2), 6.81 (d, J = 9 Hz, 2H, Ar), 7.44 (dd, J = 8, 2 Hz, 1H, Ar), 7.58 (d, J = 9 Hz, 1H, Ar), 7.72 (d, J = 9 Hz, 2H, Ar), 7.98 (d, J = 8 Hz, 1H, Ar), 8.20 (d, J = 9 Hz, 1H, Ar), 8.29 (d, J = 7 Hz, 1H, Glu NH), 9.76 (s, 1H, Ar), 12.48 (s, 1H, N^2H); mass spectrum (CI-CH_4) m/z 559 ((M + 1)$^+$, 100). Anal. ($C_{31}H_{34}N_4O_6$) C, H, N.

***N*-(4-(((1,2-Dihydro-3-methyl-1-oxobenzo[*f*]quinazolin-9-yl)methyl)methylamino)benzoyl)-L-glutamic acid (3b)** (0.58 g, 94% from diester (0.65 g, 1.2 mmol)): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.83–2.14 (m, 2H, Glu CH_2), 2.32 (t, J = 7 Hz, 2H, Glu CH_2), 2.42 (s, 3H, C^3-CH_3), 3.18 (s, 3H, NCH_3), 4.30–4.42 (m, 1H, Glu CH), 4.91 (s, 2H, C^9-CH_2), 6.81 (d, J = 9 Hz, 2H, Ar), 7.44 (dd, J = 8, 2 Hz, 1H, Ar), 7.58 (d, J = 9 Hz, 1H, Ar), 7.73 (d, J = 9 Hz, 2H, Ar), 7.98 (d, J = 8 Hz, 1H, Ar), 8.18 (d, J = 8 Hz, 1H, GluNH), 8.19 (d, J = 9 Hz, 1H, Ar), 9.76 (s, 1H, Ar), 12.32 (br s, 2H, CO_2H's), 12.50 (br s, 2H, N^2H). Anal. ($C_{27}H_{26}N_4O_6$·1.4H_2O) C, H, N.

Diethyl (*S*)-2-(4-Nitrophthalimido)glutarate (11). Diisopropylethylamine (24 mL, 0.138 mol) (Aldrich) was added to a suspension of 4-nitrophthalic anhydride (25 g, 0.13 mol) (Tokyo Kasei) and L-glutamic acid diethyl ester hydrochloride (35 g,

0.146 mole) (Aldrich) in toluene (130 mL). The reaction mixture was stirred at reflux under a Dean-Stark trap for 2.5 h. After cooling, the solution was diluted with diethyl ether (300 mL), washed with water (75 mL) and saturated $NaHCO_3$ solution (50 mL), dried ($MgSO_4$), and concentrated *in vacuo* at 70 °C to give 11 as an oil that solidified to a white solid on standing (35.8 g, 73%): mp 65.5–66.5 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.12 (t, J = 7 Hz, 3H, ester CH_3), 1.14 (t, J = 7 Hz, 2H, ester CH_3), 2.2–2.5 (m, 4H, Glu CH_2CH_2), 3.96 (q, J = 7Hz, 2H, ester CH_2), 4.08–4.19 (m, 2H, ester CH_2), 4.97-5.04 (m, 1H, Glu CH), 8.19 (dd, J = 8, 0.5 Hz, 1H, Ar), 8.56 (dd, J = 2, 0.5 Hz, 1H, Ar), 8.68 (dd, J = 8, 2 Hz, 1H, Ar); mass spectrum (CI-CH_4) m/z 379 ((M + 1)$^+$, 28.8), 333 (71.6), 305 (100). Anal. ($C_{17}H_{18}N_2O_8$) C, H, N.

