Benzoquinazoline Inhibitors of Thymidylate Synthase: Enzyme Inhibitory Activity and Cytotoxicity of Some Sulfonamidobenzoylglutamate and Related Derivatives

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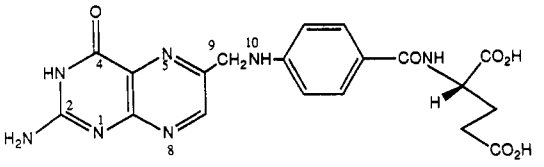
Several folate-like thymidylate synthase inhibitors are described in which the pteridine nucleus of the folic acid molecule is replaced by a benzoquinazoline moiety, which in turn is attached to the benzoylglutamate side chain by a sulfonamide link. The most potent compounds had K_i values as low as 2.5 nM against the human enzyme, were good substates for the cellular reduced folate transport system and for folylpolyglutamate synthetase, and had IC_{50} values for growth inhibition of tumor cell lines as low as 70 nM.

Variation of the C9–N10 link in analogues of folic acid (1) has been a fertile area for generating inhibitors of a number of folate-utilizing enzymes including dihydrofolate reductase (DHFR), thymidylate synthase (TS), and glycineamide ribonucleotide transformylase (GAR-T).[1] Thus in folate analogues of general type **2**, the homo (X–Y = CH_2CH_2NR),[2-4] iso (X–Y = $NRCH_2$),[5-10] and 10-deaza (X–Y = CH_2CH_2)[11-13] linkages, along with oxa[3,4,14,15] and thia variants[3,4,16,17] of these have often been profitably employed in attachment of a variety of heterocycles to the benzoylglutamate side chain. The sulfonamido (SO_2NR) link, however, has received relatively little attention, despite a report of the antimalarial activity of a series of 2,4-diamino-6-quinazolinesulfonamides **3**,[18] some of which were at least equipotent with cycloguanil or pyrimethamine; glutamate derivatives were not examined in the latter study, presumably because of the lack of any active folate transport mechanism in the target organisms.[19]



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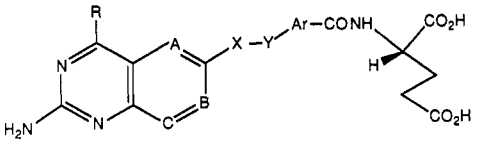
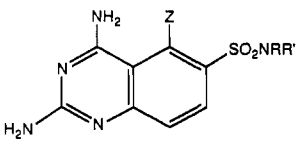


2: R = OH, NH_2;
A, B, C = CH, N;
X-Y = CH_2CH_2NR', $NR'CH_2$, CH_2CH_2, CH_2O, CH_2S.



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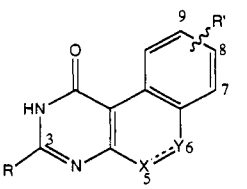
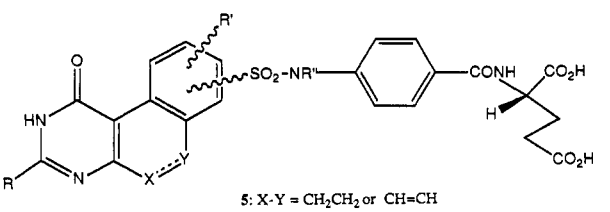
The enzyme thymidylate synthase (TS) catalyzes the transfer of a one-carbon unit from the pteridine cofactor 5,10-methylenetetrahydrofolic acid during the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) and represents the only *de novo* pathway for the synthesis of this essential component of DNA. We have previously shown that a series of unusual analogs of the cofactor, simple substituted benzoquinazoline derivatives of type **4**, had I_{50} values as low as 0.020 μM against human TS, but were relatively poor *in vitro* cytotoxic agents due to inefficient cell penetration.[20] The compounds were extremely insoluble, making them difficult to formulate for *in vivo* testing, and were poorly bioavailable. Therefore we set out to synthesize the sulfonamide-linked folate analogs **5** for the following reasons: (a) as part of a general strategy to improve the physical properties of the benzoquinazolines by substitution in the benzene moiety by hydrophilic groups; (b) to probe any specific (*p*-aminobenzoyl)-glutamate binding area at the enzyme active site, possibly in synergy with the already-potent binding ability of the benzoquinazoline moiety; and (c) to exploit the active folate transport processes of human tumor cells, and the activation and retention of the inhibitors via polyglutamylation, both of which mechanisms require a folate-like side chain in the substrate.[21]

Chlorosulfonylation of benzoquinazolines **4** followed by amidation with (*p*-aminobenzoyl)glutamate derivatives offered a convenient synthetic entry into this area. We here report the synthesis of several ((benzo[*f*]quinazolinesulfonamido)benzoyl)glutamates of general structure **5**, with potent thymidylate synthase activity, which, due to their ability to act as substrates for the reduced folate



4: X-Y = CH_2CH_2 or CH=CH



5: X-Y = CH_2CH_2 or CH=CH

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transport system and for folylpolyglutamate synthetase, also showed significant activity against human tumor cell lines.

Chemistry

The required (chlorosulfonyl)benzoquinazolines were prepared by reaction of the appropriate benzoquinazoline (**4a–e**) with chlorosulfonic acid. The chlorosulfonyl intermediates (**6a–e**)were treated with a diethyl L-(4-aminobenzoyl)glutamate derivative (**7a–c**), either by fusion with an excess of the amine, or in pyridine as solvent, followed by hydrolysis of the diesters (**8a–i**) in aqueous base to yield the free glutamic acid derivatives (**5a-i**)-(Scheme I).

Chlorosulfonylation of the 3-amino-5,6-dihydrobenzoquinazoline **4a** yielded exclusively the 8-substituted derivative **6a**, which was elaborated into the sulfonamidobenzoylglutamates **5a** and **5b** as shown in Scheme I. A 9-substituted derivative **5d** was obtained by blocking the 8-position with a bromo substituent, which was subsequently removed from the glutamate diester intermediate **8c** by hydrogenolysis over palladium, prior to hydrolysis of the diethyl glutamate **8d** to the free acid. In the 3-methyl-5,6-dihydro derivative **4c** the weaker electron-donating ability of the methyl substituent was considerably attenuated at the 8-position; consequently the 6-methylene group directed electrophilic attack exclusively to the 9-position to yield **6c**, ultimately leading to derivatives **5e** and **5f** by the sequence described above.

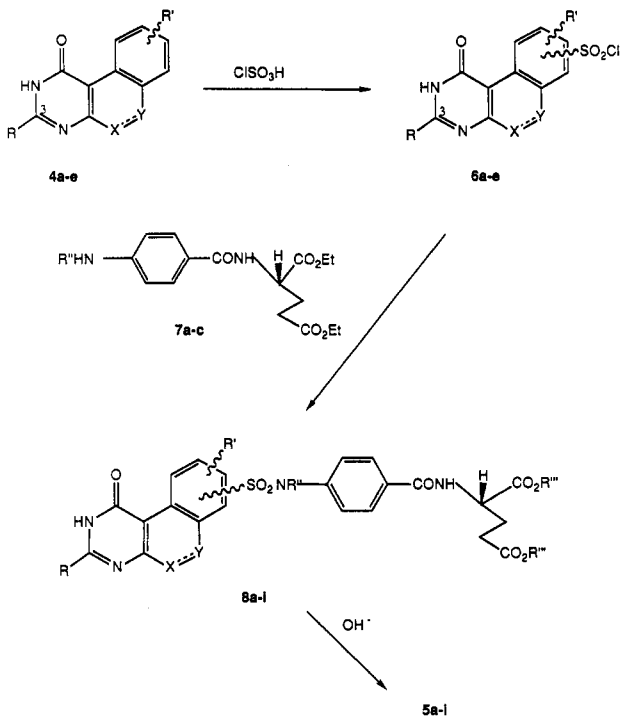
Preferably chlorosulfonation was carried out on the 5,6-dihydro compounds **4** rather than on the fully aromatic derivatives **5**, which were much less soluble and less selective in their substitution behavior.[20] However this lack of selectivity offered an opportunity in one case to obtain a 7-isomer which was not accessible via the dihydro route. Thus reaction of the fully aromatic 3-methyl compound **4d** with chlorosulfonic acid gave a mixture of chlorosulfonyl derivatives from which **6d** was not separated but treated directly with diethyl (*p*-aminobenzoyl)-glutamate; multiple chromatographic separations of the resulting mixture of glutamate diester derivatives gave a low yield of the 7-isomer **8g**. Although the corresponding 9-isomer **8h** was present in the mixture, the preferred route to this derivative was via catalytic dehydrogenation of the dihydro diester **8e** over a palladium–carbon catalyst in diglyme. The 9-bromo-8-sulfonamido derivative **5i** was also prepared through chlorosulfonylation of a fully aromatic derivative, 9-bromobenzoquinazoline **4e**.

