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10-Formyl-5,8,10-trideazafolic Acid (10-Formyl-TDAF): A Potent Inhibitor of Glycinamide Ribonucleotide Transformylase

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Abstract—The synthesis of 10-formyl-5,8,10-trideazafolic acid (**3**) as a potential inhibitor of glycinamide ribonucleotide transformylase (GAR Tfase) is reported. The target compound was prepared by a convergent synthesis utilizing the alkylation of hydrazone **5** with benzylic bromide **6** to construct the core heterocycle **7**. The aldehyde **3** and related agents were evaluated as inhibitors of *pur*N GAR Tfase and avian AICAR Tfase. Compound **3** exhibited potent inhibition of GAR Tfase with a K_i of 0.26 ± 0.05 μM. In contrast, **3** exhibited more moderate inhibition of aminoimidazole carboxamide ribonucleotide transformylase (AICAR Tfase), with K_i of 7.6 ± 1.5 μM. © 1997 Elsevier Science Ltd.

Introduction

Glycinamide ribonucleotide transformylase (GAR Tfase) is an enzyme central to de novo purine biosynthesis.[1–12] Since purines play a critical role as required components of DNA and RNA, inhibition of enzymes in the purine biosynthetic pathway has been proposed to be an effective approach for antineoplastic intervention.[13] The disclosure that (6*R*)-5,10-dideazatetrahydrofolate (Lometrexol, DDATHF), originally prepared as a potential dihydrofolate reductase inhibitor in the pyrimidine biosynthetic pathway, is an efficacious antitumor agent that acts as an effective inhibitor of GAR Tfase (K_i = 0.1 μM) established inhibition of purine biosynthesis and GAR Tfase as viable targets for antineoplastic intervention.[14–23] GAR Tfase uses (6*R*)-N^{10}-formyltetrahydrofolate (**1**) to transfer a formyl group to the primary amine of its substrate, glycinamide ribonucleotide (**2a**, GAR; Fig. 1). This one carbon transfer constitutes the incorporation of the C-8 carbon of the purines and is the first of two formyl transfer reactions. The second formyl transfer reaction is catalyzed by the enzyme aminoimidazole carboxamide ribonucleotide transformylase (AICAR Tfase) which also employs (6*R*)-N^{10}-formyltetrahydrofolate (**1**) to transfer a formyl group to the C5-amine of its substrate, aminoimidazole carboxamide ribonucleotide (**2b**, AICAR; Fig. 1).[1,24–27]

Here, we detail the preparation and evaluation of 10-formyl-5,8,10-trideazafolic acid (**3**)[28] and a series of structurally related agents as potential inhibitors of GAR and AICAR Tfase. In the accompanying articles,

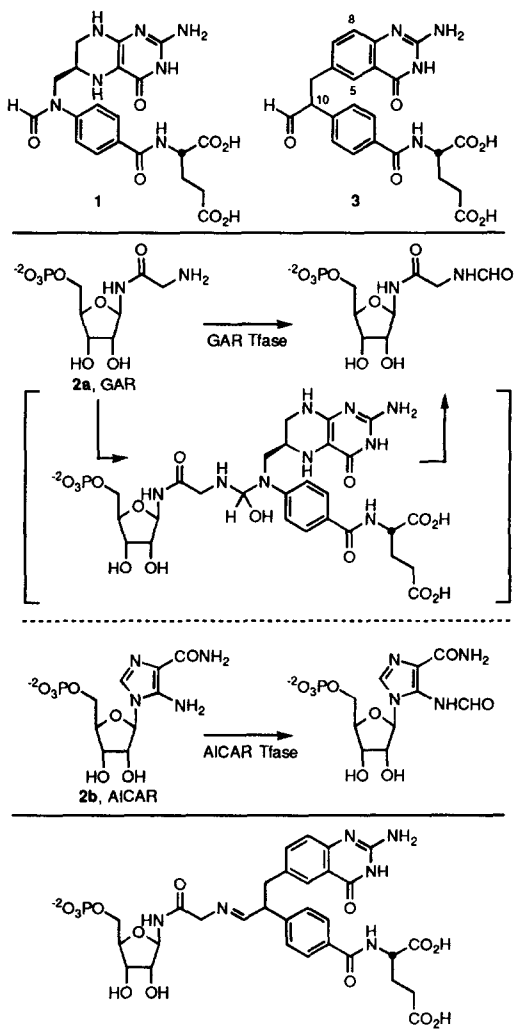


Figure 1.

we report the extension of these studies to related inhibitors.[29]

Inhibitor Design

A folate-based inhibitor incapable of transferring the formyl group was the initial focus of our efforts. Replacement of N-10 with a carbon prevents the breakdown of the tetrahedral intermediate with elimination of the cofactor and was expected to provide an enzyme-assembled tight binding inhibitor of GAR or AICAR Tfase by virtue of imine formation with the substrate (Fig. 1).[28-31] In the absence of imine formation with the substrate, less effective competitive inhibition was expected to convey a degree of selectivity for GAR and AICAR Tfase over other folate-dependent enzymes not directly involved in a formyl transfer reaction. Subsequent crystallographic examination[32] of the binary and ternary complexes of **3** with GAR or AICAR Tfase in the absence and presence of the substrates was expected to reveal key interactions and spatial requirements at the substrate and cofactor binding sites as well as key elements of enzymatic catalysis useful for future inhibitor design. 10-Formyl-5,8,10-trideazafolic acid (**3**) was designed to take advantage not only of the nontransferable aldehyde, but also the trideazafolate core in which the pterin was replaced with a quinazoline ring system. The potential effectiveness of this substitution was based on the previous work of Benkovic in which it was found to constitute an efficient alternative cofactor for GAR Tfase (Fig. 2).[33]

Although the N-8 amino group in the pterin ring has been established to play a role in the recognition or binding affinity of the natural cofactor as part of an array of three specific hydrogen bonds with the GAR Tfase peptide backbone,[32] modeling studies (MacroModel, AMBER force field) suggested that its removal may not significantly affect the orientation or binding interactions of 10-formyl-5,8,10-trideazafolic acid within the active site of GAR Tfase (Fig. 3).

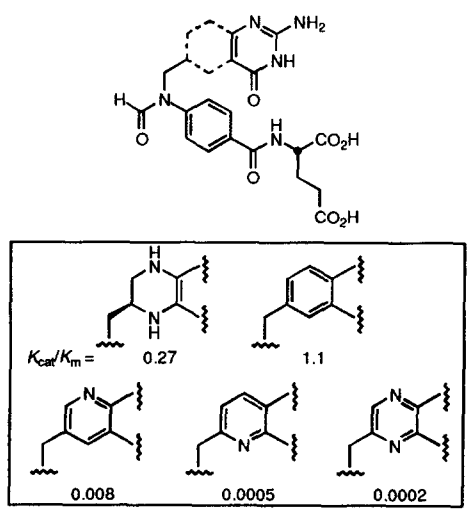


Figure 2.

Chemistry

The synthesis of 10-formyl-5,8,10-trideazafolic acid was accomplished in a convergent manner through the alkylation[34] of the *N,N*-dimethylhydrazone **5** of the known aldehyde **4**[35] with **6**.[36] This was best accomplished upon LDA deprotonation of **5** (THF, −78 °C, 30 min) and subsequent treatment with **6** (THF–HMPA, −78 °C, 6 h, 93%; Scheme 1). Because of the unusually sensitive nature of the free aldehyde, the *N,N*-dimethylhydrazone was not removed at this stage but rather maintained in order to protect the aldehyde. Treatment of **7** with LiOH (2–4 equiv, THF:CH_3OH:-H_2O 3:1:1, 25 °C, 24–48 h, 86%) cleanly provided the carboxylic acid **8** which was coupled with di-*tert*-butyl L-glutamate hydrochloride (1.2 equiv) on activation with DPPA (1.2 equiv, THF–DMF 9:1, 3 equiv Et_3N, 0 °C, 18 h, 64%) to provide **10** as a 1:1 mixture of inseparable diastereomers. Subsequent hydrolysis of the dimethylhydrazone was effectively accomplished to provide the sensitive aldehyde **11**[37] by treatment with $CuCl_2$[38] (5.0 equiv, 0 °C, 1 h, 46%) in THF–H_2O

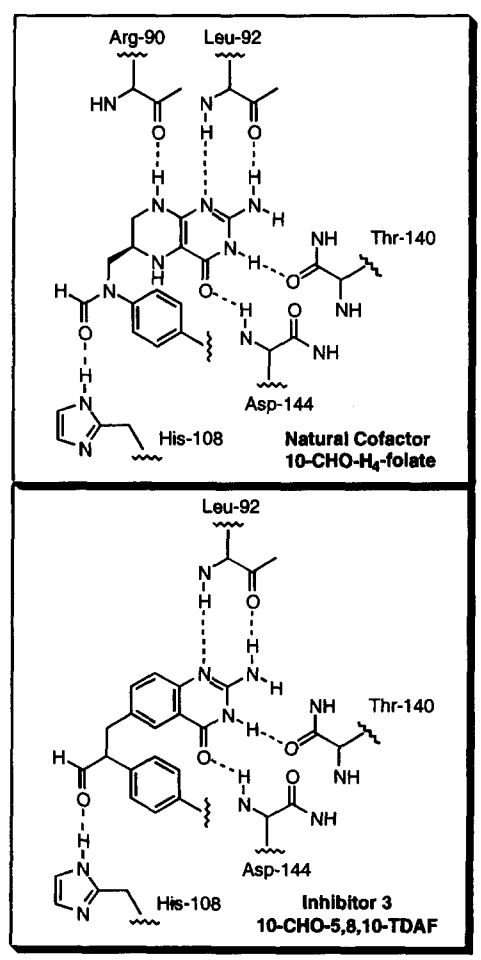
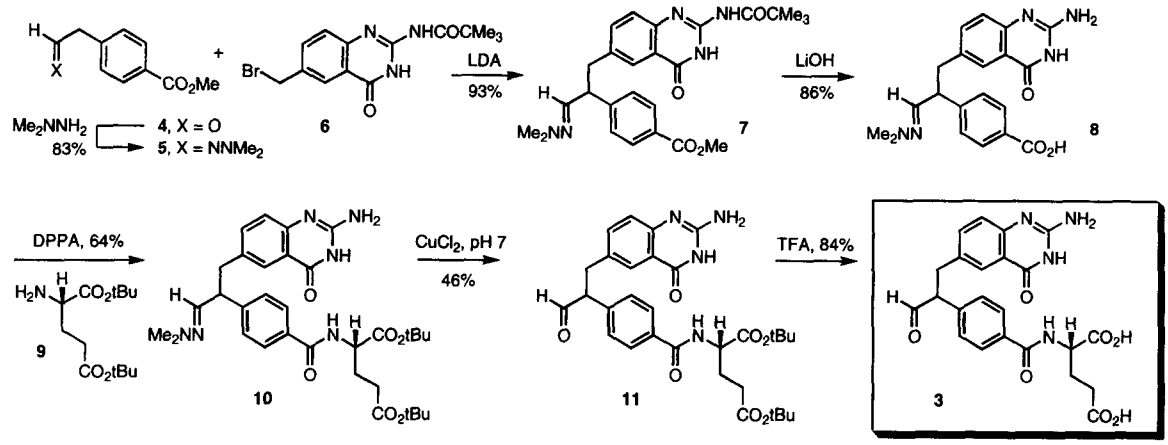


Figure 3.