Diethyl (*S*)-2-(4-Aminophthalimido)glutarate (12). A suspension of 11 (35.6 g, 94.1 mmol) and 10% palladium on carbon (0.5 g) (Aldrich) in ethanol (200 mL) was shaken under a hydrogen atmosphere (40–50 psi) for 26 h. The solution was filtered through Celite and concentrated *in vacuo*. The residue was purified by chromatography on silica gel (250 g), eluting with diethyl ether–hexane (4:1) to give 12 as a viscous yellow oil (29.1 g, 89%): ^1H NMR (DMSO-d_6, 300 MHz) δ 1.10 (t, J = 7 Hz, 3H, ester CH_3), 1.12 (t, J = 7 Hz, 3H, ester CH_3), 2.17–2.39 (m, 4H, Glu CH_2CH_2), 3.87–3.98 (m, 2H, ester CH_2), 4.05–4.18 (m, 2H, ester CH_2), 4.75–4.82 (m, 1H, Glu CH), 6.57 (br s, 2H, NH_2), 6.82 (dd, J = 8, 2 Hz, 1H, Ar), 6.93 (d, J = 2 Hz, 1H, Ar), 7.51 (d, J = 8 Hz, 1H, Ar); mass spectrum (CI-CH_4) m/z 349 ((M + 1)$^+$, 40.8), 303 (60.1), 275 (100). Anal. ($C_{17}H_{20}N_2O_6$) C, H, N.

Diethyl (*S*)-2-(5-Amino-1-oxo-2-isoindolinyl)glutarate (8e). A solution of 12 (10.5 g, 30.2 mmol) in ethanol (150 mL) was cooled in an acetonitrile/CO_2 bath. Concentrated HCl (25 mL) was added, followed by 30-mesh granular Zn (10.5 g, 0.161 mole) (Fisher) when the internal temperature had reached −40 °C. The reaction mixture was stirred for 1.5 h at this temperature and a further 1 h at −10 °C. The excess of Zn was filtered from the solution, 10% palladium on carbon (1.0 g) was added, and the solution shaken under hydrogen at (30–50 psi) overnight. The catalyst was removed by filtration through Celite and the filtrate concentrated *in vacuo*. The residue was made basic by the addition of saturated $NaHCO_3$ solution (~300 mL) and extracted with diethyl ether (3 × 100 mL), and the ether extracts were dried (K_2CO_3) and concentrated *in vacuo*. The residue was absorbed onto silica gel (15 g) and purified by chromatography on silica gel (400 g), eluting with ethyl acetate–methylene chloride (1:4) to give 8e as a viscous oil (4.14 g, 41%): ^1H NMR (DMSO-d_6, 300 MHz) δ 1.12 (t, J = 7 Hz, 3H, ester CH_3), 1.17 (t, J = 7 Hz, 3H, ester CH_3), 1.98–2.33 (m, 4H, Glu CH_2CH_2), 3.92–4.03 (m, 2H, ester CH_2), 4.11 (q, J = 7 Hz, 2H, ester CH_2), 4.26 (very strongly coupled AB pair, 2H, $ArCH_2N$), 4.77–4.84 (m, 1H, Glu CH), 5.83 (br s, 2H, NH_2), 6.58–6.65 (m, 2H, Ar), 7.32 (d, J = 8 Hz, 1H, Ar); mass spectrum (CI-CH_4) m/z 335 ((M + 1)$^+$, 100). Anal. ($C_{17}H_{22}N_2O_5$) C, H, N.

9-(Bromomethyl)-3-methylbenzo[*f*]quinazolin-1(2*H*)-one (6). To a hot solution of 4 (4.00 g, 17.9 mmol) in benzene (2000 mL) under nitrogen was added *N*-bromosuccinimide (4.00 g, 22.5 mmol). The reaction mixture was stirred just below reflux for 30 min and then at a gentle reflux for 30 min. The resulting suspension was allowed to cool for 2 h, and the solid was filtered and dried at 70 °C under reduced pressure to give 6 (4.32 g, 83% purity by NMR): ^1H NMR (DMSO-d_6, 300 MHz) δ 2.45 (s, 3H, CH_3), 4.96 (s, 2H, CH_2), 7.65 (d, J = 9 Hz, 1H, Ar), 7.70 (dd, J = 8, 2 Hz, 1H, Ar), 8.05 (d, J = 8 Hz, 1H, Ar), 8.16 (d, J = 9 Hz, 1H, Ar), 9.89 (s, 1H, Ar), 12.7 (br s, 1H, NH).