Treatment of the 3-(pivaloylamino)-9-aminoquinazolinone **9** [20] with 4-(chlorosulfonyl)benzoic acid gave the pteroic acid analogue **10**; the acid was not purified but heated with methanolic hydrogen chloride solution which both esterified the carboxylate and removed the pivaloyl protecting group to yield **11**. Hydrolysis to the free acid **12**, coupling with L-diethyl glutamate to yield the diester **13**, followed by alkaline hydrolysis as above gave the desired 9-substituted "reversed-bridge" analogue **14** (Scheme II).

Biological Testing

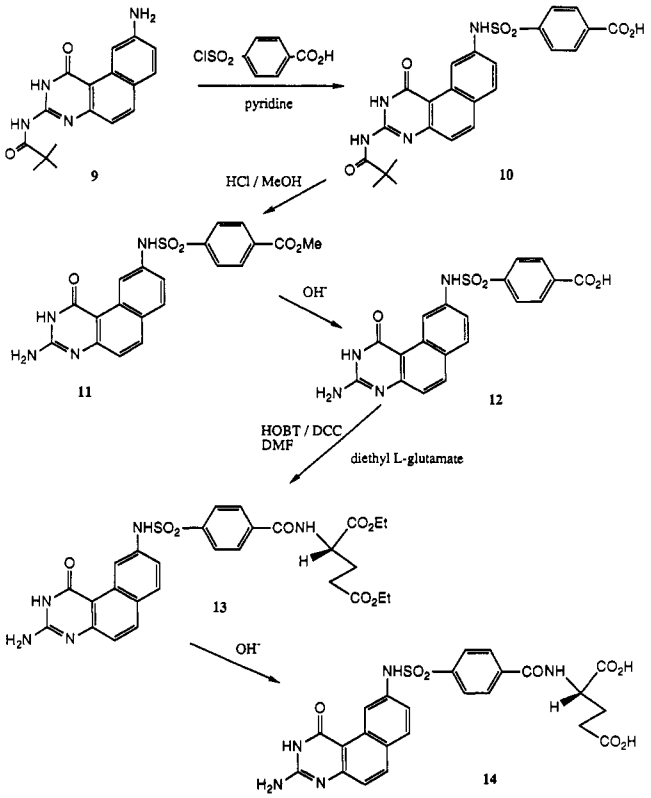
The target diacids (**5a–i** and **14**) were tested as inhibitors of purified human thymidylate synthase (TS) isolated from an *Escherichia coli* harboring a plasmid with *thy* A gene cloned from SV40 transformed human fibroblast cells.[22] The enzyme was assayed, and the extent of inhibition of the enzyme by the various compounds was determined by the tritium release assay of Roberts[23] as modified by Dev *et al.*[24]. Values of the inhibition constant (K_i) were estimated by the method of Henderson[25] for tight-binding noncompetitive inhibitors.

Scheme I



Cpd #	R	R'	R''	R'''	Position -SO_2-	X-Y
4a	NH_2	H	–	–	–	CH_2CH_2
4b	NH_2	8-Br	–	–	–	CH_2CH_2
4c	CH_3	H	–	–	–	CH_2CH_2
4d	CH_3	H	–	–	–	CH=CH
4e	NH_2	9-Br	–	–	–	CH=CH
5a	NH_2	H	H	H	8	CH_2CH_2
5b	NH_2	H	propargyl	H	8	CH_2CH_2
5c	NH_2	8-Br	H	H	9	CH_2CH_2
5d	NH_2	H	H	H	9	CH_2CH_2
5e	CH_3	H	H	H	9	CH_2CH_2
5f	CH_3	H	CH_3	H	9	CH_2CH_2
5g	CH_3	H	H	H	7	CH=CH
5h	CH_3	H	H	H	9	CH=CH
5i	NH_2	9-Br	H	H	8	CH=CH
6a	NH_2	H	–	–	8	CH_2CH_2
6b	NH_2	8-Br	–	–	9	CH_2CH_2
6c	CH_3	H	–	–	9	CH_2CH_2
6d	CH_3	H	–	–	7	CH=CH
6e	NH_2	9-Br	–	–	8	CH=CH
7a	–	–	H	Et	–	–
7b	–	–	propargyl	Et	–	–
7c	–	–	Me	Et	–	–
8a	NH_2	H	H	Et	8	CH_2CH_2
8b	NH_2	H	propargyl	Et	8	CH_2CH_2
8c	NH_2	8-Br	H	Et	9	CH_2CH_2
8d	NH_2	H	H	Et	9	CH_2CH_2
8e	CH_3	H	H	Et	9	CH_2CH_2
8f	CH_3	H	CH_3	Et	9	CH_2CH_2
8g	CH_3	H	H	Et	7	CH=CH
8h	CH_3	H	H	Et	9	CH=CH
8i	CH_3	9-Br	H	Et	8	CH=CH

Scheme II



For growth inhhibition studies, SW480 colon adenocarcinoma, MCF-7 breast adenocarcinoma, and MOLT-4 T-cell leukemia cells were maintained in folic acid-free RPMI 1640 (Gibco) containing (6*R*,*S*)-5-formyltetrahydrofolic acid (10 nM) as the folate source and 10% charcoal-dialyzed fetal bovine serum (JRH Biosciences). Determinations of cell culture cytotoxicity were carried out in 96-well plates using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye reduction assay as described previously.[26]

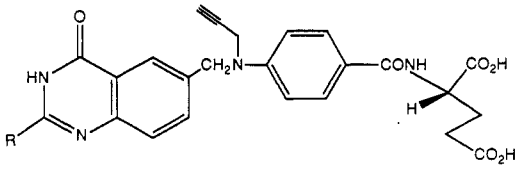
The ability of the compounds to enter tumor cells by the reduced folate transport system was assessed by measurement of their inhibition of the uptake by MOLT-4 T-cell leukemia cells of [^{3}H]methotrexate, itself a substrate for this transport system.[27] The compounds were also examined for their ability to function as substrates for partially purified hog liver folylpolyglutamate synthetase (FPGS)[28] to provide an estimate of their relative capacity for intracellular polyglutamylation. Dihydrofolate reductase inhibitory activity of **5e** was determined as previously described.[28]

Discussion

Attachment of the sulfonamidobenzoylglutamate side chain to the benzoquinazoline nucleus gave significant enhancements of aqueous solubility of these molecules. For example while the solubilities of precursor molecules **4c** and **4d** in 0.05 M phosphate buffer (pH 7) were each less than 0.1 mg/mL, solubilities of the corresponding sulfonamides **5e** and **5h** were 9.0 and 10.0 mg/mL, respectively.