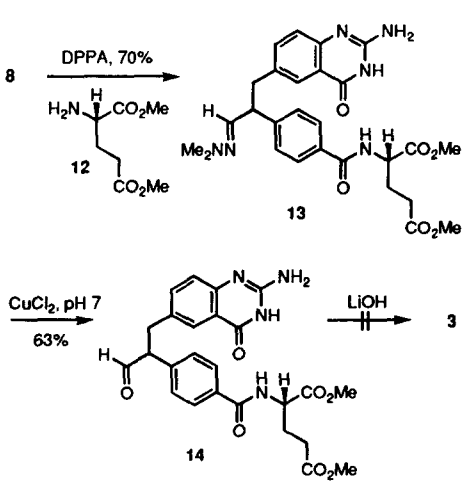


Scheme 1.

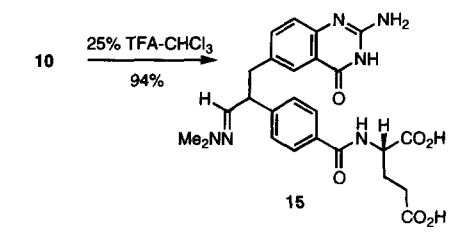
buffered to pH 7. The sensitivity of the aldehyde to oxidative deformylation and the rapid epimerization precluded efforts to separate and evaluate the two diastereomers. Final acid-catalyzed deprotection of the *tert*-butyl esters (25% CF_3CO_2H–$CHCl_3$, 0–25 °C, 12–14 h, 84%) cleanly provided **3**.[37]

Comparable efforts to prepare **3** through coupling of **8** with dimethyl L-glutamate[39] (**12**, 1.2 equiv, 1.1 equiv DPPA, 3 equiv Et_3N, 0 °C, 18 h, 70%) followed by hydrolysis of the dimethylhydrazone **13** by treatment with $CuCl_2$[38] (5 equiv, 0 °C, 1 h, 63%) in pH 7 buffered THF–H_2O cleanly provided the sensitive aldehyde **14** (Scheme 2). However, efforts to convert the dimethyl ester **14** to **3** under conventional basic conditions proved problematic because of the sensitive nature of the aldehyde.

Because of the sensitivity of the aldehyde **3** and with the availability of the stable dimethyl hydrazones **10** and **13**, **10** was also converted to **15** by acid-catalyzed deprotection of the di-*tert*-butyl esters (25% TFA–$CHCl_3$, 0–25 °C, 12 h, 25 °C, 12 h, 94%) for direct comparison with **3** (eq 1). Although in principle deprotection of **15** could also serve to provide **3**, efforts to remove the dimethylhydrazone provided impure **3** which was much more difficult to purify than **11**.

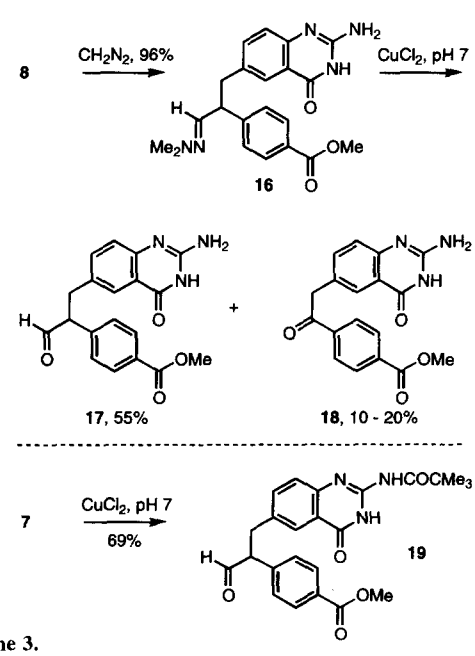


Scheme 2.



(1)

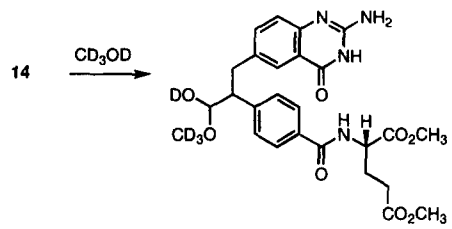
For a similar comparison with **3**, the aldehydes **17** and **19** lacking the glutamic acid side-chain and **22** lacking the entire benzoyl glutamic acid subunit were also prepared. Esterification of the carboxylic acid **8** (excess CH_2N_2, DMF–Et_2O, 25 °C, 96%) followed by hydrolysis of the dimethylhydrazone **16** employing $CuCl_2$[38] (5.0 equiv) under pH 7 buffered reaction conditions (THF–buffer, 0 °C, 1 h) provided **17** (55%) and smaller amounts of the oxidative deformylation product **18** (10–20%; Scheme 3). Similar deprotection of **7** provided **19** (69%).



Scheme 3.

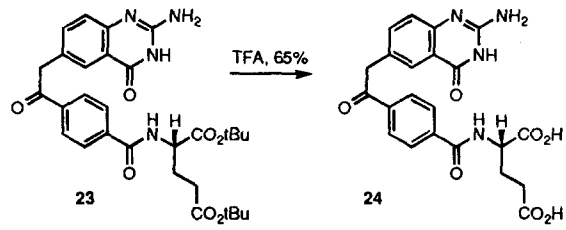
The aldehyde **22** lacking the full benzoyl glutamic acid subunit was prepared by alkylation of the lithium anion of acetaldehyde *N,N*-dimethylhydrazone[34] with **6** (THF–HMPA, −78 °C, 16 h, 69%) followed by sequential hydrolysis of the labile pivaloyl amide (2–4 equiv LiOH, $THF:CH_3OH:H_2O$ 3:1:1, 25 °C, 24–48 h, 61%) and the dimethylhydrazone (5.0 equiv $CuCl_2$, 0 °C, 1 h; Scheme 4).

Consistent with expectations of the electrophilic aldehyde and required of enzyme-assembled imine formation with GAR, the aldehyde was found to exist in the aldehyde versus enol tautomer (^1H NMR) and to react effectively with nucleophiles. This was clear on ^1H NMR spectroscopic examination of **3**, **11**, **14**, and **17**, each of which exhibited the characteristic aldehyde signal at 9.83, 9.83, 9.82 and 9.84 (DMF-d_7), respectively. In addition, exposure of **11** to CD_3OD (25 °C, <5 min) resulted in complete hemiacetal (100%) formation illustrating that the aldehyde is sufficiently electrophilic to permit the projected enzyme-assembled imine formation to occur (eq 2). This was confirmed upon imine formation of aldehydes **11**, **17** and **19** with glycinamide (CH_2Cl_2–CH_3OH, Na_2CO_3, 4 Å MS, 25 °C, 8 h, 75–85%; Scheme 5).



(2)

Nonetheless, the inhibitor **3** and the related aldehydes **11**, **14**, and **17** proved sensitive and prone to oxidative deformylation[40] especially in the presence of base and O_2. In fact, attempts to cleave the dimethylhydrazones under oxidative as well as acid-catalyzed hydrolysis[34] conditions failed to provide the corresponding aldehydes and only the pH 7 buffered $CuCl_2$-promoted hydrolysis was found to afford the desired products. Even under these conditions a minor amount of the oxidative deformylation product **23** was always obtained on purification of the sensitive aldehyde **11** by SiO_2 chromatography. Simply exposing **11** to a slurry of SiO_2 in $CHCl_3$ for 3 h (25 °C) with no special precautions to exclude air or light provided **23** in 86% yield.[40] Acid-catalyzed deprotection of **23** provided **24**, an additional interesting inhibitor capable of potential enzyme-assembled tight binding inhibition of GAR or AICAR Tfase by virtue of imine formation with the substrate (eq 3).



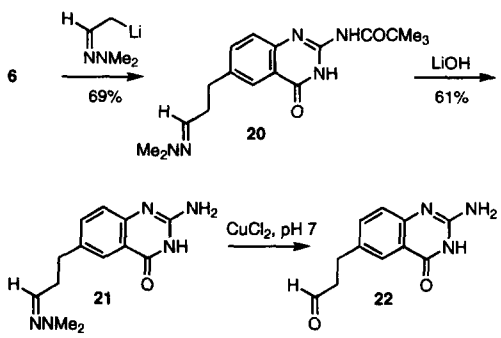
(3)

This propensity for oxidative deformylation was even more facile with **17**. On simply standing in DMF-d_7 (0.03 M) with no special precautions to exclude air or light, **17** was completely converted to **18** in 2–3 h.

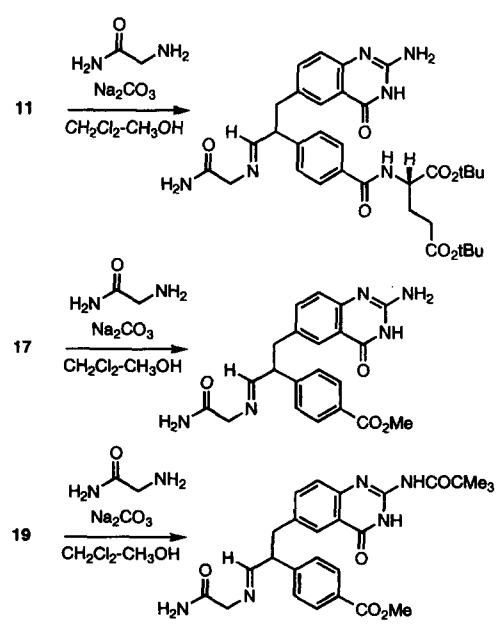
Inhibitor Studies

GAR Tfase inhibition

10-Formyl-5,8,10-trideazafolic acid (**3**) closely resembles the cofactor used in the kinetic studies of GAR Tfase, 10-formyl-5,8-dideazafolate (fDDF). However, substitution of the N-10 nitrogen by a carbon results in the formyl moiety at the C-10 position being non-transferable, and should produce low K_i values even if a multisubstrate adduct does not form. Traditional inhibition kinetics were used to determine K_i values, using saturating substrate GAR while varying cofactor fDDF at different concentrations of the inhibitors. Compound **3** demonstrated mixed type inhibition, with K_i = 0.26 ± 0.05 μM (Table 1). Although this binding



Scheme 4.



Scheme 5.