Diethyl (*S*)-2-(5-(((1,2-Dihydro-3-methyl-1-oxobenzo[*f*]-quinazolin-9-yl)methyl)amino)-1-oxo-2-isoindolinyl)glutarate (9e). A mixture of crude 6 (4.32 g, 12 mmol), 8e (4.0 g, 12 mmol), and $NaHCO_3$ (2.0 g, 24 mmol) in DMF (30 mL) was stirred under nitrogen at 105 °C for 1.5 h. After cooling, acetic acid (1 mL, 17 mmol) was added, the reaction mixture was transferred to a larger round-bottom flask with ethanol, and then the mixture was concentrated *in vacuo* onto silica gel (30 g). The absorbed material was purified by chromatography on silica gel, eluting with methanol–methylene chloride (1:24) followed by precipitation of the solid from methylene chloride (~20 mL) with ethyl acetate (~45 mL) and methanol (~5 mL). The white solid was filtered under nitrogen and dried under vacuum to give 9e (3.27 g, 49%): ^1H NMR (DMSO-d_6, 300 MHz), δ 1.10 (t, J = 7 Hz, 3H, ester CH_3), 1.15 (t, J = 7 Hz, 3H, ester CH_3), 1.93–2.33 (m, 4H, Glu CH_2CH_2), 2.43 (s, 3H, C^3-CH_3), 3.87–4.03 (m, 2H, ester CH_2), 4.09 (q, J = 7 Hz, 2H, ester CH_2), 4.25 (very strongly coupled AB pair, 2H, Glu NCH_2Ar), 4.58 (d, J = 6 Hz, 2H, C^9-CH_2N), 4.74–4.83 (m, 1H, Glu CH), 6.71 (br d, J = 2 Hz, 1H, Ar), 6.74 (dd, J = 8, 2 Hz, 1H, Ar), 7.20 (t, J = 6 Hz, 1H, ArNH), 7.37 (d, J = 8 Hz, 1H Ar), 7.59 (d, J = 9 Hz, 1H, Ar), 7.63 (dd, J = 8, 2 Hz, 1H, Ar), 8.00 (d, J = 8 Hz, 1H, Ar), 8.21 (d, J = 9 Hz, 1H, Ar), 9.87 (s, 1H, Ar), 12.54 (s, 1H, N^2H); mass spectrum (CI-CH_4) m/z 557 ((M + 1)$^+$, 100). Anal. ($C_{31}H_{32}N_4O_6$) C, H, N.

(*S*)-2-(5-(((1,2-Dihydro-3-methyl-1-oxobenzo[*f*]quinazolin-9-yl)methyl)amino)-1-oxo-2-isoindolinyl)glutaric Acid (3e). A solution of 9e (3.20 g, 5.75 mmol) in 0.2 N NaOH (140 mL) was stirred under nitrogen for 3 h at room temperature. The solution was then slowly adjusted to pH 3 with 1 N HCl and the resulting suspension allowed to stir briefly. The white solid was filtered under nitrogen, washed with water, and dried under vacuum to give 3e (2.85 g, 94%) as a white solid: ^1H NMR (DMSO-d_6/D_2O, 300 MHz) δ 1.86–2.34 (m, 4H, Glu CH_2CH_2), 2.44 (s, 3H, CH_3), 4.25 (very strongly coupled AB pair, 2H, Glu NCH_2Ar), 4.58 (s, 2H, C^9-CH_2), 4.67–4.75 (m, 1H, Glu CH), 6.69–6.77 (m, 2H, Ar), 7.37 (d, J = 8 Hz, 1H, Ar), 7.59 (d, J = 9 Hz, 1H, Ar), 7.64 (dd, J = 8, 2 Hz, 1H, Ar), 8.01 (d, J = 8 Hz, 1H, Ar), 8.22 (d, J = 9 Hz, 1H, Ar), 9.85 (s, 1H, Ar). Anal. ($C_{27}H_{24}N_4O_6$·1.6H_2O) C, H, N.

By a similar sequence of reactions, the *N*-methyl derivative (3f) of the foregoing compound was prepared by condensation of the (bromomethyl)benzoquinazoline (6) (2 g, ~5.2 mmol) with diethyl (*S*)-2-(5-(methylamino)-1-oxo-2-isoindolinyl)glutarate (8f) (1.8 g, 5.1 mmol). The preparation of the latter, and physical data on the final benzoquinazoline product and diester intermediate are given below.