In vitro activities of the benzoquinazoline inhibitors **5a–i** and **14** are shown in Table I. With the exception of the fully aromatic 9-bromo compound **5i**, introduction of the N-4′-unsubstituted sulfonamidobenzoylglutamate side chain into this series of benzoquinazolines gave significant increases in thymidylate synthase inhibitory activity (up to 2800-fold) compared with the precursor heterocyles; thus these inhibitors seem to probe an area of the enzyme capable of binding a folate cofactor-like (*p*-aminobenzoyl)-glutamate residue. The greater activity of the 9-substituted derivatives **5d** and **5h** compared with the 8- and 7-isomers **5a** and **5g**, respectively, paralleled the greater activity of the 9-substituted isomers in a series of compounds of type **4a** bearing compact lipophilic substituents in the corresponding benzene ring.[20] However an attempt to enhance the activity of an 8-sulfonamido compound by addition of a small lipophilic substituent in the 9-position was unsuccessful; TS inhibition by the 9-bromo-8-sulfonamido compound **5i** was 820-fold lower than that of the precursor benzoquinazoline (**4e**) and 290-fold lower than that of the 8-sulfonamido compound **5a**. In the absence of any X-ray data on the enzyme–inhibitor complex, it is not clear whether this is due to a constraint by the bromo substituent of an obligatory conformation of the side chain, an overcrowded pocket on the enzyme close to the 8- and 9- positions, or a reflection of a generally different mode of binding for the simple heterocycles from that of the derivatives with the folate-like side chain. In a detailed study, which will be published elsewhere,[29] of the kinetics of inhibition and equilibrium binding with various folate analogs, it was shown how the two catalytic sites of the TS dimer may each exhibit different binding affinities depending on the state of ligation of the other, and how this differential binding affects the observed kinetics. Inhibition of TS by compounds of type **5a–i** was noncompetitive with the folate cofactor, while inhibition by the simple derivatives of type **4** was mixed competitive/noncompetitive.

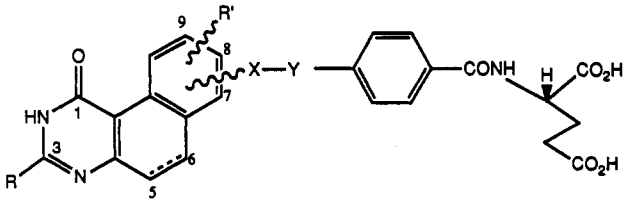
Replacement of the 3-amino group of **5d** by 3-methyl (**5e**) resulted in a 7-fold decrease in enzyme inhibitory activity. Cytotoxic potency, however, held steady (MCF-7) or showed a slight increase (2-fold on SW480, 12-fold on MOLT-4), a qualitatively similar result to that observed in a related series of quinazoline inhibitors (for **15**, I_{50}(TS) = 0.02 μM; IC_{50} (L1210 cells) = 3.4 μM: for **16**, I_{50}(TS) = 0.04 μM; IC_{50} (L1210 cells) = 0.09 μM).[30] The greater cytotoxic potency of 2-methylquinazolines of type **16** relative to the corresponding 2-amino derivatives **15** has been attributed to an increased affinity for the cellular reduced folate active transport system and/or enhanced intracellular polyglutamylation.[5] A greater affinity of the 3-methylbenzoquinazoline **5e** for this transporter relative to that of the corresponding amino compound **5d** was confirmed in this case; however, the lower activity of **5e** as a substrate for FPGS (4-fold decrease in V/K compared with that of **5d**) would suggest that intracellular polyglutamylation makes less of a contribution to the cytotoxicity of these dihydrobenzoquinazolines.



15: R = NH_2
16: R = CH_3

Aromatization of the 3-methyl-5,6-dihydro derivative **5e** to **5h** resulted in a 3-fold increase in TS activity, a trend we had previously found in many of the simple

Table I. *In Vitro* Activities of Sulfonamidobenzoquinazolines: Inhibition of Human Thymidylate Synthase (TS), Cell Culture Cytotoxicity (CCCT), Methotrexate Uptake (MTXupt), and Substrate Activity for Folylpolyglutamate Synthetase (FPGS)



compd	R	R'	bridge X–Y	K_i[a](TS) (μM)	CCCT[b] (μM) MCF-7	SW480	MOLT-4	MTXupt[c] K_i (μM) or % [at μM]	V_m rel %	FPGS[d] K_m (μM)	V/K
					5,6-Dihydro						
5a	NH_2	H	8-SO_2NH	0.057	25	nd[e]	nd	23 [30]	102	1.8	56.5
5b	NH_2	H	8-SO_2 Npropargyl	0.66	>50	>50	>50	30 [30]	nd	nd	
5c	NH_2	8-Br	9-SO_2NH	0.079	>100	>100	nd	0 [10]	28.9	nd	
5d	NH_2[f]	H	9-SO_2NH	0.0025	0.65	7.9	2.4	42	115	7.0	16.4
5e	CH_3[f]	H	9-SO_2NH	0.018	0.65	4.1	0.2	15	109	26.8	4.1
5f	CH_3	H	9-SO_2NMe	16.7	>50	>50	nd	1.8	nd	nd	
					Fully Aromatic						
5g	CH_3	H	7-SO_2NH	7.37	50	>100	nd	72 [30]	55.2	7.6	7.3
5h	CH_3[f]	H	9-SO_2NH	0.0055	0.07	2.4	0.12	4.9	185	3.11	59.6
5i	NH_2	9-Br	8-SO_2NH	16.4	>50	>50	nd	45 [30]	nd	nd	
					Reversed Bridge						
14	NH_2	H	9-$NHSO_2$	0.1	nd	>100	nd	42 [30]	nd	nd	
					Parent Benzoquinazolines (for Comparison)						
4a	NH_2	H		7[g]	>100						
4b	NH_2	8-Br		9[g]							
4c	CH_3	H		35.6[g]	>100						
4d	CH_3[h]	H		5.4[g]	>100						
4e	NH_2[h]	9-Br		0.02[g]	10						

[a] Inhibition constant *vs* purified recombinent human TS. [b] Concentration for 50% reduction in the growth rate upon continuous drug exposure for 72 h (SW480 and MOLT-4) 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] nd = not determined. [f] Reference 33. [g] I_{50} values from ref 20. [h] Fully aromatic.

benzoquinazolines.[20] The even greater increase in cytotoxicity (up to 9.5-fold) suggested that there might also be a positive effect of aromatization on cellular uptake, and indeed the greater affinity for the transporter and the enhanced substrate activity for FPGS (15-fold increase in V/K) support this.

Alkyl substitution on the *p*-ABA nitrogen (**5a** *vs* **5b** and **5e** *vs* **5f**) was detrimental to both enzymic and cytotoxic activity, which contrasts sharply with observations in the aforementioned series of quinazoline inhibitors; similar substitutions in compounds of type **15** and **16** resulted in increases in TS activity of up to 120-fold relative to the corresponding bridge-NH derivatives,[5,31] though gains in cytotoxicity were much less.[30,31]

In those cases where an $IC_{50} < 50$ μM was determined (Table I), cytotoxicity in purine- and pyrimidine-free media was shown to be completely reversed by thymidine alone (20 μM), indicating TS as the sole locus of action. One of the compounds (**5e**) was shown to be a poor inhibitor of human dihydrofolate reductase ($I_{50} = 8.4 \times 10^{-4}$ M).

TS inhibitory activities of the more potent compounds in Table I are comparable with that of *N*-((5-(((3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl)methylamino)-2-thienyl)carbonyl)-L-glutamic acid[32] (ICI D1694 (K_i = 60 nM)), but the latter was up to 7000-fold more inhibitory of cell growth than the most potent of the sulfonamides, **5h**. A comparison of **5d**, **5e**, and **5h** with the aminomethylene-linked benzoquinazoline derivative (*S*)-2-(5-(((1,2-dihydro-3-methyl-1-oxobenzo[*f*]quinazolin-9-yl)methyl)-amino)-1-oxo-2-isoindolinyl)glutaric acid(BW1843U89) showed that the latter was not only a more potent inhibitor of TS (I_{50}(TS) = 0.09 nM), but by virtue of its superior uptake was also a more potent inhibitor of cell growth (IC_{50}(MCF-7) = 0.2 nM; SW480 = 0.7 nM).[33] Synthetic studies directed toward optimization of the side-chain structure of benzoquinazolines of the latter type will be described in the next paper in this series.