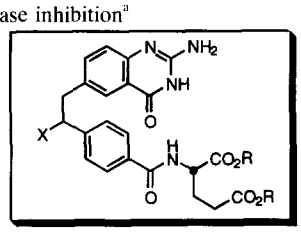
constant is nearly two orders of magnitude better than the K_m of 17 μM for fDDF and comparable to that of DDATHF (Table 1), it is three orders of magnitude higher than multisubstrate adduct inhibitors (250–100 pM).[30,31]

Although the effect of the aldehyde inhibitor **3** was not as great as might be expected of an enzyme-assembled tight binding inhibitor, it is significantly more potent than related agents in the series. With the exception of **22**, the inhibitors bearing an aldehyde were 5–20 times more potent than the corresponding inhibitors containing the *N,N*-dimethylhydrazone. This is clear from the comparisons of **3** with **15**, **14** with **13**, and **17** with **8** and **16**. Moreover, these observations proved consistent with those made in the accompanying articles where, for example, the corresponding free alcohols also proved significantly less effective than the corresponding aldehyde and comparable in potency to the *N,N*-dimethylhydrazones detailed here. These observations might suggest that a rapid, but readily reversible imine formation with GAR provides some enhancement of binding affinity but fails to provide a classical tight binding inhibitor. Alternatively, the carbonyl oxygen of **3** may be specifically interacting through hydrogen bonds with the carboxamide and imidazole of the Asn-106 and His-108 residues. Any alteration in the position or functionality of the aldehyde likely disrupts these interactions. Similarly, the aldehyde may be reacting with one of these active site nucleophiles to provide a readily reversible hemiacetal type adduct which displays slightly tighter binding.

Clear from the studies is the expected potentiation of the binding and inhibition by the glutamate side-chain. The comparisons of **3** with **14**, **15** with **13**, and **24** with **23** indicates a 20–30-fold increase in inhibitor potency with its presence as the free acid. Removal of the entire glutamate side-chain has the same effect as its conversion to the methyl ester indicating that it is the carboxylates that contribute to the binding affinity and that little additional binding affinity is derived from the remainder of the residue. This is clear in the comparisons of **14** with **17**, and **13** with **16** and **8**. Further removal of the benzoyl subunit resulted in two interesting observations. In the case of the comparisons of **20** or **21** with **16**, **8**, or **13**, little or no contribution of the benzoyl subunit to the binding affinity and inhibitor potency was observed. In contrast, aldehyde **22** was inactive as an inhibitor of GAR Tfase and substantially less effective than **14** and **17**. In this latter case, the benzoyl subunit is clearly important.

Finally, the oxidative deformylation product **24** exhibited substantially weaker inhibition than **3** indicating that it is not only a poorer competitive inhibitor of GAR Tfase, but that it is not forming an enzyme assembled inhibitor through reaction with GAR.

Table 1. GAR Tfase inhibition[a]



Agent	K_i	X	R
3	0.26 ± 0.05 μM	CHO	H
11	ND[b]	CHO	*t*-Bu
14	4.90 ± 0.3 μM	CHO	CH_3
15	1.40 ± 0.2 μM	HC=NNMe$_2$	H
10	ND	HC=NNMe$_2$	*t*-Bu
13	40 ± 9 μM	HC=NNMe$_2$	CH_3
24	19 ± 2 μM	=O	H
23	> 100 μM	=O	*t*-Bu
(6*R,S*)-DDATHF	0.12 ± 0.02[22]		
(6*R*)-DDATHF	0.029 ± 0.012[22]		
(6*S*)-DDATHF	0.10 ± 0.22[22]		
Related agents			
7	ND		
8	19 ± 3 μM		
16	60 ± 20 μM		
17	3.1 ± 0.2 μM		
18	>100 μM		
19	ND		
20	39 ± 6 μM		
21	41 ± 9 μM		
22	>100 μM		

[a] *pur*N GAR Tfase.
[b] Not soluble and not determined.

Time-dependent inhibition studies on GAR Tfase

If the compounds in this study reacted with substrate GAR at the active site of the enzyme slowly to form multisubstrate adducts, a substantial decrease in K_i could be observed over time. The best inhibitor from the classical inhibition studies was **3**, with K_i = 0.26 ± 0.05 μM. At the concentration examined (2 nM enzyme, 10 μM inhibitor), **3** resulted in near complete inhibition of GAR Tfase which did not change with time. No significant subsequent decreases in activity were observed for **15**, **22**, or **24** over a 6-h time course, making it unlikely that stable adducts are forming (Table 2).

In efforts to further distinguish possibilities with **3**, the time-dependent enzyme inhibition at a concentration of **3** that provided partial inhibition was examined under a

Table 2. Time-dependent GAR Tfase inhibition[a]

Inhibitor	*t* = 3 min	*t* = 15 min	*t* = 6 h
None	100	100	92
3	2.6	2.6	9
15	52	52	40
24	84	82	69
22	100	100	96

[a] 2 nM *pur*N GAR Tfase, 10 μM inhibitor, 50 μM GAR, percent enzyme activity vs time.

Table 3. Time-dependent GAR Tfase inhibition[a]

Incubation conditions	t = 1 min	4 min	10 min	30 min	60 min	120 min	180 min
E + fDDF (control)	99	100	98	104	97	103	98
E + GAR + **3**	41	43	46	53	58	58	63
E + **3**	39	37	37	39	42	41	41
GAR + **3**	46	49	50	45	56	56	58
E + fDDF + **3**	46	51	48	51	63	64	66
fDDF + **3**	50	51	51	55	63	64	62

[a]3 nM *pur*N GAR Tfase (E), 10 μM inhibitor (**3**), 50 μM fDDF, 50 μM GAR, % enzyme activity vs time.

variety of incubation conditions (Table 3). No apparent distinctions were observed when the enzyme–inhibitor incubation was carried out in the presence or absence of GAR, fDDF, or with preincubation of **3** with GAR and **3** with fDDF. Thus, the inclusion of fDDF failed to offer any temporal protection from inhibition and the exclusion of GAR failed to lower the extent of enzyme inhibition. If anything, the exclusion of GAR from the incubation mixture seemed to provide results in which the potency of chemically sensitive inhibitor was maintained over the 3-h period. Although this might be indicative of the formation of a hemiacetal type adduct with the enzyme that prevents inhibitor degradation over the time course of the assay and that this adduct is reversibly and competitively displaced in the presence of both GAR and fDDF, this is unlikely at the concentrations examined (3 nM enzymes, 10 μM inhibitor). With a K_i that appears to be two orders of magnitude better than fDDF binding, it is possible that an adduct between **3** and GAR could be rapidly and transiently forming, followed by hydrolysis. However, compound **3** has a very similar K_i to that of 5,10-dideazatetrahydrofolate (Lometrexol, DDATHF), which has K_i = 0.12 μM and is incapable of forming an adduct with GAR. Since they have comparable K_i values, it is possible that **3** is simply functioning as a competitive inhibitor and not forming a multisubstrate adduct. However, the fact that **3**, which lacks the N-8 amine of Lometrexol, binds with a comparable K_i suggests that the aldehyde of **3** compensates in part for the lost N-8 amine hydrogen bond present in the Lometrexol binding. Whether this is provided by analogous noncovalent contacts with the enzyme active site (i.e., hydrogen bonds) or is achieved by a covalent but rapidly reversible imine formation with the substrate or a hemiacetal-type adduct with the enzyme is not yet known.

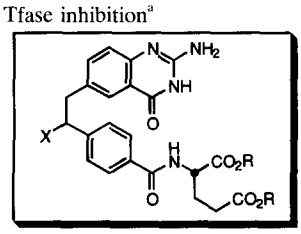
AICAR Tfase inhibition

The same inhibitors were examined for their ability to inhibit AICAR Tfase (Table 4). The results obtained were analogous to those observed with GAR Tfase with aldehyde **3** being the most potent in the series, K_i = 7.6 ± 1.5 μM. However, the inhibition was typically weaker and the distinctions between classes of inhibitors (e.g., **3** versus **14**, **15** versus **13**, or **3** versus **15**) less pronounced. The exception to this generalization is **24** which proved equipotent against both GAR Tfase (19 μM) and AICAR Tfase (15 μM). Agent **24**, as well as **3**, are the more potent AICAR Tfase inhibitors in this and our related[29] series. In fact, both are more potent than 10-formyl-5,8-dideazafolate (K_i = 29 μM) which is one of the better anti-folate inhibitors of AICAR Tfase.[40] Because these inhibitors were designed based on the crystal structure of GAR Tfase, it is not surprising they show tighter binding to GAR Tfase than AICAR Tfase. Without the benefit of structural information, it is not presently possible to interpret these comparisons in terms of active site interactions with AICAR Tfase.

Time-dependent AICAR Tfase inhibition

The time dependence for inhibition of AICAR Tfase was examined with the full range of inhibitors including the aldehydes capable of putative imine formation with

Table 4. AICAR Tfase inhibition[a]



Agent	K_i	X	R
3	7.6 ± 1.5 μM	CHO	H
11	ND[b]	CHO	*t*-Bu
14	66 ± 17 μM	CHO	CH_3
15	22 ± 7 μM	HC=NNMe$_2$	H
10	ND	HC=NNMe$_2$	*t*-Bu
13	47 ± 15 μM	HC=NNMe$_2$	CH_3
24	15 ± 4 μM	=O	H
23	ND	=O	*t*-Bu
Related agents			
7	ND		
8	66 ± 21 μM		
16	77 ± 22 μM		
17	>100 μM		
18	>100 μM		
19	77 ± 20 μM		
20	>100 μM		
21	>100 μM		
22	>100 μM		

[a]Avian AICAR Tfase.
[b]Not soluble and not determined.
[c]K_i for 10-formyl-5,8-dideazafolate = 29 μM.

the substrate resulting in an enzyme-assemble tight binding inhibitor. None of the inhibitors exhibited time-dependent inhibitory properties (Table 5). This is perhaps not surprising in that the reacting amine of AICAR is much less nucleophilic and much less likely to form a stable imine with the aldehyde of **3** or related potential inhibitors.

Cytotoxic Activity

The agents were examined for cytotoxic activity both in the presence (+) and absence (−) of added hypoxanthine against both L-1210 and CCRF-CEM cell lines cultured in a medium in which purines were removed from the FBS supplement by dialysis (Table 6). The key aldehyde **3** exhibited a cytotoxic potency that was at least two orders of magnitude higher than its ability to inhibit GAR Tfase and this activity was insensitive to the presence or absence of purine in the culture medium. Similarly, the key inhibitors **15**, **22**, and **24** exhibited only modest cytotoxic activity and only **15** exhibited a modest fourfold sensitivity to the absence of medium purines in the CCRF-CEM, but not L-1210, cell line. Of the related agents examined, only **11, 13** and **18** exhibited both cytotoxic activity and a modest CCRF-CEM sensitivity (3–9 times) to the absence of medium purines and this same modest sensitivity was not observed with L-1210. Consequently, the agents in general do not appear to express their cell growth inhibition by a mechanism consistent with selective GAR or AICAR Tfase inhibition.