Diethyl (*S*)-2-(5-(Methylamino)-1-oxo-2-isoindolinyl)glutarate (8f). To a solution of 8e (3.1 g, 9.3 mmol), 37% aqueous formaldehyde (0.81 g, 10 mmol), and acetic acid (0.5 mL) in ethanol (30 mL) was added sodium cyanoborohydride (0.63 g, 10 mmol). After the reaction mixture was stirred for 45 min, additional acetic acid (0.5 mL) and sodium borohydride (0.32 g, 5 mmol) were added, and after a further 15 min additional 37% aqueous formaldehyde (0.30 g, 3.7 mmol) was added. The reaction mixture was stirred for 1 h, water (1 mL) was added, and then the mixture was concentrated *in vacuo*.

Purification by chromatography on silica gel eluting with ethyl acetate–methylene chloride (1:6) gave 8f (1.8 g, 55%) as a viscous oil: ^1H NMR (DMSO-d_6, 300 MHz) δ 1.12 (t, J = 7 Hz, 3H, ester CH_3), 1.17 (t, J = 7 Hz, 3H, ester CH_3), 1.95–2.35 (m, 4H, Glu CH_2's), 2.74 (d, J = 5 Hz, 3H, NCH_3), 3.90–4.03 (m, 2H, ester CH_2), 4.11 (q, J = 7 Hz, 2H, ester CH_2), 4.30 (very strongly coupled AB pair, 2H, $ArCH_2$), 4.77–4.84 (m, 1H, Glu CH), 6.43 (br q, J = 5 Hz, 1H, NH), 6.58–6.64 (m, 2H, Ar), 7.38 (d, J = 9 Hz, 1H, Ar). Anal. ($C_{18}H_{24}N_2O_5$·0.33H_2O) C, H, N.

Diethyl (*S*)-2-(5-(((1,2-dihydro-3-methyl-1-oxobenzo[*f*]-quinazolin-9-yl)methyl)methylamino)-1-oxo-2-isoindolinyl)glutarate (9f) (1.5 g, 51%): ^1H NMR (DMSO-d_6, 300 MHz) δ 1.10 (t, J = 7 Hz, 3H, ester CH_3), 1.15 (t, J = 7 Hz, 3H, ester CH_3), 1.97–2.35 (m, 4H, Glu CH_2's), 2.42 (s, 3H, C^3-CH_3), 3.23 (s, 3H, NCH_3), 3.89–4.02 (m, 2H, ester CH_2), 4.10 (q, J = 7 Hz, 2H, ester CH_2), 4.30 (very strongly coupled AB pair, 2H, Glu NCH_2 Ar), 4.77–4.84 (m, 1H, Glu CH), 4.94 (s, 2H, C^9-CH_2), 6.84–6.12 (m, 2H, Ar), 7.45 (d, J = 8.5 Hz, 2H, Ar), 7.58 (d, J = 9 Hz, 1H, Ar), 7.99 (d, J = 8.5 Hz, 1H, Ar), 8.20 (d, J = 9 Hz, 1H, Ar), 9.77 (s, 1H, Ar), 12.49 (s, 1H, N^2H). Anal. ($C_{32}H_{34}N_4O_6$·0.25H_2O) C, H, N.

(*S*)-2-(5-(((1,2-Dihydro-3-methyl-1-oxobenzo[*f*]quinazolin-9-yl)methyl)methylamino)-1-oxo-2-isoindolinyl)glutaric acid (3f) (1.31 g, 91% from 1.44 g, 2.5 mmol of 9f): ^1H NMR (DMSO-d_6, 300 MHz) δ 1.88–2.35 (m, 4H, Glu CH_2's), 2.43 (s, 3H, C^3-CH_3), 3.22 (s, 3H, NCH_3), 4.31 (s, 2H, Glu NCH_2 Ar), 4.67–4.76 (m, 1H, Glu CH), 4.93 (s, 2H, C^9-CH_2), 6.87 (dd, J = 8.5, 2 Hz, 1H, Ar), 6.91 (s, 1H, Ar), 7.44 (d, J = 8.5 Hz, 1H, Ar), 7.46 (dd, J = 8.5, 1.5 Hz, 1H, Ar), 7.58 (d, J = 9 Hz, 1H, Ar), 7.99 (d, J = 8.5 Hz, 1H, Ar), 8.20 (d, J = 9 Hz, 1H, Ar), 9.76 (s, 1H, Ar), 11.8–13.0 (br s, 2H, CO_2H's), 12.52 (br s, 1H, N^2H). Anal. ($C_{28}H_{26}N_4O_6$·2H_2O) C, H, N.