Conclusions

Attachment of the sulfonamidobenzoylglutamate side chain onto the benzoquinazoline nucleus resulted in significant increases in TS inhibitory activity and tumor cell growth inhibition compared with the parent heterocycle.[20] Though some attenuation of TS inhibition in these sulfonamides was observed on exchanging a 3-amino substituent for 3-methyl, the effect on cytotoxicity was more than offset by enhanced cellular uptake. Alkylation of the sulfonamide nitrogen was detrimental to TS and cell growth inhibition, in contrast with recent observations in a series of quinazoline-based folate analogs.[5,31] Oxidation of a 3-methyl-5,6-dihydro derivative (**5e**) to the corresponding fully aromatic compound **5h** enhanced not only TS inhibition, but also uptake by the reduced folate transporter, and intracellular polyglutamylation, resulting in an IC_{50} for tumor cell growth inhibition (MCF-7 cells) of 70 nM.

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. Mass spectra were determined by Oneida Research Services, Whitesboro, NY, on a Finnegan 4500 instrument. Analytical samples of intermediates moved as single spots on TLC and were run on Whatman MK6F silica gel plates. The

diesters **8a–i** and **13** were rigorously purified prior to hydrolysis in dilute sodium hydroxide, allowing the corresponding free acids to be isolated without further purification. Column chromatography was carried out on silica gel 60 (E. Merck, Darmstadt, Germany). The sulfonamides generally did not have sharp melting points, but decomposed gradually above 220 °C. They were also very tenacious of water and alcohols of crystallization, and in cases where the analysis indicated the presence of these solvents, the ^{1}H NMR spectrum in rigorously-dried DMSO-d_6 also showed the presence of these substances. Analyses were performed by Atlantic Microlab, Inc.; all values were within 0.4% of theory.

3-Amino-1,2,5,6-tetrahydro-1-oxobenzo[*f*]quinazoline-8-sulfonyl Chloride(6a). Chlorosulfonic acid (25 mL, Aldrich), cooled to 5°C in ice, was stirred during its addition to 3-amino-1,2,5,6-tetrahydro-1-oxobenzo[*f*]quinazoline (**4a**) (5 g, 0.025 mol) contained in a beaker immersed in an ice bath. The solution was removed from the ice bath, stirred for a further 20 min, and then poured onto ice (1000 g). The solid product was removed by filtration, washed with water, and dried under high vacuum at room temperature. The sulfonyl chloride (5.1 g) was used without further purification.

3-Amino-8-bromo-1,2,5,6-tetrahydro-1-oxobenzo[*f*]quinazoline-9-sulfonyl Chloride (6b). Chlorosulfonic acid (100 g) was added to 3-amino-8-bromo-1,2,5,6-tetrahydro-1-oxobenzo[*f*]-quinazoline (**4b**) (5.20 g, 17.8 mmol), and the solution was stirred overnight at room temperature. The reaction mixture was poured over ice, and the collected solid was washed with water and dried under high vacuum to give the sulfonyl chloride **6b** (7.25 g, 90%). ^{1}H NMR DMSO-d_6, 200 MHz): δ 2.67–2.75 (m, 2H, Ar CH_2), 2.81–2.88 (m, 2H, Ar CH_2), 7.43 (s, 1H, Ar), 8.23 (br s, 2H, NH_2), 8.91 (s, 1H, Ar), 9.90–10.50 (v br s, 1H, NH). Anal. ($C_{12}H_9N_3O_3S\cdot 0.5H_2O\cdot 0.45H_2SO_4$) C, H, N.

1,2,5,6-Tetrahydro-3-methyl-1-oxobenzo [*f*]quinazoline-9-sulfonyl Chloride (6c). 1,2,5,6-tetrahydro-3-methyl-1-oxobenzo[*f*]quinazoline (**4c**) (5 g, 0.024 mol) was added to chlorosulfonic acid (50 mL, Aldrich) and stirred for 12 h at room temperature. The reaction mixture was poured over ice (750 g), and the dark brown solid was collected by filtration. The solid was washed with water and suspended in water (500 mL), and the pH of the suspension was adjusted to 5.00 by addition of sodium bicarbonate. The suspension was filtered, and the product was washed with water and dried under high vacuum at room temperature to give 1,2,5,6-tetrahydro-3-methyl-1-oxobenzo[*f*]-quinazoline-9-sulfonyl chloride as a light brown solid (3.054 g, 41%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.60 (s, 3H, CH_3), 2.90 (s, 4H, Ar CH_2), 7.22 (d, J = 9 Hz, 1H, Ar), 7.53 (d, J = 9 Hz, 1H, Ar), 8.75 (s, 1H, Ar).

Diethyl *N*-(4-(3-Amino-1,2,5,6-tetrahydro-1-oxobenzo[*f*]-quinazoline-8-sulfonamido)benzoyl)-L-glutamate (8a). Diethyl *N*-(4-aminobenzoyl)-L-glutamate (**7a**) (3.2 g, 0.01 mol) (Aldrich) and 3-amino-1,2,5,6-tetrahydro-1-oxobenzo[*f*]quinazoline-8-sulfonyl chloride (**6a**) (0.62 g, 0.002 mol) were fused at 150 °C until a clear melt was obtained (~10 min). A solution of the crude product in methylene chloride was subjected to chromatography on silica, eluting with methanol–methylene chloride (1:4). Fractions containing the product were evaporated, and the residue was recrystallized from ethanol and dried under high vacuum to yield the diethyl ester **8a** (0.48 g, 40.2% based on sulfonyl chloride). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.00–1.2 (overlapping t, 6H, CH_2CH_3), 1.88–2.15 (m, 2H, Glu CH_2), 2.32–2.45 (m, 2H, Glu CH_2), 2.45–2.60 (m, 2H, $ArCH_2$), 2.72–2.86 (m, 2H, $ArCH_2$), 3.92–4.12 (overlapping q, 4H, CH_2CH_3), 4.26–4.11 (m, 1H, Glu CH), 6.66–7.00 (br s, 2H, NH_2), 7.16 (d, J = 8.6 Hz, 2H, Ar), 7.52–7.63 (m, 2H, Ar), 7.71 (d, J = 8.6 Hz, 1H, Ar), 8.54 (d, J = 7.2 Hz, 1H, Glu NH), 8.54 (d, J = 9 Hz, 1H, Ar), 10.55 (br s, 1H, SO_2NH), 11.03 (br s, 1H, N^2H). Anal. ($C_{28}H_{31}N_5O_8S$) C, H, N, S.

N-(4-(3-Amino-1,2,5,6-tetrahydro-1-oxobenzo[*f*]quinazoline-8-sulfonamido)benzoyl)-L-glutamic Acid (5a). A solution of the diester **8a** (0.15 g, 7.6 mmol) in a mixture of 2 N NaOH (3 mL) and ethanol (6 mL) was stored at room temperature for 14 h. The ethanol was evaporated and the pH of the solution adjusted to 2 with 1 N HCl. The solid was collected by filtration, washed with water, and dried at 60 °C under vacuum. The product was crystallized from ethanol and redried as above to yield a white solid (0.12 g, 85%). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.75–2.18 (m, 2H, Glu CH_2), 2.20–2.40 (m, 2H, Glu CH_2), 2.54–2.64 (m, 2H, $ArCH_2$), 2.70–2.89 (m, 2H, $ArCH_2$), 4.23–4.40 (m, 1H, Glu CH), 6.70–7.08 (br s, 2H, NH_2), 7.16 (d, J = 8.64 Hz, 2H, Ar), 7.56–7.61 (m, 2H, Ar), 7.72 (d, J = 8.63 Hz, 2H, Ar), 8.42 (d, J = 7.81 Hz, 1H, Glu NH), 8.54 (d, J = 8.99 Hz, 1H, Ar), 10.55 (s, 1H, SO_2NH), 10.91–11.24 (br s, 1H, N^2H), 11.98–12.63 (v br s, 2H, CO_2H), shows presence of water and EtOH. Anal. ($C_{24}H_{23}N_5O_8S\cdot 0.6H_2O\cdot 0.2EtOH$) C, H, N, S.