Experimental

Methyl 4-(3-Dimethylhydrazonoethyl)benzoate (5). A slurry of 4-(methoxycarbonyl)phenylacetaldehyde[35] (**4**, 364 mg, 1.40 mmol) and $MgSO_4$ (494 mg, 4.1 mmol) in Et_2O (3.4 mL) was treated with *N,N*-dimethylhydrazine (0.15 mL, 2.0 mmol) and the mixture was stirred at 25 °C for 2 h under N_2. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. PCTLC (SiO_2, 1 mm plate, 15% EtOAc–hexane) gave **5** (375 mg, 83%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.95 (2H, d, J = 8.2 Hz), 7.28 (2H, d, J = 8.1 Hz), 6.62 (1H, t, J = 5.6 Hz, CH), 3.88 (3H, s, OCH_3), 3.60 (2H, d, J = 5.6 Hz, CH_2), 2.75 (6H, s, $N(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.8, 144.0, 134.8, 129.7, 129.5, 128.6, 51.8, 42.8, 39.3; IR (neat) ν_{max} 2948, 2854, 2784, 1721, 1610 cm^{-1}; FABHRMS (NBA) *m/z* 221.1294 (M^+ + H, $C_{12}H_{16}N_2O_2$ requires 221.1290).

6-(Bromomethyl)-2-(trimethylacetimido)-3,4-dihydro-4-oxo-quinazoline (6). A mixture containing 4-hydroxy-6-methyl-2-(trimethylacetimido)quinazoline[36] (500 mg, 1.93 mmol), *N,N*-dibromo-5,5-dimethylhydantoin (411 mg, 1.44 mmol), and a catalytic amount of dibenzoyl peroxide were added to a flame-dried flask fitted with a reflux condenser. $CHCl_3$ (40 mL, 0.04 M) was passed through a plug of basic alumina and added to the reaction vessel. The solution was illuminated with a Kenmore 275 W sunlamp for 3 h. Additional *N,N*-dibromo-5,5-dimethylhydantoin (411 mg, 1.44 mmol) was added, and the reaction was illuminated for an additional 6 h. The solvent was removed under reduced pressure and the residue was washed with H_2O and recrystallized from $CHCl_3$ to give **6**[36] (526 mg, 80%) as an off-white powder: mp 205–207 °C ($CHCl_3$), lit.[36] mp 205–207 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.34 (1H, br s, NH), 8.22 (1H, d, J = 1.9 Hz, H-5), 7.87 (1H, dd, J = 2.0, 8.5 Hz, H-7), 7.64 (1H, d, J = 1.9 Hz, H-8), 5.93 (1H, br s, NH), 4.53 (2H, s, CH_2Br), 1.41 (9H, s, $COC(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.2, 159.1, 148.1, 136.4, 136.2, 134.0, 127.5, 123.6, 119.2, 40.8, 31.8, 26.6; IR (KBr) ν_{max} 3262, 2969, 1771, 1723, 1656 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 469.9480 (M^+ + Cs, $C_{14}H_{16}N_3O_2Br$ requires 469.9485).

Table 5. Time-dependent AICAR Tfase inhibition[a]

Inhibitor	t = 3 min	t = 3 h	t = 6 h
None	100	100	100
3	58	—	46
14	88	—	90
15	81	—	86
13	90	—	89
24	66	65	61
8	94	92	92
16	93	90	94
17	93	94	92
19	95	—	98
20	100	—	99
21	95	95	97
22	95	—	—

[a]100 nM avian AICAR Tfase, 10 μM inhibitor, percent enzyme activity vs time.

Table 6. Cytotoxic activity (IC_{50}, μM)[a]

Agent	L-1210	CCRF-CEM
(6*R*)-DDATHF	>225, 0.07	>225, 0.05
Methotrexate	0.05, 0.05	0.06, 0.07
3	60, 50	860, 430
15	60, 40	250, 60
24	56, 60	80, 40
22	200, 160	370, 330
7	50, 50	100, 100
8	250, 250	>200, >200
10	30, 30	50, 50
11	70, 30	50, 7
13	>200, >200	90, 10
14	>200, 50	40, 10
16	50, 50	80, 90
17	25, 25	50, 50
18	35, 35	230, 70
19	40, —	—, —
20	4, 6	30, 30
21	150, 120	170, 130
23	40, —	—, —

[a]Dialyzed FBS, RPMI-1640 medium, with + hypoxanthine, − hypoxanthine.

Methyl (2*R)-4-[1-Dimethylhydrazono-3-(2-trimethylacetimido-3,4-dihydro-4-oxo-quinazolin-6-yl)-prop-2-yl]benzoate (7).** A solution of freshly prepared LDA (6 mL of 0.3 M, 1.8 mmol) cooled to −78 °C was treated with **5** (362 mg, 1.64 mmol) in THF (3.30 mL) and the resulting solution was stirred at −78 °C for 30 min. A solution of **6** (157 mg, 0.46 mmol) dissolved in a minimal amount of HMPA was added and the mixture was stirred at −78 °C for 6 h under N_2. The reaction mixture was warmed to 25 °C and quenched by the dropwise addition of a saturated aqueous NH_4Cl (10 mL). This mixture was poured into saturated aqueous LiCl (30 mL). The product was extracted into EtOAc (3 × 40 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. PCTLC (SiO_2, 2 mm plate, 5% EtOAc–hexane) afforded **7** (205 mg, 93%) as a yellow solid: mp 72–74 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.91 (1H, d, J = 1.9 Hz, H-5), 7.87 (2H, d, J = 8.3 Hz), 7.29 (1H, dd, J = 2.1, 6.3 Hz, H-7), 7.18 (1H, d, J = 8.5 Hz, H-8), 7.18 (2H, d, J = 8.3 Hz), 6.65 (1H, d, J = 5.5 Hz, HC=N), 3.85 (3H, s, OCH_3), 3.86 (1H, m, CH), 3.38 (1H, dd, J = 7.1, 13.8 Hz, C*H*H), 3.05 (1H, dd, J = 8.1, 13.8 Hz, CH*H*), 2.70 (6H, s, $N(CH_3)_2$), 1.29 (9H, s, $COC(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.0, 166.8, 160.8, 147.3, 146.6, 145.7, 137.6, 136.9, 136.2, 129.8, 128.4, 128.0, 126.9, 125.2, 119.9, 51.9, 50.6, 43.0, 40.0, 26.9, 20.6; IR (KBr) ν_{max} 3222, 2955, 2862, 1716, 1670, 1650 cm^{-1}; FABHRMS (NBA) *m/z* 478.2454 (M^+ + H, $C_{26}H_{31}N_4O_3$ requires 478.2454). Anal. calcd for $C_{26}H_{31}N_4O_3$: C, 65.39; H, 6.54; N, 14.66. Found: C, 65.62; H, 6.55; N, 14.61.

(2*R)-4-[3-(2-Amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-1-(dimethylhydrazono)prop-2-yl]benzoic acid (8).** A solution of **7** (20.4 mg, 0.04 mmol) in THF–H_2O–CH_3OH (3:1:1, 0.10 mL, 0.4 M) was treated with aqueous 1 N LiOH (64 μL, 0.06 mmol) and the mixture was stirred at 25 °C for 24 h. Additional aqueous 1 N LiOH (64 μL, 0.6 mmol) was added and the solution was stirred at 25 °C for an additional 24 h. The mixture was diluted with H_2O (5 mL) and the aqueous layer was washed with EtOAc (3 × 5 mL) and acidified with the addition of 10% aqueous HCl (0.2 mL, pH = 4). The precipitated product was collected by filtration to give **8** (14 mg, 86%) as an off-white solid: mp > 250 °C; ^{1}H NMR (DMF-d_7, 400 MHz) δ 7.93 (2H, d, J = 8.1 Hz), 7.75 (1H, d, J = 1.7 Hz, H-5), 7.42 (2H, d, J = 8.1 Hz), 7.39 (1H, dd, J = 1.8, 8.4 Hz, H-7), 7.09 (1H, d, J = 8.3 Hz, H-8), 6.91 (2H, br s, NH_2), 6.87 (1H, d, J = 6.1 Hz, HC=N), 3.88 (1H, dt, J = 6.4, 7.4 Hz, CH), 3.30 (1H, dd, J = 7.9, 13.6 Hz, C*H*H), 3.09 (1H, dd, J = 7.4, 13.6 Hz, CH*H*), 2.65 (6H, s, $N(CH_3)_2$); IR (KBr) ν_{max} 3384, 1717, 1651, 1394 cm^{-1}; ^{13}C NMR (DMF-d_7, 100 MHz) δ 162.0, 156.3, 156.2, 146.8, 142.1, 132.0, 129.5, 127.4, 124.3, 123.7, 122.1, 120.4, 117.0, 111.2, 45.0, 36.5, 34.2; FABHRMS (NBA–NaI) *m/z* 380.1723 (M^+ + H, $C_{20}H_{21}N_5O_5$ requires 380.1723). Anal. calcd for $C_{22}H_{21}N_5O_5$: C, 63.31; H, 5.58; N, 18.46. Found: C, 63.33; H, 5.19; N, 18.62.

Di-*tert*-butyl (2*RS*)-*N*-[4-{(2-Amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-1-(dimethylhydrazono)-prop-2-yl}benzoyl]-L-glutamate (10). A slurry of **8** (29.7 mg, 0.07 mmol) and **9**[39] (28 mg, 0.09 mmol) in 10% DMF–THF (7.0 mL) cooled to 0 °C was treated with Et_3N (34 μL, 0.24 mmol) followed by diphenylphosphoryl azide (DPPA, 20 μL, 0.09 mmol). The reaction mixture was stirred at 0 °C for 18 h before the solvent was removed under reduced pressure. PCTLC (SiO_2, 1 mm plate, 10% CH_3OH–$CHCl_3$) afforded **10** (31.0 mg, 64%) as a yellow solid as a mixture of inseparable diastereomers: mp > 300 °C; ^{1}H NMR (CD_3OD, 400 MHz) diastereomer A: δ 7.92 (2H, d, J = 8.4 Hz), 7.73 (1H, s, H-5), 7.35 (2H, d, J = 8.2 Hz), 7.34 (1H, m, H-7), 7.10 (1H, d, J = 8.3 Hz, H-8), 6.86 (1H, d, J = 6.4 Hz, HC=N), 4.46 (1H, m, NHC*H*$CO_2C(CH_3)_3$), 3.86 (1H, m, CH), 3.30 (1H, m, C*H*H), 3.09 (1H, dd, J = 4.4, 7.8 Hz, CH*H*), 2.67 (6H, s, $N(CH_3)_2$), 2.37 (2H, t, J = 7.3 Hz), 2.14 (1H, m), 2.01 (1H, m), 1.46 (9H, s, $CO_2C(CH_3)_3$), 1.41 (9H, s, $CO_2C(CH_3)_3$); diastereomer B: δ 7.74 (2H, d, J = 8.2 Hz), 7.75 (1H, s, H-5), 7.36 (1H, dd, J = 2.0, 8.3 Hz, H-7), 7.32 (2H, d, J = 8.4 Hz), 7.11 (1H, d, J = 8.3 Hz, H-8), 6.91 (1H, d, J = 6.5 Hz, HC=N), 4.46 (1H, m, NHC*H*$CO_2C(CH_3)_3$), 3.86 (1H, m, CH), 3.30 (1H, m), 3.13 (1H, dd, J = 4.5, 7.9 Hz, CH*H*), 2.67 (6H, s, $N(CH_3)_2$), 2.37 (2H, t, J = 7.3 Hz), 2.14 (1H, m), 2.10 (1H, m), 1.46 (9H, s, $CO_2C(CH_3)_3$), 1.40 (9H, s, $CO_2C(CH_3)_3$); ^{13}C NMR (CD_3OD, 100 MHz) δ 173.8, 173.5, 172.6, 170.2, 167.7, 154.7, 150.9, 147.6, 146.6, 141.6, 140.7, 137.0, 135.9, 135.7, 133.5, 132.2, 130.7, 130.3, 129.5, 129.2, 128.8, 127.8, 121.7, 117.6, 82.9, 81.9, 54.5, 52.2, 52.0, 43.4, 43.3, 41.2, 41.1, 32.8, 28.3, 28.2, 27.5; IR (neat) ν_{max} 3325, 3163, 2980, 1724, 1657, 1609 cm^{-1}; FABHRMS (NBA) *m/z* 621.3405 (M^+ + H, $C_{33}H_{44}N_4O_6$ requires 621.3401).