Diethyl *N*-((5-(((1,2-dihydro-3-methyl-1-oxobenzo[*f*]-quinazolin-9-yl)methyl)amino)-2-thienyl)carbonyl)-L-glutamate (9d) (0.46 g, 31%) was similarly prepared by condensation of 6 (1.1 g, 80% pure, 2.9 mmol) with diethyl (5-

amino-2-thenoyl)-L-glutamate[18] (8d) (0.85 g, 2.6 mmol): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.15 (t, J = 7 Hz, 3H, ester CH_3), 1.17 (t, J = 7 Hz, 3H, ester CH_3), 1.82–2.10 (m, 2H, Glu CH_2), 2.38 (t, J = 7.5 Hz, 2H, Glu CH_2), 2.43 (s, 3H, C^3-CH_3), 4.03 (q, J = 7 Hz, 2H, ester CH_2), 4.08 (q, J = 7 Hz, 2H, ester CH_2), 4.26–4.36 (m, 1H, Glu CH), 4.51 (d, J = 5.5 Hz, 2H, C^9-CH_2), 5.90 (d, J = 4 Hz, 1H, Ar), 7.46 (d, J = 4 Hz, 1H, Ar), 7.60 (d, J = 9 Hz, 1H, Ar), 7.63 (dd, J = 8, 1.5 Hz, 1H, Ar), 7.70 (t, J = 5.5 Hz, 1H, ArNH), 8.01 (d, J = 8.5 Hz, 1H, Ar), 8.14 (d, J = 7.5 Hz, 1H, Glu NH), 8.22 (d, J = 9 Hz, 1H, Ar), 9.84 (s, 1H, Ar), 2.55 (s, 1H, N^2H); mass spectrum (CI-CH_4) m/z 551 ((M + 1)$^+$, 90.3), 329 (100). Anal. ($C_{28}H_{30}N_4O_6S$·1.33H_2O) C, H, N, S.

N-((5-(((1,2-Dihydro-3-methyl-1-oxobenzo[*f*]quinazolin-9-yl)methyl)amino)-2-thienyl)carbonyl)-L-glutamic acid (3d) (0.37 g, 91%) was prepared from the foregoing diester (0.45 g, 0.78 mmol) by base hydrolysis as described above: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.77–2.08 (m, 2H, Glu CH_2), 2.30 (t, J = 7.5 Hz, Glu CH_2), 2.43 (s, 3H, CH_3), 4.22–4.32 (m, 1H, Glu CH), 4.51 (d, J = 5.5 Hz, 2H, C^9-CH_2), 5.89 (d, J = 4 Hz, 1H, Ar), 7.46 (d, J = 4 Hz, 1H, Ar), 7.58–7.70 (m, 3H, 2 Ar & ArNH), 8.01 (d, J = 8 Hz, 1H, Ar), 8.03 (d, J = 7.5 Hz, 1H, Glu NH), 8.22 (d, J = 9 Hz, 1H, Ar), 9.84 (s, 1H, Ar), 12.34 (br s, 2H, CO_2H's), 12.55 (s, 1H, N^2H). Anal. ($C_{24}H_{22}N_4O_6S$·1.33H_2O) C, H, N, S.