A similar reaction of **diethyl *N*-(4-(prop-2-ynylamino)-benzoyl)-L-glutamate (7b)**[34] with the sulfonyl chloride (**7a**) and subsequent hydrolysis of the diester intermediate (**8b**) gave ***N*-(4-((((3-amino-1,2,5,6-tetrahydro-1-oxobenzo[*f*]quinazolin-8-yl)sulfonyl)(prop-2-ynyl)amino)benzoyl)-L-glutamic acid (5b)** (8.3% from sulfonyl chloride). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.82–2.20 (m, 2H, Glu CH_2), 2.30–2.38 (m, 2H, Glu CH_2), 2.56–2.65 (m, 2H, $ArCH_2$), 2.83–2.87 (m, 2H, $ArCH_2$), 3.23 (t, J = 2 Hz, 1H, propynyl CH), 4.34–4.40 (m, 1H, Glu CH), 4.53 (d, J = 2 Hz, 2H, propynyl CH_2), 7.03 (br s, 2H, NH_2), 7.29 (d, J = 8.6 Hz, 2H, Ar), 7.37 (dd, J = 2.5, 8.6 Hz, 1H, Ar), 7.45 (d, J = 2.5 Hz, 1H, Ar), 7.83 (d, J = 8.6 Hz, 2H, Ar), 8.56 (d, J = 8.6 Hz, 1H, Ar), 8.64 (d, J = 7.8 Hz, 1H, Glu NH), 10.94–11.56 (v br s, 1H, N^2H), 11.80–12.90 (v br s, 1H, CO_2H), shows presence of H_2O. Anal. ($C_{27}H_{25}N_5O_8S\cdot 2.3H_2O$) C, H, N.

Diethyl *N*-(4-(((3-Amino-8-bromo-1,2,5,6-tetrahydro-1-oxobenzo[*f*]quinazolin-9-yl)sulfonyl)amino)benzoyl)-L-glutamate (8c). A mixture of the sulfonyl chloride (**6b**) (2.00 g, 4.50 mmol) and the amine **7a** (7.26 g, 22.5 mmol) (Aldrich) was melted at 175°C. The mixture was heated for 80 min, and the cooled residue was suspended in methylene chloride and filtered to remove undissolved solid. The filtrate was evaporated to dryness, and the residue was subjected to chromatography on a Waters Prep 500 instrument (silica column, eluting with methanol/methylene chloride (1:24)). The combined fractions containing product were evaporated, and the residue was dried under high vacuum. The solid was suspended in boiling ethanol (900 mL), and the suspension was cooled to room temperature and filtered to give **8c** (0.794 g, 26%). Mp > 250 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.12 (t, J = 7 Hz, 3H, ester CH_3), 1.14 (t, J = 7 Hz, 3H, ester CH_3), 1.89–2.09 (m, 2H, Glu CH_2), 2.37 (t, J = 7 Hz, 2H, Glu CH_2), 2.48–2.59 (m, 2H, Ar CH_2), 2.77–2.85 (m, 2H, Ar CH_2), 3.99 (q, J = 7 Hz, 2H, ester CH_2), 4.05 (q, J = 7 Hz, 2H, ester CH_2), 4.29–4.40 (m, 1H, Glu CH), 6.84 (br s, 2H, NH_2), 7.14 (d, J = 9 Hz, 2H, Ar), 7.54 (s, 1H, Ar), 7.69 (d, J = 9 Hz, 2H, Ar), 8.48 (d, J = 8 Hz, 1H, NH), 9.37 (s, 1H, Ar), 10.87 (br s, 1H, NH), 11.04 (br s, 1H, NH). Anal. ($C_{28}H_{30}BrN_5O_8S\cdot 0.25H_2O$) C, H, Br, N, S.

Diethyl *N*-(4-(((3-Amino-1,2,5,6-tetrahydro-1-oxobenzo-[*f*]quinazolin-9-yl)sulfonyl)amino)benzoyl)-L-glutamate (8d). The foregoing bromo derivative (**8c**) (0.3 g, 0.44 mmol) was dissolved in boiling ethanol (250 mL), the solution was cooled to room temperature, and 10% palladium on carbon (0.20 g) was added. The mixture was shaken under a hydrogen atmosphere for 35 h. Additional 10% palladium on carbon (0.20 g) was added to the reaction mixture which was shaken under hydrogen for a further 15 h. Ethanol (750 mL) was added, and the reaction mixture was heated to reflux and filtered while hot through Celite. Water (33 mL) was added, and the solution was neutralized with ammonium hydroxide. The solvent was removed in vacuo, the solid suspended in water, and the pH of the mixture adjusted to 7 with dilute acetic acid and dilute ammonium hydroxide. The resulting solid was collected by filtration and air-dried. The crude product was passed through a pad of silica gel, eluting with methanol–methylene chloride. Combined fractions containing product were evaporated, and the solid residue was suspended in a small amount of methanol, filtered, and dried under high vacuum to give the diester **8d** (0.095 g). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.12 (t, J = 7 Hz, 3H, CH_3), 1.14 (t, J = 7 Hz, 3H, CH_3), 1.85–2.15 (m, 2H, Glu CH_2), 2.38 (t, J = 7 Hz, 2H, Glu CH_2), 2.48–2.61 (m, 2H, Ar CH_2), 2.75–2.87 (m, 2H, Ar CH_2), 4.00 (q, J = 7 Hz, 2H, ester CH_2), 4.06 (q, J = 7 Hz, 2H, ester CH_2), 4.28–4.42 (m, 1H, Glu CH), 6.78 (br s, 2H, NH_2), 7.16 (d, J = 9 Hz, 2H, Ar), 7.27 (d, J = 8 Hz, 1H, Ar), 7.46 (dd, J = 8, 2 Hz, 1H, Ar), 7.70 (d, J = 9 Hz, 2H, Ar), 8.52 (d, J = 7 Hz, 1H, Glu NH), 9.06 (d, J = 2 Hz, 1H, Ar), 10.66 (br s, 1H, SO_2NH), 11.03 (br s, 1H, N^2H). Anal. ($C_{28}H_{31}N_5O_8S$) C, H, N.

N-(4-(3-Amino-1,2,5,6-tetrahydro-1-oxobenzo[f]quinazoline-9-sulfonamido)benzoyl)-L-glutamic acid (5d) (0.043 g, 52%) was obtained by hydrolysis of the foregoing diester (0.088 g, 0.15 mmol) in sodium hydroxide as described for **5a** above. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.74–2.16 (m, 2H, Glu CH_2), 2.29 (t, J = 7 Hz, 2H, Glu CH_2), 2.48–2.60 (m, 2H, $ArCH_2$), 2.72–2.86 (m, 2H, $ArCH_2$), 4.23–4.38 (m, 1H, Glu CH), 6.78 (br s, 2H, NH_2), 7.16 (d, J = 9 Hz, 2H, Ar), 7.26 (d, J = 8 Hz, 1H, Ar), 7.45 (dd, J = 8, 2 Hz, 1H, Ar), 7.70 (d, J = 9 Hz, 2H, Ar), 8.42 (br d, J = 8 Hz, 1H, Glu NH), 9.06 (d, J = 2 Hz, 1H, Ar), 10.65 (br s, 1H, SO_2NH), 11.05 (br s, 1H, N^2H), 12.33 (br s, 2H, CO_2H). Anal. ($C_{24}H_{23}N_5O_8S·H_2O$) C, H, N.