Di-*tert*-butyl (1*RS*)-*N*-[4-{2-(2-Amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-1-formyl-1-ethyl}benzoyl]-L-glutamate (11). A solution of **10** (20.9 mg, 0.03 mmol) in THF (511 μL) and pH 7 aqueous phosphate buffer (102 μL) cooled to 0 °C was treated with a solution of $CuCl_2$ (28.7 mg, 0.17 mmol) in H_2O (169 μL). The reaction mixture was stirred at 0 °C for 1 h before it was quenched by the dropwise addition of a pH 8 saturated aqueous NH_4Cl–NH_4OH (30 mL). The solution was extracted with $CHCl_3$ (3 × 25 mL) purged with N_2, dried (Na_2SO_4), filtered, and the solvent was removed under reduced pressure. PCTLC (SiO_2, 1 mm plate, 10% CH_3OH–$CHCl_3$) afforded **11** (9.0 mg, 46%) as a white solid: mp > 300 °C; ^{1}H NMR (DMF-d_7, 400 MHz) δ 9.83 (1H, d, J = 5.1 Hz, CHO), 7.96 (2H, d, J = 8.3 Hz), 7.81 (1H, d, J = 2.0 Hz, H-5), 7.46 (1H, dd, J = 2.1, 8.2 Hz, H-7), 7.41 (2H, d, J = 8.4 Hz), 7.14 (1H, d, J = 8.4 Hz, H-8), 6.88 (2H, br s, NH_2), 4.50 (1H, m, NHC*H*$CO_2C(CH_3)_3$), 4.25 (1H, t, J = 7.0 Hz, CH), 3.56 (1H, dd, J = 6.6, 14.3 Hz, C*H*H), 3.15 (1H, dd, J = 8.5, 14.0 Hz, CH*H*), 2.44 (2H, t, J = 7.9 Hz, $CH_2CH_2CO_2C(CH_3)_3$), 2.15 (1H, m), 2.04 (1H, m), 1.43 (9H, s, $CO_2C(CH_3)_3$), 1.41 (9H, s, $CO_2C(CH_3)_3$); IR (neat) ν_{max} 3388, 2961, 2920, 2859,

2737, 1722, 1651, 1482 cm^{-1}; FABHRMS (NBA) *m/z* 579.2829 (M^+ + H, $C_{31}H_{35}N_4O_7$ requires 579.2819).

(10*RS*)-10-Formyl-5,8,10-trideazafolate (3). A solution of **11** (10.1 mg, 0.017 mmol) in $CHCl_3$ (99 μL) cooled to 0 °C was treated with 33 μL of trifluoroacetic acid. The solution was stirred at 0 °C for 2 h and 25 °C for 12 h. Et_2O (1 mL) was added to the reaction mixture and a white precipitate formed. The precipitate was triturated with Et_2O (3 × 1 mL) and dried in vacuo to give **3**·CF_3CO_2H (8.5 mg, 84%) as a white solid: mp > 300 °C; ^1H NMR (DMF-d_7, 400 MHz) δ 9.83 (1H, d, *J* = 1.4 Hz, CHO), 7.98 (2H, d, *J* = 8.7 Hz), 7.91 (1H, s, H-5), 7.68 (1H, d, *J* = 7.6 Hz, H-7), 7.44 (2H, d, *J* = 8.2 Hz), 7.28 (1H, d, *J* = 8.3 Hz, H-8), 4.61 (1H, m, NHC*H*$CO_2C(CH_3)_3$), 4.30 (1H, t, *J* = 7.8 Hz, CH), 3.60 (1H, dd, *J* = 6.5, 14.8 Hz, C*H*H), 3.22 (1H, dd, *J* = 8.5, 13.7 Hz, CH*H*), 2.50 (2H, t, *J* = 7.5 Hz, $CH_2CH_2CO_2H$), 2.25 (1H, m), 2.08 (1H, m); IR (KBr) ν_{max} 3304, 2932, 2742, 2612, 1702, 1681, 1639 cm^{-1}; FABHRMS (NBA) *m/z* 467.1590 (M^+ + H, $C_{23}H_{22}N_4O_7$ requires 467.1567).

Dimethyl (2*RS*)-*N*-[4-{3-(2-Amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-1-(dimethylhydrazono)prop-2-yl}benzoyl]-L-glutamate (13). A slurry of **8** (17.4 mg, 0.04 mmol) and **12**[39] (17 mg, 0.05 mmol) in 10% DMF–THF (4.6 mL) cooled to 0 °C was treated with Et_3N (20 μL, 0.14 mmol) followed by diphenylphosphoryl azide (DPPA, 12 μL, 0.05 mmol) and the reaction mixture was stirred at 0 °C for 18 h. The solvent was removed under reduced pressure. PCTLC (SiO_2, 1 mm plate, 10% CH_3OH–$CHCl_3$) afforded **13** (17.3 mg, 63%) as a yellow solid as a 1:1 mixture of separable diastereomers: mp > 300 °C; ^1H NMR (CD_3OD, 400 MHz) diastereomer A: δ 7.92 (2H, d, *J* = 8.5 Hz), 7.74 (1H, s, H-5), 7.37 (2H, d, *J* = 8.2 Hz), 7.36 (1H, m, H-7), 7.11 (1H, d, *J* = 8.3 Hz, H-8), 6.87 (1H, d, *J* = 6.4 Hz, HC=N), 4.61 (1H, m, C*H*CO_2CH_3), 3.88 (1H, m, CH), 3.72 (3H, s, OCH_3), 3.65 (3H, s, OCH_3), 3.30 (1H, m), 3.11 (1H, dd, *J* = 8.1, 13.6 Hz, C*H*H), 2.68 (6H, s, $N(CH_3)_2$), 2.45 (2H, t, *J* = 7.1 Hz, $CH_2CH_2CO_2CH_3$), 2.01 (2H, m); diastereomer B: (DMF-d_7, 400 MHz) δ 7.90 (2H, d, *J* = 8.2 Hz), 7.76 (1H, d, *J* = 1.9 Hz, H-5), 7.39 (1H, dd, *J* = 2.0, 8.4 Hz, H-7), 7.41 (2H, d, *J* = 8.1 Hz), 7.07 (1H, d, *J* = 8.3 Hz, H-8), 6.85 (1H, d, *J* = 6.1Hz, HC=N), 4.61 (1H, m, NHC*H*CO_2CH_3), 3.86 (1H, dt, *J* = 6.7, 7.7 Hz, CH), 3.72 (3H, s, OCH_3), 3.62 (3H, s, OCH_3), 3.29 (1H, C*H*H), 3.09 (1H, dd, *J* = 7.5, 13.7 Hz, CH*H*), 2.65 (6H, s, $N(CH_3)_2$), 2.53 (2H, t, *J* = 7.6 Hz, $CH_2CO_2CH_3$), 2.21 (1H, m), 2.10 (1H, m); ^{13}C NMR (DMF-d_7, 100 MHz) diastereomer A: δ 172.5, 162.8, 151.5, 138.7, 138.0, 136.1, 133.7, 130.0, 129.4, 129.2, 129.0, 128.6, 128.3, 126.8, 124.8, 117.8, 52.9, 52.2, 51.7, 51.5, 51.4, 43.09, 43.05, 40.5, 39.1, 26.8; diastereomer B: δ 173.5, 173.1, 167.3, 162.9, 147.7, 138.7, 136.1, 134.0, 132.9, 132.7, 131.5, 129.4, 128.6, 128.3, 126.8, 124.8, 117.8, 57.8, 52.9, 52.2, 51.7, 51.4, 46.6, 43.1, 40.6, 26.8; IR (neat) ν_{max} 3344, 2943, 1827, 1647 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 669.1430 (M^+ + Cs, $C_{27}H_{32}N_6O_6$ requires 669.1438). Anal. calcd for $C_{27}H_{32}N_6O_6$: C, 60.44; H, 6.01; N, 15.66. Found: C, 60.61; H, 6.09; N, 15.78.

Dimethyl (1*RS*)-*N*-[4-{2-(2-Amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-1-formyl-1-ethyl}benzoyl]-L-glutamate (14). A solution of **13** (7.8 mg, 0.008 mmol) in THF (136 μL) and pH 7 aqueous phosphate buffer (30 μL) cooled to 0 °C under N_2 was treated with $CuCl_2$ (7.6 mg, 0.04 mmol) in H_2O (45 μL) and the reaction mixture was stirred at 0 °C for 1 h. The reaction was quenched by the dropwise addition of a pH 8 solution of saturated aqueous NH_4Cl–NH_4OH (20 mL). The solution was extracted with $CHCl_3$ (3 × 25 mL) purged with N_2, dried (Na_2SO_4), filtered, and the solvent was removed under reduced pressure. PCTLC (SiO_2, 1 mm plate, 10% CH_3OH–$CHCl_3$) afforded **14** (4.5 mg, 63%) as a yellow solid: mp > 300 °C; ^1H NMR (DMF-d_7, 400 MHz) δ 9.82 (1H, d, *J* = 1.4 Hz, CHO), 7.95 (2H, d, *J* = 8.2 Hz), 7.77 (1H, s, H-5), 7.42 (2H, d, *J* = 8.2 Hz), 7.41 (1H, dd, *J* = 2.2, 8.3 Hz, H-7), 7.08 (1H, d, *J* = 8.4 Hz, H-8), 6.47 (2H, br s, NH_2), 4.60 (1H, m, NHC*H*CO_2CH_3), 4.23 (1H, t, *J* = 7.8 Hz, CH), 3.68 (3H, s, CO_2CH_3), 3.61 (3H, s, CO_2CH_3), 3.54 (1H, dd, *J* = 6.4, 14.3 Hz, C*H*H), 3.12 (1H, dd, *J* = 8.7, 14.2 Hz, CH*H*), 2.53 (2H, t, *J* = 7.3 Hz, $CH_2CO_2CH_3$), 2.12 (1H, m, C*H*H$CH_2CO_2CH_3$), 2.01 (1H, m, CH*H*$CH_2CO_2CH_3$); IR (neat) ν_{max} 2957, 2917, 2849, 1726, 1637 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 627.0870 (M^+ + Cs, $C_{25}H_{26}N_4O_7$ requires 627.0856).