2,2'-Dithiobis(4-nitrobenzoic acid) (15).[33] A suspension of 4-nitroanthranilic acid (32.7 g, 180 mmol) (Aldrich) in water (100 mL) and concentrated HCl (35 mL) was stirred for 30 min at room temperature and then chilled in an ice bath. A solution of sodium nitrite (12.4 g, 180 mmol) in water (25 mL) was added in small aliquots below the surface of the suspension via pipette. Crushed ice was added during the addition to maintain an internal temperature below 5 °C. The reaction mixture was then stirred for 1 h at 0 °C. In a 1-L flask, sulfur (6.4 g, 0.20 mol) and Na_2S·9H_2O (48 g, 0.20 mol) were dissolved in hot water (100 mL). A solution of NaOH (7.2 g, 0.18 mol) in water (40 mL) was added and the resulting solution cooled in an ice bath. The solution of diazonium salt was then added in aliquots (15–25 mL) along with crushed ice to maintain an internal temperature below 5 °C. The reaction mixture was stirred for 2 h at room temperature and filtered, and the filtrate was adjusted to neutral pH with acetic acid. The solution was treated with activated charcoal (5 g), filtered, and adjusted to pH 2.5–3.0 with concentrated HCl, and the resulting precipitate was filtered and washed with water. The solid was dissolved in hot ethanol (200 mL), treated with activated charcoal (5 g), filtered, concentrated *in vacuo*, then resuspended in methanol (~80 mL), filtered, and dried under reduced pressure at 110 °C to give crude 2,2'-dithiobis(4-nitrobenzoic acid) as a white solid (9.9 g, ~28%). An analytical sample was prepared by recrystallization from methanol: gradually decomped >120 °C; H NMR (DMSO-d_6, 300 MHz) δ 8.15 (dd, J = 9, 2 Hz, 1H, Ar), 8.27 (d, J = 9 Hz, 1H, Ar), 8.39 (d, J = 2 Hz, 1H, Ar). Anal. ($C_{14}H_8N_2O_6S_2$) C, H, N, S.

Diethyl (*S*)-2-(2,3-Dihydro-6-nitro-3-oxo-1,2-benzisothiazol-2-yl)glutarate (16). A solution of crude **15** (8.35 g, ~21 mmol) in $SOCl_2$ (50 mL) was stirred at reflux for 1.5 h and then concentrated *in vacuo*. The residual solid was suspended in methylene chloride (50 mL), Cl_2 (3.3 g, 47 mmol) was bubbled in, and the solution was stirred for 1.5 h at room temperature. Nitrogen was then bubbled into the solution until moistened starch-iodine paper indicated the absence of Cl_2. Diethyl L-glutamate hydrochloride (6.5 g, 27 mmol) (Aldrich) was then added, followed by the dropwise addition of diisopropylethylamine (~10 mL, ~57 mmol) (Aldrich), and the reaction mixture was stirred under nitrogen for 45 min. Additional diisopropylethylamine was added dropwise until formation of white amine hydrochloride fumes above the solution ceased. After a further 30 min of stirring, the reaction mixture was concentrated *in vacuo* onto silica gel (40 g); chromatography on silica gel (200 g), eluting with ethyl acetate–hexane (1:2), gave **16** (4.0 g, 50%) as an oil: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.14 (t, J = 7 Hz, 3H, ester CH_3), 1.18 (t, J = 7 Hz, 3H, ester CH_3), 2.12–2.46 (m, 4H, Glu CH_2CH_2), 4.01 (q, J = 7 Hz, 2H, ester CH_2), 4.17 (q, J = 7 Hz, 2H, ester CH_2), 5.22–5.29 (m, 1H, Glu CH), 8.11 (d, J = 9 Hz, 1H, Ar), 8.21 (dd, J = 9, 2 Hz, 1H, Ar), 9.03 (d, J = 2 Hz, 1H, Ar). Anal. ($C_{18}H_{18}N_2O_7S$) C, H, N, S.

Diethyl (*S*)-2-(6-Amino-2,3-dihydro-3-oxo-1,2-benzisothiazol-2-yl)glutarate (8g). A solution of **16** (4.0 g, 10 mmol) and suspended iron (1.0 g, 18 mmol) in acetic acid (100 mL) was stirred under nitrogen for 1 h at 55 °C. Additional iron (3 × 0.25 g, 13 mmol) was added at intervals of 1, 1.25, and 1.75 h. The reaction mixture was stirred for 30 min after the last addition, filtered, and concentrated *in vacuo*, and the residue was absorbed onto silica gel (20 g) from a methylene chloride solution. Purification by chromatography on silica gel (150 g) eluting with ethyl acetate–hexane (1:1 → 2:1) gave **8g** (3.3 g, 90%) as an oil: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.15 (t, J = 7 Hz, 3H, ester CH_3), 1.17 (t, J = 7 Hz, 3H, ester CH_3), 1.95–2.38 (m, 4H, Glu CH_2CH_2), 4.02 (q, J = 7 Hz, 2H, ester CH_2), 4.13 (q, J = 7 Hz, 2H, ester CH_2), 5.08–5.15 (m, 1H, Glu CH), 6.09 (br s, 2H, NH_2), 6.62 (dd, J = 9, 2 Hz, 1H, Ar), 6.84 (d, J = 2 Hz, 1H, Ar), 7.50 (d, J = 9 Hz, 1H, Ar); mass spectrum (CI-CH_4) m/z 353 ((M + 1)$^+$, 100). Anal. ($C_{18}H_{20}N_2O_5S$) C, H, N, S.