Diethyl N-(4-(1,2,5,6-Tetrahydro-3-methyl-1-oxobenzo[f]quinazoline-9-sulfonamido)benzoyl)-L-glutamate (8e). *N*-(4-Aminobenzoyl)-L-glutamic acid diethyl ester (6.06 g, 0.0188 mol) (Aldrich) and the sulfonyl chloride **6c** (5.84 g, 0.0188 mol) were dissolved in pyridine (55 mL), and the reaction mixture was stirred at room temperature for 3.5 h. The pyridine was removed *in vacuo*, the residue was washed with water, and the pink solid was collected by filtration. The crude product was dried under high vacuum, and then subjected to chromatography on a Waters Prep 500 instrument (silica cartridge, eluting with methanol–methylene chloride (1:4). The product was recrystallized from ethanol and dried under high vacuum to yield the diethyl ester **8e** (5.68 g, 51%). ^{1}H NMR (DMSO-d_6,300 MHz): δ 1.14 (t, J = 7 Hz, 3H, CH_2CH_3), 1.16 (t, J = 7 Hz, 3H, CH_2CH_3), 1.88–2.13 (m, 2H, Glu CH_2), 2.32 (s, 3H, CH_3), 2.40 (t, J = 8 Hz, 2H, Glu CH_2), 2.71 (m, 2H, Ar CH_2), 2.89 (m, 2H, Ar CH_2), 4.02 (q, J = 7 Hz, 2H, CH_2CH_3), 4.08 (q, J = 7 Hz, 2H, CH_2CH_3), 4.33–4.41 (m, 1H, CH), 7.20 (d, J = 9 Hz, 2H, Ar), 7.39 (d, 1H, J = 8 Hz, Ar), 7.62 (dd, J = 8, 2 Hz, Ar), 7.73 (d, J = 9 Hz, 2H, Ar), 8.55 (d, J = 8 Hz, 1H, Glu NH), 9.21 (d, 2 Hz, 1H, Ar), 10.74 (s, 1H, NH), 12.72 (s, 1H, NH). Anal. ($C_{29}H_{32}N_4O_8S·0.1EtOH·0.75H2O$) C, H, N, S.

N-(4-(1,2,5,6-Tetrahydro-3-methyl-1-oxobenzo[f]quinazoline-9-sulfonamido)benzoyl)-L-glutamic Acid (5e). The diester **9e** (4.53 g, 7.6 mmol) was dissolved in in 1 N NaOH (64 mL), and the solution was stirred at room temperature for 4 h. The pH of the solution was adjusted to 3.00 with 1 N HCl, and the solid was collected by filtration, washed with water, and dried under high vacuum to yield **10e** as an off-white solid (3.94 g, 96%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.84–1.96 (m, 1H, Glu CH), 2.00–2.10 (m, 1H, Glu CH), 2.32 (s, 3H, CH_3, superimposed over t, 2H, Glu CH_2), 2.71 (m, 2H, Ar CH_2), 2.89 (m, 2H, Ar CH_2), 4.28–4.36 (m, 1H, Glu CH), 7.20 (d, J = 9 Hz, 2H, Ar), 7.39 (d, J = 8 Hz, 1H, Ar), 7.62 (dd, J = 8, 2 Hz, 1H, Ar), 7.74 (d, J = 9 Hz, 2H, Ar), 8.44 (d, J = 8 Hz, 1H, Glu NH), 9.21 (d, J = 2 Hz, 1H, Ar), 10.73 (s, 1H, SO_2NH), 12.36 (br s, 2H, CO_2H), 12.72 (br s, 1H, NH). Anal. ($C_{25}H_{24}N_4O_8S·1.5H_2O$) C, H, N, S.

A similar sequence of reactions using diethyl *N*-[4-(methylamino)benzoyl]glutamate (**7c**)[35] (2.0 g, 6.0 mmol) with the sulfonyl chloride **6c** (2.0 g, 6.4 mmol) gave the corresponding product bearing a methyl substituent on the sulfonamido nitrogen atom; data on the product and intermediate are given below.

Diethyl N-(4-(methyl((1,2,5,6-tetrahydro-3-methyl-1-oxobenzo[f]quinazolin-9-yl)sulfonyl)amino)benzoyl)-L-glutamate (8f) (1.47 g, 40%). Mp: 168–170.5 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.14 (t, J = 7 Hz, 3H, ester CH_3), 1.16 (t, J = 7 Hz, 3H, ester CH_3), 1.85–2.20 (m, 2H, Glu CH_2), 2.30 (s, 3H, C^3-CH_3), 2.42 (t, J = 7 Hz, 2H, Glu CH_2), 2.67–2.80 (m, 2H, $ArCH_2$), 2.85–2.99 (m, 2H, $ArCH_2$), 3.17 (s, 3H, NCH_3), 4.02 (q, J = 7 Hz, 2H, ester CH_2), 4.08 (q, J = 7 Hz, 2H, ester CH_2), 4.32–4.48 (m, 1H, Glu CH), 7.22 (dd, J = 8, 2 Hz, 1H, Ar), 7.28 (d, J = 9 Hz, 2H, Ar), 7.38 (d, J = 8 Hz, 1H, Ar), 7.82 (d, J = 9 Hz, 2H, Ar), 8.73 (d, J = 7 Hz, 1H, Glu NH), 9.00 (d, J = 2 Hz, 1H, Ar), 12.68 (br s, 1H, N^2H). Anal. ($C_{30}H_{34}N_4O_8S·0.33H_2O$) C, H, N, S.

N-(4-(Methyl((1,2,5,6-tetrahydro-3-methyl-1-oxobenzo[f]quinaolin-9-yl)sulfonyl)amino)benzoyl)-L-glutamic Acid (5f) (0.89 g, 99% from diester **9f** (1.0 g, 1.6 mmol)). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.80–2.20 (m, 2H, Glu CH_2), 2.31 (s, 3H, C^3-CH_3), 2.34 (t, J = 7 Hz, 2H, Glu CH_2), 2.67–2.80 (m, 2H, $ArCH_2$), 2.85–2.98 (m, 2H, $ArCH_2$), 3.17 (s, 3H, NCH_3), 4.30–4.43 (m, 1H, Glu CH), 7.22 (dd, J = 8, 2 Hz, 1H, Ar), 7.28 (d, J = 9 Hz, 2H, Ar), 7.38 (d, J = 8 Hz, 1H, Ar), 7.83 (d, J = 9 Hz, 2H, Ar), 8.62 (d, J = 8 Hz, 1H, Glu NH), 9.00 (d, J = 2 Hz, 1H, Ar), 12.39 (br s, 2H, CO_2H's), 12.69 (br s, 1H, N^2H). Anal. ($C_{26}H_{26}N_4O_8S·0.15H_2O$) C, H, N, S.

Diethyl N-(4-(1,2-Dihydro-3-methyl-1-oxobenzo[f]quinazoline-9- sulfonamido)benzoyl)-L-glutamate (8h). A solution of diethyl *N*-(4-(1,2,5,6-tetrahydro-3-methyl-1-oxobenzo[*f*]quinazoline-9-sulfonamido)benzoyl)-L-glutamate (0.50 g, 0.84 mmol) in diglyme (10 mL) was stirred with 10% palladium on carbon (0.25 g) (Aldrich) under nitrogen at reflux for 3 h. The solution was diluted with diglyme (20 mL), filtered hot through Celite, and concentrated under high vacuum. The resulting solid was suspended in hot methanol (50 mL), stirred overnight at room temperature, filtered, and dried under high vacuum to give the fully aromatic derivative **8h** as a white solid (0.25 g). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.10 (t, J = 7 Hz, 3H, ester CH_3), 1.12 (t, J = 7 Hz, 3H, ester CH_3), 1.78–2.15 (m, 2H, Glu CH_2), 2.35 (t, J = 7 Hz, 2H, Glu CH_2), 2.43 (s, 3H, C^3-CH_3), 3.98 (q, J = 7 Hz, 2H, ester CH_2), 4.04 (q, J = 7 Hz, 2H, ester CH_2), 2.35–4.39 (m, 1H, Glu CH), 7.21 (d, J = 9 Hz, 2H, Ar), 7.69 (d, J = 9 Hz, 2H, Ar), 7.76 (d, J = 9 Hz, 1H, Ar), 7.91 (dd, J = 9, 2 Hz, 1H, Ar), 8.19 (d, J = 9 Hz, 1H, Ar), 8.29 (d, J = 9 Hz, 1H, Ar), 8.51 (d, J = 7 Hz, 1H, Glu NH), 10.44 (d, J = 2 Hz, 1H, Ar), 10.88 (br s, 1H, SO_2NH), 12.75 (br s, 1H, N^2H). Mass spectrum (CI-CH_4): m/z 595 ($(M + 1)^+$, 24.1). Anal. ($C_{29}H_{30}N_4O_8S$) C, H, N, S.