(2*RS*)-*N*-[4-{3-(2-Amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-1-(dimethylhydrazono)prop-2-yl}-benzoyl]-L-glutamic Acid (15). A solution of **10** (16.5 mg, 0.026 mmol) in $CHCl_3$ (150 μL) cooled to 0 °C was treated with 50 μL of trifluoroacetic acid. The solution was stirred at 0 °C for 2 h and 25 °C for 12 h. Et_2O (1 mL) was added to the reaction mixture and a white precipitate formed. The precipitate was triturated with Et_2O (3 1 mL) and dried in vacuo to give **15**·CF_3CO_2H (18.5 mg, 94%) as a yellow solid and a mixture of diastereomers: mp > 300 °C; ^1H NMR (CD_3OD, 400 MHz) diastereomer A: δ 7.93 (2H, d, *J* = 8.3 Hz), 7.86 (1H, s, H-5), 7.57 (1H, dd, *J* = 1.7, 8.4 Hz, H-7), 7.38 (2H, d, *J* = 6.7 Hz), 7.27 (1H, d, *J* = 8.4 Hz, H-8), 7.11 (1H, d, *J* = 6.0 Hz, HC=N), 4.61 (1H, m, NHC*H*CO_2H), 3.95 (1H, dt, *J* = 6.1, 7.6 Hz, CH), 3.38 (1H, m, C*H*H), 3.28 (1H, m, CH*H*), 2.78 (6H, s, $N(CH_3)_2$), 2.46 (2H, t, *J* = 7.3 Hz, $CH_2CH_2CO_2H$), 2.27 (1H, m, C*H*HCH_2CO_2H), 2.08 (1H, m, C*H*HCH_2CO_2H); diastereomer B: δ 7.78 (2H, d, *J* = 8.4 Hz), 7.85 (1H, s, H-5), 7.57 (1H, dd, *J* = 1.7, 8.4 Hz, H-7), 7.34 (2H, d, *J* = 8.3 Hz), 7.27 (1H, d, *J* = 8.4 Hz, H-8), 7.22 (1H, d, *J* = 6.1 Hz, HC=N), 4.61 (1H, m, NHC*H*CO_2H), 3.95 (1H, dt, *J* = 6.1, 7.6 Hz, CH), 3.38 (1H, m, C*H*H), 3.28 (1H, m, CH*H*), 2.76 (6H, s, $N(CH_3)_2$), 2.46 (2H, t, *J* = 7.3 Hz, $CH_2CH_2CO_2H$), 2.27 (1H, m, C*H*HCH_2CO_2H), 2.08 (1H, m, CH*H*CH_2CO_2H); IR (neat) ν_{max} 3323, 3189, 3035, 1723, 1692 cm^{-1}; FABHRMS (NBA) *m/z* 509.2145 (M^+ + H, $C_{25}H_{28}N_6O_6$ requires 509.2149).

Methyl (2*R)-4-[3-(2-Amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-1-(dimethylhydrazono)prop-2-yl]benzoate (16).** A solution of **8** (10 mg, 0.02 mmol) in DMF (10 mL) was treated with excess CH_2N_2 in Et_2O at 25 °C for 24 h before the solvent was removed under reduced pressure. PCTLC (SiO_2, 1 mm plate, 10% CH_3OH–$CHCl_3$) gave **16** (10 mg, 96%) as a yellow oil: ^1H NMR (DMF-d_7, 400 MHz) δ 11.09 (1H, br s, NH), 7.89 (2H, d, J = 8.2 Hz), 7.73 (1H, d, J = 1.5 Hz, H-5), 7.46 (2H, d, J = 8.3 Hz), 7.39 (1H, dd, J = 2.1, 8.4 Hz, H-7), 7.08 (1H, d, J = 8.3 Hz, H-8), 6.86 (1H, d, J = 6.0 Hz, HC=N), 6.50 (2H, br s, NH_2), 3.90 (1H, m, CH), 3.85 (3H, s, OCH_3), 3.29 (1H, dd, J = 7.8, 13.7 Hz, C*H*H), 3.08 (1H, dd, J = 7.7, 13.7 CH*H*), 2.65 (6H, s, $N(CH_3)_2$); ^{13}C NMR (DMF-d_7, 100 MHz) δ 167.0, 162.9, 153.6, 149.6, 138.3, 136.1, 133.7, 130.1, 130.0, 129.3, 129.0, 128.7, 126.8, 124.8, 117.8, 52.2, 51.5, 43.0, 40.6; IR (neat) ν_{max} 3333, 1620, 1415, 1276 cm^{-1}; FABHRMS (NBA) m/z 394.1875 (M^+ + H, $C_{22}H_{22}N_4O_4$ requires 394.1879).

Methyl (2*R)-4-[2-(2-Amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-1-formyl-1-ethyl]benzoate (17).** A solution of **16** (11.3 mg, 0.03 mmol) in THF (436 μL) and pH 7 aqueous phosphate buffer (87 μL) cooled to 0 °C was treated with $CuCl_2$ (25 mg, 0.14 mmol) in H_2O (144 μL) and the reaction mixture was stirred at 0 °C for 1 h. The reaction was quenched by the addition of a pH 8 solution of saturated aqueous NH_4Cl–NH_4OH (20 mL) and extracted with $CHCl_3$ (3 × 20 mL) purged with N_2. The combined organic extracts were dried, filtered, and the solvent was removed under reduced pressure. PCTLC (SiO_2, 1 mm plate, 10% CH_3OH–$CHCl_3$) afforded **17** (5.5 mg, 55%) as a yellow oil: ^1H NMR (DMF-d_7, 400 MHz) δ 9.84 (1H, d, J = 1.5 Hz, CHO), 7.94 (2H, d, J = 8.4 Hz), 7.74 (1H, d, J = 1.9 Hz, H-5), 7.47 (2H, d, J = 6.5 Hz), 7.39 (1H, dd, J = 2.2, 8.4 Hz, H-7), 7.07 (1H, d, J = 8.4 Hz, H-8), 6.58 (2H, br s, NH_2), 4.27 (1H, m, CH), 3.83 (3H, s, OCH_3), 3.55 (1H, dd, J = 6.6, 14.3 Hz, C*H*H), 3.12 (1H, dd, J = 8.8. 14.0 Hz, CH*H*); IR (KBr) ν_{max} 3155, 2928, 2837, 1722, 1645 cm^{-1}; FABHRMS (NBA) m/z 352.1288 (M^+ + H, $C_{19}H_{17}N_3O_4$ requires 352.1297).

For **18**: off-white solid, mp > 300 °C; ^1H NMR (DMF-d_7, 400 MHz) δ 8.23 (2H, d, J = 8.6 Hz), 8.12 (2H, d, J = 8.6 Hz), 7.92 (1H, d, J = 2.0 Hz, H-5), 7.57 (1H, dd, J = 2.1, 8.4 Hz, H-7), 7.24 (1H, d, J = 8.4 Hz, H-8), 4.58 (2H, s, CH_2), 3.93 (3H, s, OCH_3); IR (neat) ν_{max} 2959, 1682, 1611, 1479, 1261 cm^{-1}; FABHRMS (NBA) m/z 338.1135 (M^+ + H, $C_{18}H_{15}N_3O_4$ requires 338.1141).

Methyl (2*R)-4-[1-Formyl-2-(2-trimethylacetimido-3,4-dihydro-4-oxo-quinazolin-6-yl)-1-ethyl]benzoate (19).** A solution of **7** (27.4 mg, 0.05 mmol) in THF (0.87 mL) and pH 7 aqueous phosphate buffer (191 μL) cooled to 0 °C was treated with $CuCl_2$ (49 mg, 0.28 mmol) in H_2O (287 μL) and the reaction mixture was stirred at 0 °C for 1 h. The reaction was quenched by the addition of a pH 8 solution of saturated aqueous NH_4Cl–NH_4OH (40 mL) and extracted with $CHCl_3$ (3 × 30 mL) purged with N_2. The combined organic extracts were dried (Na_2SO_4), filtered, and the solvent was removed under reduced pressure. PCTLC (SiO_2, 1 mm plate, 10% CH_3OH–$CHCl_3$) afforded **19** (17 mg, 69%) as a yellow oil: ^1H NMR (DMF-d_7, 400 MHz) δ 9.74 (1H, d, J = 1.2 Hz, CHO), 7.96 (2H, d, J = 8.4 Hz), 7.91 (1H, d, J = 2.1 Hz, H-5), 7.29 (1H, dd, J = 2.0, 8.4 Hz, H-7), 7.24 (1H, d, J = 7.2 Hz, H-8), 7.16 (2H, d, J = 10.1 Hz), 3.91 (1H, m, CH), 3.89 (3H, s, OCH_3), 3.55 (1H, dd, J = 6.4, 14.1 Hz, CH*H*), 3.03 (1H, dd, J = 8.4, 14.2 Hz, C*H*H), 1.31 (9H, s, $CO(CH_3)_3$); ^{13}C NMR (DMF-d_7, 100 MHz) δ 198.5, 180.0, 166.5, 140.1, 136.5, 136.2, 130.3, 129.9, 129.8, 129.0, 128.3, 126.8, 124.8, 119.8, 60.5, 52.2, 45.0, 40.4, 35.6, 27.0; IR (neat) ν_{max} 3202, 29611, 2877, 2719, 1719, 1668, 1565 cm^1; FABHRMS (NBA) m/z 436.1870 (M^+ + H, $C_{24}H_{25}N_3O_5$ requires 436.1872).

6-[3-(1-Dimethylhydrazono)prop-1-yl]-2-(trimethylacetimido)-3,4-dihydro-4-oxo-quinazoline (20). A solution of freshly prepared LDA (13.5 mL of 0.3 M, 4.0 mmol) cooled to 0 °C under N_2 was treated with the *N*,*N*-dimethylhydrazone of acetaldehyde[34] (304 mg, 3.68 mmol) and the resulting solution was stirred at 0 °C for 30 min. The solution was cooled to −78 °C and a solution of **6**[36] (415 mg, 1.22 mmol) dissolved in a minimal amount of HMPA was added and the solution was stirred at −78 °C for 16 h under N_2. The reaction mixture was warmed to 25 °C and quenched by the dropwise addition of a saturated aqueous NH_4Cl (30 mL). This mixture was poured into saturated aqueous LiCl (30 mL). The product was extracted with EtOAc (3 × 40 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Chromatography (SiO_2, 65 mm × 250 mm, 10% CH_3OH–$CHCl_3$) gave **20** (300 mg, 69%) as an off-white solid: mp 185–186 °C; ^1H NMR (CD_3OD, 400 MHz) δ 7.95 (1H, d, J = 1.7 Hz, H-5), 7.62 (1H, dd, J = 2.1, 8.4 Hz, H-7), 7.46 (1H, d, J = 8.3 Hz, H-8), 6.79 (1H, t, J = 5.3 Hz, HC=N), 2.91 (2H, t, J = 7.3 Hz, CH_2), 2.66 (6H, s, $N(CH_3)_2$), 2.56 (2H, q, J = 7.8 Hz, CH_2), 1.32 (9H, s, $C(CH_3)_3$); ^{13}C NMR (CD_3OD, 100 MHz) δ 182.9, 162.9, 148.6, 140.5, 136.8, 127.2, 126.5, 120.4, 98.8, 79.5, 43.4, 41.3, 35.2, 34.3, 27.0, 21.1; IR (neat) ν_{max} 3206, 2927, 1664, 1632 cm^{-1}; FABHRMS (NBA) m/z 344.2087 (M^+ + H, $C_{18}H_{25}N_5O_2$ requires 344.2087).