Diethyl (*S*)-2-(2,3-dihydro-6-(((1,2-dihydro-3-methyl-1-oxobenzo[*f*]quinazolin-9-yl)methyl)-amino)-3-oxo-1,2-benzisothiazol-2-yl)glutarate (9g) (0.34 g, 9.7%) was prepared by condensation of the foregoing ester (3.2 g, 9.1 mmol) with **6** (2.3 g, 6.1 mmol) essentially as described for **9e**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.12 (t, J = 7 Hz, 3H, ester CH_3), 1.14 (t, J = 7 Hz, 3H, ester CH_3), 1.93–2.34 (m, 4H, Glu CH_2CH_2), 2.42 (s, 3H, C^3-CH_3), 4.00 (q, J = 7 Hz, 2H, ester CH_2), 4.11 (q, J = 7 Hz, 2H, ester CH_2), 4.59 (d, J = 6 Hz, 2H, C^9-CH_2), 5.06–5.14 (m, 1H, Glu CH), 6.79 (dd, J = 9, 2 Hz, 1H, Ar), 6.92 (d, J = 2 Hz, 1H, Ar), 7.44 (t, J = 6 Hz, 1H, ArNH), 7.53 (d, J = 9 Hz, 1H, Ar), 7.59 (d, J = 9 Hz, 1H, Ar), 7.63 (dd, J = 8, 2 Hz, 1H, Ar), 8.01 (d, J = 8 Hz, 1H, Ar), 8.21 (d, J = 9 Hz, 1H, Ar), 9.86 (s, 1H, Ar), 2.54 (s, 1H, N^2H); mass spectrum (CI-CH_4) m/z 575 ((M + 1)$^+$, 36.5). Anal. ($C_{30}H_{30}N_4O_6S$) C, H, N, S.

(*S*)-2-(2,3-Dihydro-6-(((1,2-dihydro-3-methyl-1-oxobenzo[*f*]quinazolin-9-yl)methyl)amino)-3-oxo-1,2-benzisothiazol-2-yl)glutaric acid (3g) (25 mg, 31%) was obtained by hydrolysis of the foregoing diester essentially as described for **3e**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.88–2.38 (m, 4H, Glu CH_2CH_2), 2.43 (s, 3H, CH_3), 4.60 (br d, J = 5 Hz, 2H, C^9-CH_2), 4.98–5.08 (m, 1H, Glu CH), 6.78 (dd, J = 9,2 Hz, 1H, Ar), 6.92 (d, J = 2 Hz, 1H, Ar), 7.41 (br t, J = 6 Hz, 1H, ArNH), 7.53 (d, J = 9 Hz, 1H, Ar), 7.59 (d, J = 9 Hz, 1H, Ar), 7.63 (dd, J = 8, 2 Hz, 1H, Ar), 8.02 (d, J = 8 Hz, 1H, Ar), 8.22 (d, J = 9 Hz, 1H, Ar), 9.86 (s, 1H, Ar), 11.8–13.3 (3H, CO_2H's and N^2H). Anal. ($C_{28}H_{22}N_4O_6S$·2H_2O) C, H, N, S.