N-(4-(1,2-Dihydro-3-methyl-1-oxobenzo[f]quinazoline-9-sulfonamido)benzoyl)-L-glutamic Acid (5h). A solution of the foregoing diester **8h** (0.22 g, 0.37 mmol) in ethanol (3 mL) and 0.25 N NaOH (12 mL) was stirred at room temperature for 3 h. The solution was slowly acidified to pH 3 with 1 N HCl, and the resulting precipitate was filtered, washed with water, and dried under high vacuum to give the diacid **5h** as a white solid (0.20 g). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.73–2.15 (m, 2H, Glu CH_2), 2.27 (t, J = 7 Hz, 2H, Glu CH_2), 2.43 (s, 3H, CH_3), 4.22–4.36 (m, 1H, Glu CH), 7.20 (d, J = 9 Hz, 2H, Ar), 7.69 (d, J = 9 Hz, 2H, Ar), 7. 76 (d, J = 9 Hz, 1H, Ar), 7.90 (dd, J = 9, 2 Hz, 1H, Ar), 8.18 (d, J = 9 Hz, 1H, Ar), 8.28 (d, J = 9 Hz, 1H, Ar), 8.39 (d, J = 8 Hz, 1H, Glu NH), 10.44 (d, J = 2 Hz, 1H, Ar), 10.86 (br s, 1H, SO_2NH), 12.32 (br s, 2H, CO_2H's), 12.75 (br s, 1H, N^2H). Anal. ($C_{25}H_{22}N_4O_8S·0.8H_2O$) C, H, N, S.

Diethyl N-(4-(1,2-Dihydro-3-methyl-1-oxobenzo[f]quinazoline-7-sulfonamido)benzoyl)-L-glutamate (8g). 3-Methylbenzo[*f*]quinazolin-1(2*H*)-one (**4d**) (2.6 g, 12.4 mmol) was treated with chlorosulfonic acid (15 mL) essentially as described for the analogous 3-amino-5,6-dihydro compound (**4a**) above to obtain a mixture of 1,2-dihydro-3-methyl-1-oxobenzo[*f*]quinazoline-7-, 8-, and -9-sulfonyl chlorides (2.91 g). The crude sulfonyl chlorides were added to a solution of *N*-(4-aminobenzoyl)-L-glutamic acid diethyl ester (3.85 g, 11.9 mmol) in dry pyridine (30 mL) and stirred under a nitrogen atmosphere at room temperature for 19 h. The solvent was evaporated under reduced pressure to leave a gummy residue which was suspended in water (100 mL) with vigorous stirring and sonication, filtered, and dried. The crude mixture of products was subjected to six successive silica gel column chromatography separations using methanol–methylene chloride (1:24 to 3:47) to obtain the 7-isomer **8g** as an off-white solid (0.22 g, 3%). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.10 (t, J = 7 Hz, 3H, ester CH_3), 1.12 (t, J = 7 Hz, 3H, ester CH_3), 1.80–2.15 (m, 2H, Glu CH_2), 2.35 (t, J = 7.6 Hz, 2H, Glu CH_2), 2.43 (s, 3H, pyr CH_3), 3.98 (q, J = 7 Hz, 2H, ester CH_2), 4.03 (q, J = 7 Hz, 2H, ester CH_2), 4.32 (m, 1H, Glu CH), 7.08 (d, J = 8.6 Hz, 2H, Ar), 7.64 (d, J = 8.4 Hz, 2H, Ar), 7.83 (t, J = 8.2 Hz, 1H, Ar), 7.86 (d, J = 8.6 Hz, 1H, Ar), 8.33 (d, J = 7.5 Hz, 1H, Glu-NH), 8.48 (d, J = 7.3 Hz, 1H, Ar), 9.06 (d, J = 9.3 Hz, 1H, Ar), 10.18 (d, J = 8.6 Hz, 1H, Ar), 11.15 (br s, 1H, NH), 12.73 (br s, 1H, NH). Anal. ($C_{29}H_{30}N_4O_8S·0.25H_2O$) C, H, N, S.

N-(4-(1,2-Dihydro-3-methyl-1-oxobenzo[f]quinazoline-7-sulfonamido)benzoyl)-L-glutamic Acid (5g). A solution of diethyl *N*-(4-(1,2-dihydro-3-methyl-1-oxobenzo[*f*]quinazoline-7-sulfonamido)benzoyl)-L-glutamate (0.16 g, 0.3 mmol) in 0.1 N NaOH (10 mL) was stirred at room temperature under a nitrogen atmosphere for 48 h, and then the solution was acidified (pH 3.5) with acetic acid. The precipitate was collected, washed with water, and dried to give *N*-(4-(1,2-dihydro-3-methyl-1-oxobenzo[*f*]quinazoline-7-sulfonamido)benzoyl)-L-glutamic acid as an off-white solid (0.12 g, 86%). ^{1}H NMR (DMSO-d_6, 200 MHz): δ

1.72–2.08 (m, 2H, Glu CH_2), 2.26 (t, J = 7 Hz, 2H, Glu CH_2), 2.43 (s, 3H, CH_3), 4.18–4.35 (m, 1H, Glu CH), 7.07 (d, J = 8.4 Hz, 2H, Ar), 7.63 (d, J = 8.3 Hz, 2H, Ar), 7.82 (t, J = 7.3 Hz, 1H, Ar), 7.84 (d, J = 8.9 Hz, 1H, Ar), 8.32 (m, 2H, Glu NH + Ar), 9.07 (d, J = 9.3 Hz, 1H, Ar), 10.16 (d, J = 8.8 Hz, 1H, Ar), 12.71 (br s, 1H, NH). Anal. ($C_{25}H_{22}N_4O_8S\cdot1.75H_2O$) C, H, N.

N-(4-(3-Amino-9-bromo-1,2-dihydro-1-oxobenzo[f]quinazoline-8-sulfonamido)benzoyl)-L-glutamic Acid (5i) was prepared from 3-amino-9-bromo-1,2-dihydro-1-oxobenzoquinazoline (4e, 6 g), essentially as described for 5e above; yields and analytical data on the product and intermediates are given below.

3-Amino-9-bromo-1,2-dihydro-1-oxobenzo[f]quinazoline-8-sulfonyl Chloride (6e) (3 g, 36%). ^{1}H NMR (DMSO-d_6,200 MHz): δ 7.59 (d, J = 9 Hz, 1H, Ar), 8.44 (d, J = 8 Hz, 1H, Ar), 8.54 (s, 1H, Ar), 9.75 (s, 1H, Ar), 11.30 (v br s, H_2O + exchangeable H's).