2-Amino-6-[3-(dimethylhydrazono)prop-1-yl]-3,4-dihydro-4-oxo-quinazoline (21). A solution of **20** (17.7 mg, 0.05 mmol) in THF:H_2O:CH_3OH (3:1:1, 0.12 mL) was treated with aqueous 1 N LiOH (0.1 mL, 0.1 mmol) and the solution was stirred at 25 °C for 24 h. Additional aqueous 1 N LiOH (0.1 mL, 0.1 mmol) was added and the solution was stirred at 25 °C for an additional 24 h. The mixture was diluted with H_2O (0.5 mL) and the aqueous layer was washed with EtOAc (3 × 5 mL) and acidified with the addition of 10% aqueous HCl (0.2 mL, pH 4). The precipitated product was collected by filtration to give **21** (8.2 mg, 61%) as an off-white solid: mp > 300 °C; ^1H NMR (CD_3OD, 400 MHz) δ 7.84 (1H, d, J = 2.0 Hz, H-5),

7.49 (1H, dd, J = 2.0, 8.3 Hz, H-7), 7.20 (1H, d, J = 8.4 Hz, H-8), 6.79 (1H, t, J = 5.4 Hz, HC=N), 2.86 (2H, t, J = 7.8 Hz, CH_2), 2.65 (6H, s, $N(CH_3)_2$), 2.54 (2H, q, J = 7.9 Hz, CH_2); IR (KBr) ν_{max} 3159, 1625, 1480 cm^{-1}; FABHRMS (NBA–NaI) m/z 282.1323 (M^+ + Na, $C_{13}H_{17}N_5O$ requires 282.1331).

3-(2-Amino-3,4-dihydro-4-oxo-quinazolin-6-yl)propanal (22). A solution of **21** (18.3 mg, 0.07 mmol) in THF (1.07 mL) and pH 7 aqueous phosphate buffer (235 μL) cooled to 0 °C was treated with a solution of $CuCl_2$ (60 mg, 0.35 mmol) in H_2O (353 μL). The reaction mixture was stirred at 0 °C for 18 h before it was quenched by the dropwise addition of pH 8 saturated aqueous NH_4Cl–NH_4OH (30 mL). The solution was extracted with 20% CH_3OH–$CHCl_3$ (3 × 25 mL) purged with N_2, dried (Na_2SO_4), filtered, and the solvent was removed under reduced pressure. PCTLC (SiO_2, 1 mm plate, 15% CH_3OH–$CHCl_3$) afforded **22** (3.1 mg, 20%) as an off-white solid: mp > 300 °C; ^1H NMR (DMF-d_7, 400 MHz) δ 11.03 (1H, br s, NH), 9.79 (1H, t, J = 1.3 Hz, CHO), 7.79 (1H, d, J = 2.1 Hz, H-5), 7.47 (1H, dd, J = 2.2, 8.4 Hz, H-7), 7.16 (1H, d, J = 8.4 Hz, H-8), 6.48 (2H, br s, NH_2), 2.91 (2H, q, J = 7.3 Hz, CH_2), 2.83 (2H, t, J = 7.3 Hz, CH_2); IR (neat) ν_{max} 3375, 2915, 2725, 1629, 1597 cm^{-1}; Electrospray MS (NBA) m/z 218 (M^+).

Imine formation of 19 with glycinamide: methyl 4-[1-(*N*-amidomethyl)iminomethylene-2-(2-trimethylacetimido-3,4-dihydro-4-oxo-quinazolin-6-yl)-1-ethyl]-benzoate. A slurry of **19** (7.8 mg, 0.01 mmol), glycinamide (4 mg, 0.03 mmol) and Na_2CO_3 (1.3 mg, 0.01 mmol) in 25% CH_3OH–CH_2Cl_2 (0.13 mL) was stirred at 25 °C for 8 h. The solids were removed by filtration and the filtrate concentrated under reduced pressure to afford the imine (7.4 mg, 84%) as a yellow oil: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.99 (1H, d, J = 1.5 Hz, H-5), 7.95 (2H, d, J = 10.1 Hz), 7.81 (1H, d, J = 4.4 Hz, HCN), 7.31 (1H, dd, J = 1.9, 8.3 Hz, H-7), 7.24 (1H, d, J = 6.5 Hz, H-8), 7.20 (2H, d, J = 8.4 Hz), 6.48 (1H, br s, NH), 5.65 (1H, br s, NH), 4.02 (2H, s, CH_2), 3.88 (3H, s, OCH_3), 3.88 (1H, m, CH), 3.46 (1H, dd, J = 7.7, 14.1 Hz, C*H*H), 3.13 (1H, dd, J = 7.6, 14.2 Hz, CH*H*), 1.30 (9H, s, $CO_2C(CH_3)_3$); IR (neat) ν_{max} 3195, 2969, 1714, 1662 cm^{-1}; FABHRMS (NBA) m/z 492.2256 (M^+ + H, $C_{26}H_{29}N_5O_5$ requires 492.2247).

Di-*tert*-butyl *N*-[4-{2-(2-Amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-1-oxo-1-ethyl}benzoyl]-L-glutamate (23). A slurry of **11** (2.5 mg, 0.004 mmol) and SiO_2 (20 mg) in $CHCl_3$ (500 μL, 0.008 M) was stirred at 25 °C for 3 h. Chromatography through a plug of SiO_2 (10% CH_3OH–$CHCl_3$) afforded **23** (2.1 mg, 86%) as a yellow oil: $[\alpha]^{23}_D$ +4.5 (c 0.09, $CHCl_3$); ^1H NMR (DMF-d_7, 400 MHz) δ 8.20 (2H, d, J = 8.5 Hz), 8.09 (2H, d, J = 8.6 Hz), 7.90 (1H, s, H-5), 7.54 (1H, dd, J = 2.0, 8.3 Hz, H-7), 7.20 (1H, d, J = 8.3 Hz, H-8), 6.55 (2H, br s, NH_2), 4.54 (2H, s, CH_2), 4.52 (1H, m, $HNCHCO_2C(CH_3)_3$), 2.45 (2H, t, J = 7.6 Hz, $CH_2CO_2C(CH_3)_3$), 2.17 (1H, m, $CHHCH_2CO_2C(CH_3)_3$), 2.05 (1H, m, $CHHCH_2CO_2C(CH_3)_3$), 1.43 (9H, s, $CO_2C(CH_3)_3$), 1.40 (9H, s, $CO_2C(CH_3)_3$); ^{13}C NMR (DMF-d_7, 100 MHz) δ 198.1, 172.4, 171.8, 166.9, 141.9, 139.5, 138.9, 136.5, 134.5, 130.1, 129.5, 129.1, 128.4, 127.5, 125.2, 118.1, 81.4, 53.7, 45.2, 45.1, 32.2, 28.0, 27.0; IR (neat) ν_{max} 3326, 2933, 1726, 1655 cm^{-1}; FABHRMS (NBA–NaI) m/z 565.2670 (M^+ + H, $C_{30}H_{36}N_4O_7$ requires 565.2662).

***N*-[4-{2-(2-Amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-1-oxo-1-ethyl}benzoyl]-L-glutamic acid (24).** A solution of **23** (3.8 mg, 0.006 mmol) in $CHCl_3$ (37 μL) cooled to 0 °C was treated with 12 μL of trifluoroacetic acid. The solution stirred at 0 °C for 2 h and 25 °C for 12 h. Et_2O (1 mL) was added to the reaction mixture and a white precipitate formed. The precipitate was triturated with Et_2O (3 × 1 mL) and dried in vacuo to give **24**·CF_3CO_2H (3.0 mg, 65%) as a white solid: mp > 300 °C; $[\alpha]^{25}_D$ +7.8 (c 0.02, CH_3OH); ^1H NMR (DMF-d_7, 400 MHz) δ 9.01 (2H, br s, NH_2), 8.27 (1H, d, J = 8.2 Hz), 8.21 (1H, d, J = 8.3 Hz), 8.15 (1H, d, J = 8.2 Hz), 8.13 (1H, d, J = 8.3 Hz), 8.03 (1H, s, H-5), 7.80 (1H, dd, J = 1.9, 8.4 Hz, H-7), 7.43 (1H, d, J = 8.4 Hz, H-8), 4.70 (2H, d, J = 14.2 Hz, CH_2), 4.64 (1H, m, $HNCHCO_2H$), 2.53 (2H, t, J = 7.0 Hz, $CH_2CH_2CO_2H$), 2.27 (1H, m, $CHHCH_2CO_2H$), 2.11 (1H, m, $CHHCH_2CO_2H$); IR (KBr) ν_{max} 3362, 1707, 1625 cm^{-1}; FABHRMS (NBA) m/z 453.1420 (M^+ + H, $C_{22}H_{20}N_4O_7$ requires 453.1410).

GAR Tfase inhibitor screening

As an initial screen to determine approximate K_i values, 0.5 mg of each compound was dissolved in 100 μL DMSO. Aliquots of these solutions were then diluted in H_2O or assay buffer (50 mM Tris, 1 mM EDTA, pH 7.5) to 0.7 mM. The concentrations of DMSO used in these assays showed no effect on the *pur*N GAR Transformylase activity. *Pur*N GAR Transformylase (2 nM) was mixed with 10 μM of the potential inhibitors and 20 μM substrate 10-formyl-5,8-dideazafolate (fDDF) in a total volume of 1 mL assay buffer. This mixture was incubated at 26 °C for 2 min. Assays were initiated by the addition of 50 μM GAR. The assay monitors the deformylation of fDDF ($\Delta\varepsilon$ = 18.9 mM^{-1} cm^{-1} at 295 nm) resulting from the transfer of the formyl group to GAR.[33] Reaction rates were measured in triplicate using 1 mL cuvettes thermostated to 26 °C on a Gilford 252 spectrophotometer. The K_i values were determined using eq 4 where v_i is the enzyme rate in the presence of the inhibitor, v is the enzyme rate in the absence of any inhibitor, S is the concentration of fDDF, and $[I]$ is the concentration of the inhibitor.[42]

$$\frac{v_i}{v} = \frac{K_m + S}{K_m(1 + [I]/K_i) + S} \quad (4)$$

For compounds with K_i < 10 μM, a series of $1/v_i$ versus $1/S$ plots at different fixed concentrations of I were generated in order to more accurately determine K_i.