Diethyl N-(4-(((1,2-Dihydro-1-oxo-3-pivalamidobenzo[*f*]-quinazolin-9-yl)methyl)amino)benzoyl)-L-glutamate (9h). To a hot solution of *N*-(9-methyl-1,2-dihydro-1-oxobenzo[*f*]-quinazolin-3-yl)pivalamide (**5**)[18] (0.94 g, 3.0 mmol) in benzene (250 mL) under nitrogen were added *N*-bromosuccinimide (0.57 g, 3.2 mmol) (Kodak) and 2,2'-azobisisobutyronitrile (AIBN) (35 mg, 0.21 mmol) (Kodak). The solution was stirred at reflux for 1.5 h and then concentrated *in vacuo* to give crude **7**. The pivalamide and **8a** (2.5 g, 7.8 mmol) (Aldrich) in DMF (10 mL) were stirred under nitrogen at 105–110 °C for 5 min and allowed to cool. Triethylamine (0.5 mL, 3.6 mmol) was added and the solution concentrated under vacuum. The residue was purified by chromatography on silica gel (130 g) eluting with ethyl acetate–methylene chloride (1:19 → 2:3), followed by recrystallization of the solid from ethanol–diethyl ether. This was filtered and dried under vacuum to give **9h** (0.32 g) as a white solid. The filtrate was concentrated and the residue purified by chromatography and crystallization to give an additional 0.243 g (total yield = 30%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.15 (t, J = 7 Hz, 3H, ester CH_3), 1.16 (t, J = 7 Hz, 3H, ester CH_3), 1.28 (s, 9H, t-Bu), 1.87–2.13 (m, 2H, Glu CH_2), 2.39 (t, J = 7 Hz, 2H, Glu CH_2), 4.03 (q, J = 7 Hz, 2H, ester CH_2), 4.07 (q, J = 7 Hz, 2H, ester CH_2), 4.30–4.40 (m, 1H, Glu CH), 4.57 (d, J = 6 Hz, 2H, C^9-CH_2), 6.63 (d, J = 9 Hz, 2H, Ar), 7.04 (t, J = 6 Hz, 1H, ArNH), 7.53 (d, J = 9 Hz, 1H, Ar), 7.61 (dd, J = 8, 2 Hz, 1H, Ar), 7.62 (d, J = 9 Hz, 2H, Ar), 7.99 (d, J = 8 Hz, 1H, Ar), 8.21 (d, J = 8 Hz, 1H, Glu NH), 8.22 (d, J = 9Hz, 1H, Ar), 9.75 (s, 1H, Ar), 11.25 (s, 1H, N^2H), 12.30 (br s, 1H, NH); mass spectrum (CI-CH_4) m/z 630 ((M + 1)$^+$, 6.5), 243 (100). Anal. ($C_{34}H_{39}N_5O_7$) C, H, N.

N-(4-(((3-Amino-1,2-dihydro-1-oxobenzo[*f*]quinazolin-9-yl)methyl)amino)benzoyl)-L-glutamic acid (3h). A solution of **9h** (0.31 g, 0.48 mmol) in methanol (15 mL) and 1 N NaOH (5 mL) was stirred under nitrogen at reflux for 1.5 h and then allowed to cool. The solution was adjusted under nitrogen to pH 3 with 1 N HCl, and the resulting precipitate was filtered under nitrogen, washed with water, and dried under vacuum to give **3h**

(0.22 g, 90%) as a white solid: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.82–2.12 (m, 2H, Glu CH_2), 2.31 (t, J = 7 Hz, 2H, Glu CH_2), 4.27–4.38 (m, 1H, Glu CH), 4.50 (d, J = 6 Hz, 2H, C^9-CH_2), 6.55 (br s, 2H, NH_2), 6.62 (d, J = 9 Hz, 2H, Ar), 6.96 (t, J = 6 Hz, 1H, ArNH), 7.26 (d, J = 9 Hz, 1H, Ar), 7.44 (dd, J = 8, 2 Hz, 1H, Ar), 7.63 (d, J = 9 Hz, 2H, Ar), 7.84 (d, J = 8 Hz, 1H, Ar), 7.99 (d, J = 9 Hz, 1H, Ar), 8.09 (d, J = 8 Hz, 1H, Glu NH), 9.67 (s, 1H, Ar), 11.14 (br s, 1H, N^2H), 12.32 (br s, 2H, CO_2H's). Anal. ($C_{25}H_{23}N_5O_6 \cdot H_2O$) C, H, N.

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