Diethyl N-(4-(Amino-9-bromo-1,2-dihydro-1-oxobenzo[f]-quinazoline-8-yl)sulfonamido)benzoyl)-L-glutamate (8i) (0.91 g, 17%). Mp >240 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.08 (t, J = 7 Hz, 3H, ester CH_3), 1.11 (t, J = 7 Hz, 3H, ester CH_3), 1.72–2.12 (m, 2H, Glu CH_2), 2.34 (t, J = 7 Hz, Glu CH_2), 3.97 (q, J = 7 Hz, 2H, ester CH_2), 4.02 (q, J = 7 Hz, 2H, ester CH_2), 4.24-4.36 (m, 1H, Glu CH), 6.84 (br s, 2H, NH_2), 7.17 (d, J = 9 Hz, 2H, Ar), 7.39 (d, J = 9 Hz, 1H, Ar), 7.66 (d, J = 8 Hz, 2H, Ar), 8.26 (d, J = 9 Hz, 1H, Ar), 8.47 (d, J = 7 Hz, 1H, NH), 8.76 (s, 1H, Ar), 9.95 (s, 1H, Ar), 11.01 (s, 1H, NH), 11.39 (s, 1H, NH). Anal. ($C_{28}H_{28}BrN_5O_8S\cdot0.5H_2O$) C, H, Br, N, S.

N-(4-(3-Amino-9-bromo-1,2-dihydro-1-oxobenzo[f]quinazoline-8-sulfonamido)benzoyl)-L-glutamic Acid (5i) (0.68 g, 79%). Mp: 238–242 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.72–2.07 (m, 2H, Glu CH_2), 2.25 (t, J = 7 Hz, Glu CH_2), 4.24–4.29 (m, 1H, Glu CH), 6.65 (br s, 2H, NH_2), 7.17 (d, J = 9 Hz, 2H, Ar), 7.39 (d, J = 9 Hz, 1H, Ar), 7.67 (d, J = 9 Hz, 2H, Ar), 8.26 (d, J = 9 Hz, 1H, Ar), 8.36 (d, J = 8 Hz, 1H, NH), 8.78 (s, 1H, Ar), 9.95 (s, 1H, Ar), 11.00 (s, 1H, NH), 11.41 (v br s, 1H, NH), 12.30 (v br s, 2H, OH). Anal. ($C_{24}H_{20}BrN_5O_8S\cdot1.4H_2O$) C, H, N.

Methyl 4-(((3-Amino-1,2-dihydro-1-oxobenzo[f]quinazolin-9-yl)amino)sulfonyl)benzoate (11). To a solution of N-(9-amino-1,2-dihydro-1-oxobenzo[f]quinazolin-3-yl)pivalamide[20] (9) (1.38 g, 4.5 mmol) in dry pyridine (10 mL) was added 4-(chlorosulfonyl)benzoic acid (5.0 g, 22.6 mmol) at room temperature under a nitrogen atmosphere. The mixture was stirred for 3 days, and the pyridine was removed under reduced pressure to leave a gummy brown residue which was suspended in water (30 mL), filtered, washed with water, and dried to give 4-((N-(3-((*tert*-butylcarbonyl)amino)-1,2-dihydro-1-oxobenzo[f]quinazolin-9-yl)amino)sulfonyl)benzoic acid (10)(1.65 g). The crude acid was dissolved in anhydrous 5% HCl/methanol (30 mL) and heated to 50 °C with stirring under a nitrogen atmosphere for 27 h. Upon cooling of the solution, a fine precipitate formed which was filtered, washed with methanol, and dried to give the methyl ester 11 as an off-white solid (0.41 g, 27%). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 3.82 (s, 3H, OCH_3), 7.36 (dd, J = 8.8, 2 Hz, 1H, Ar), 7.39 (d, J = 8.8 Hz, 1H, Ar), 7.90 (d, J = 8.8 Hz, 1H, Ar), 7.98–8.11 (m, 6H, Ar), 8.17 (d, J = 9.1 Hz, 1H, Ar), 9.33 (d, J = 2 Hz, 1H, Ar), 11.00 (br s, 1H, NH). Anal. ($C_{20}H_{16}N_4O_5S\cdot HCl\cdot0.6CH_3OH$) C, H, Cl, N, S.

4-(((3-Amino-1,2-dihydro-1-oxobenzo[f]quinazolin-9-yl)-amino)sulfonyl)benzoic Acid (12). A solution of the methyl ester 11 (0.54 g, 1.2 mmol) in 0.1 N NaOH (30 mL) was stirred at room temperature under a nitrogen atmosphere for 19 h. The solution was acidified (pH 4) with 1 N HCl, and the precipitated product was filtered, washed with water, and dried to give the benzoic acid 12 as a light brown solid (0.46 g, 86%). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 7.28 (d, J = 8.9 Hz, 1H, Ar), 7.30 (dd, J = 8.3, 2 Hz, 1H, Ar), 7.37 (br s, 2H, NH_2), 7.82 (d, J = 8.6 Hz, 1H, Ar), 7.95–8.06 (m, 5H, Ar), 9.38 (d, J = 2 Hz, 1H, Ar), 10.85 (s, 1H, NH). Anal. ($C_{19}H_{14}N_4O_5S\cdot0.8HCl\cdot1.1H_2O$) C, H, N.

Diethyl N-(4-(((3-Amino-1,2-dihydro-1-oxobenzo[f]-quinazolin-9-yl)amino)sulfonyl)benzoyl)-L-glutamate (13). To a solution of L-glutamic acid diethyl ester (0.81 g, 4.0 mmol) and the foregoing acid 12 (0.43 g, 0.9 mmol) in dry dimethylformamide (20 mL) were added 1-hydroxybenzotriazole monohydrate (0.43 g, 3.2 mmol) and dicyclohexylcarbodiimide (0.66 g, 3.2 mmol). The solution was stirred at room temperature under a nitrogen atmosphere for 20 h, after which time the solvent was removed under reduced pressure. The residue was recrystallized successively from methylene chloride–methanol (9:1) and methanol to give the diester 13 as a white solid (0.215 g, 36%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.12 (t, J = 7 Hz, 3H, ester CH_3), 1.15 (t, J = 7 Hz, 3H, ester CH_3), 1.89–2.13 (m, 2H, Glu CH_2), 2.40 (t, J = 7.6 Hz, 2H, Glu CH_2), 4.00 (q, J = 7 Hz, 2H, ester CH_2), 4.08 (q, J = 7 Hz, 2H, ester CH_2), 6.55 (br s, 2H, NH_2), 7.16 (d, J = 8.9 Hz, 1H, Ar), 7.23 (dd, J = 8.7, 2 Hz, 1H, Ar), 7.73 (d, J = 8.7 Hz, 1H, Ar), 7.89 (d, J = 8.9 Hz, 1H, Ar), 7.96 (m, 4H, Ar), 8.89 (d, J = 7.4 Hz, 1H, Glu NH), 9.48 (d, J = 2 Hz, 1H, Ar), 10.67 (s, 1H, NH), 11.08 (s, 1H, NH). Anal. ($C_{28}H_{29}N_5O_8S$) C, H, N, S.

N-(4-(((3-Amino-1,2-dihydro-1-oxobenzo[f]quinazolin-9-yl)amino)sulfonyl)benzoyl)-L-glutamic Acid (14). A solution of the diester 13 (0.175 g, 0.3 mmol) in 1 N NaOH (12 mL) was stirred and heated to 50 °C under a nitrogen atmosphere for 24 h. The cooled solution was acidified (pH 3) with concentrated HCl, and the product was filtered, washed with water, and dried to give the diacid 14 as an off-white solid (0.18 g, 99%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.81–2.13 (m, 2H, Glu CH_2), 2.32 (t, J = 7.6 Hz, 2H, Glu CH_2), 4.34 (m, 1H, Glu CH), 6.58 (br s, 2H, NH_2), 7.16 (d, J = 8.9 Hz, 1H, Ar), 7.22 (dd, J = 8.6, 2 Hz, 1H, Ar), 7.74 (d, J = 8.7 Hz, 1H, Ar), 7.89 (d, J = 9.0 Hz, 1H, Ar), 7.96 (m, 4H, Ar), 8.78 (d, J = 7.6 Hz, 1H, Glu-NH), 9.49 (d, J = 2 Hz, 1H, Ar), 10.68 (s, 1H, NH), 11.1 (br s, 1H, NH), 12.3 (br s, 2H, $(CO_2H)_2$). Anal. ($C_{24}H_{21}N_5O_8S\cdot3H_2O$) C, H, N, S.

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