The slopes of these plots are given by eq 5.

$$\text{slope} = \frac{K_m}{V_{max}K_i}[I] + \frac{K_m}{V_{max}} \quad (5)$$

A replot of these slopes versus $[I]$ will generate a straight line with a y-intercept of K_m/V_{max} and a x-intercept of K_i.[42]

Time-dependent inhibition of GAR Tfase

In order to test for possible adduct formation, *pur*N GAR Tfase was incubated with substrate GAR and the potential inhibitors for varying lengths of time of up to 6 h. When *pur*N GAR Tfase was diluted to 2 nM in assay buffer, nearly 60% of the enzyme activity was lost over a 6-h time span. In order to help stabilize the enzyme for the duration of the experiment, the *pur*N GAR Tfase stock solutions were diluted with 0.1 mg/mL (1.5 μM) bovine serum albumin (BSA) in assay buffer, resulting in a final concentration of 30 nM BSA in the assays. Under these conditions, loss of *pur*N GAR Tfase activity was only 8% over a 6-h time span.

Stock solutions were made containing 20 nM *pur*N GAR Tfase, 300 nM BSA, 500 μM GAR, and 100 μM of the inhibitor in assay buffer. These solutions were incubated at room temperature. Aliquots of these stock solutions were taken, diluted tenfold in assay buffer, and thermostated to 26 °C on a Gilford 252 spectrophotometer. Assays were initiated by the addition of 20 μM fDDF. Time points were taken at t = 3, 15, 30, 60, 120, 180, and 360 min for each compound.

AICAR Tfase inhibitor screening

The enzyme used in all of the inhibition studies was the avian AICAR Tfase, which was fused to an N-terminal 6X histidine tag to facilitate purification. An initial screening to determine approximate K_i values for each of the potential inhibitors was done. The compounds (0.5 mg) were dissolved in 100 μL of DMSO and aliquots of these solutions were diluted in assay buffer (32.5 mM Tris–HCl, 25 mM KCl, 5 mM β-mercaptoethanol, pH 7.4) to a concentration of 1 mM. The concentrations of DMSO used in the assays did not appear to have any effect on the activity of AICAR Tfase. 100 nM of enzyme, 10 μM of inhibitor, and 50 μM of cofactor N^{10}-formyl tetrahydrofolate were mixed in assay buffer to a volume of 950 μL. This mixture was incubated at 26 °C for 2 min before initiating the reaction by the addition of 50 μM AICAR, yielding a final volume of 1 mL. The assay buffer was degassed and flushed with Ar prior to use in order to minimize oxidation of N^{10}-formyl tetrahydrofolate. The reaction was monitored by measuring the increase in absorbance due to formation of tetrahydrofolate produced by the transfer of the formyl group to product FAICAR ($\Delta\varepsilon$ = 19.7 mM^{-1} cm^{-1} at 298 nm). Rates were measured in triplicate using the same instrumentation used for the GAR Tfase measurements, and eq 4 was used to determine the K_i values.

Time-dependent inhibition of AICAR Tfase

Potential adduct formation was investigated by incubating AICAR Tfase with AICAR and each inhibitor for varying lengths of time up to 6 h. The enzyme maintained 100% activity after 6 h at room temperature. Stock solutions contained 3.33 μM of enzyme, 1.67 mM AICAR, and 0.33 mM inhibitor. These stock solutions were incubated at room temperature. Aliquots were taken and diluted in assay buffer, then thermostated to 26 °C on the Gilford spectrophotometer. The reactions were initiated by the addition of 50 μM of N^{10}-formyl tetrahydrofolate. Time points were taken at t = 3, 180, and 360 min for each of the inhibitors.

Cytotoxicity testing

The ability of the test compounds to inhibit tumor cell growth was determined using both L-1210 mouse lymphocytic leukemia cells (ATCC CCL 219) and CCRF-CEM human leukemic cells (ATCC CCL 119). They were cultivated in RPMI1640 (a medium which lacks purines and pyrimidines, or derivatives of them) supplemented with 5% FBS which had been exhaustively dialyzed against normal saline. The medium also contained 26 mM sodium bicarbonate, 100 units/mL of penicillin G and 100 μg/mL of streptomycin sulfate. Each well of a 48-well cluster dish was inoculated with 1×10^4 cells in 0.5 mL of the medium and the cultures were incubated for 24 h at 37 °C in a humidified atmosphere of 5.5% CO_2/94.5% air.

An accurately weighed sample of the test compound was dissolved in DMSO at 10 mg/mL. Identical portions of the DMSO solution were then mixed with either fresh, homologous medium or the same medium supplemented with 0.1 mM hypoxanthine, bringing the concentration of the test compound to 200 μg/mL and of DMSO to 2% v/v. Serial dilutions of these newly prepared stock solutions were made in the corresponding media and DMSO was added at each dilution to maintain its concentration at 2%. Then, 0.5 mL of each dilution was added to replicate wells of the 48-well cluster dish, each of which already contained 0.5 mL of the day-old culture. At this point, the volume in each well was 1 mL and the DMSO concentration was 1%.

The cultures were incubated for a further 48 h and then the cell population in each well was determined using a Coulter particle counter. The mean number of cells in the wells at each concentration of test compound was determined, both in the absence of hypoxanthine and in the presence of 50 μM hypoxanthine (n = 12 for the controls; n = 4 for each test concentration). The cell population was plotted (log–log) as a function of the concentration of the test compound and from the graph the concentration which reduced the control cell population by 50% was determined.

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References

1. Warren, L.; Buchanan, J. M. *J. Biol. Chem.* **1957**, *229*, 613. Buchanan, J. M.; Hartman, S. C. *Adv. Enzymol.* **1959**, *21*, 199.

2. Benkovic, S. J.; Slieker, L. J.; Daubner, S. C.; Courtney, L. F.; Dix, T. A.; Pember, S. O.; Bloom, L. M.; Fierke, C. A.; Mayer, R. J.; Chen, J.-T.; Taira, K. In *Chemistry and Biology of Pteridines*; Cooper, B. A.; Whitehead, V. M., Eds.; Walter de Gruyter: Berlin, 1986; pp 13–28. Benkovic, S. J.; Young, M. In *Enzyme Mechanisms*; Page, M. I.; Williams, A., Eds.; Royal Society of Chemistry: London, 1987; pp 429–441.

3. Inglese, J.; Johnson, D. L.; Shiau, A.; Smith, J. M.; Benkovic, S. J. *Biochemistry* **1990**, *29*, 1436.

4. Inglese, J.; Smith, J. M.; Benkovic, S. J. *Biochemistry* **1990**, *29*, 6678.

5. Aimi, J.; Qiu, H.; Williams, J.; Zalkin, H.; Dixon, J. E. *Nucleic Acids Res.* **1990**, *18*, 6665.

6. Marolewski, A.; Smith, J. M.; Benkovic, S. J. *Biochemistry* **1994**, *33*, 2531.

7. Daubner, S. C.; Schrimsher, J. L.; Schendel, F. J.; Young, M.; Henikoff, S.; Patterson, D.; Stubbe, J.; Benkovic, S. J. *Biochemistry* **1985**, *24*, 7059.

8. Daubner, S. C.; Young, M.; Sammons, R. D.; Courtney, L. F.; Benkovic, S. J. *Biochemistry* **1986**, *25*, 2951.

9. Henikoff, S.; Keene, M. A.; Sloan, J. S.; Bleskan, J.; Hards, R.; Patterson, D. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 720.

10. Rosowsky, A.; Galivan, J.; Beardsley, G. P.; Bader, H.; O'Conner, B. M.; Russello, O.; Moroson, B. A.; DeYarman, M. T.; Kerwar, S. S.; Freisheim, J. H. *Cancer Res.* **1992**, *52*, 2148.

11. Nagy, P. L.; Marolewski, A.; Benkovic, S. J.; Zalkin, H. *J. Bacteriol.* **1995**, *117*, 1292.

12. Gots, J. S.; Benson, C. E.; Jochimsen, B.; Koduri, K. R. In *Purine and Pyrimidine Metabolism*; Elliott, K.; Fitzsimons, D. W., Eds.; Elsevier: Amsterdam, 1977; 23.

13. Divekar, A. Y.; Hakala, M. T. *Mol. Pharmacol.* **1975**, *11*, 319. Moras, R. G. *Cancer Treatment and Research* **1991**, *58*, 65. Berman, E. M.; Werbel, L. M. *J. Med. Chem.* **1991**, *34*, 479.

14. Taylor, E. C.; Harrington, P. J.; Fletcher, S. R.; Beardsley, G. P.; Moran, R. G. *J. Med. Chem.* **1985**, *28*, 914.

15. Beardsley, G. P.; Taylor E. C.; Grindey, G. B.; Moran, R. G. In *Chemistry and Biology of Pteridines*; Cooper, B. A.; Whitehead, V. M., Eds.; Walter de Gruyter: Berlin, 1986; pp 953–957.

16. Taylor, E. C.; Wong, G. S. K.; Fletcher, S. R.; Harrington, P. J.; Beardsley, G. P.; Shih, C. J. In *Chemistry and Biology of Pteridines*; Cooper, B. A.; Whitehead, V. M., Eds.; Walter de Gruyter: Berlin, 1986; pp 61–64.

17. Beardsley, G. P.; Moroson, B. A.; Taylor, E. C.; Moran, R. G. *J. Biol. Chem.* **1989**, *264*, 328.

18. Moran, R. G.; Baldwin, S. W.; Taylor, E. C.; Shih, C. *J. Biol. Chem.* **1989**, *264*, 21047.

19. Taylor, E. C.; Wong, G. S. K. *J. Org. Chem.* **1989**, *54*, 3618. Taylor, E. C.; Harrington, P. M.; Warner, J. C. *Heterocycles* **1988**, *27*, 1925. Taylor, E. C.; Harrington, P. M. *J. Org. Chem.* **1990**, *55*, 3222.

20. Barnett, C. J.; Wilson, T. M.; Wendel, S. R.; Winningham, M. J.; Deeter, J. B. *J. Org. Chem.* **1994**, *59*, 7038.

21. Taylor, E. C. *J. Heterocycl. Chem.* **1990**, *27*, 1.

22. Baldwin, S. W.; Tse, A.; Gossett, L. S.; Taylor, E. C.; Rosowsky, A.; Shih, C.; Moran, R. G. *Biochemistry* **1991**, *30*, 1997.

23. For related analogues and studies see: Caperelli, C. A. *J. Med. Chem.* **1987**, *30*, 1254. Taylor, E. C.; Hamby, J. M.; Shih, C.; Grindey, G. B.; Rinzel, S. M.; Beardsley, G. P.; Moran, R. G. *J. Med. Chem.* **1989**, *32*, 1517. Kelley, J. L.; McLean, E. W.; Cohn, N. K.; Edelstein, M. P.; Duch, D. S.; Smith, G. K.; Hanlon, M. H.; Ferone, R. *J. Med. Chem.* **1990**, *33*, 561. Taylor, E. C.; Gillespie, P.; Patel, M. *J. Org. Chem.* **1992**, *57*, 3218. Taylor, E. C.; Schrader, T. H.; Walensky, L. D. *Tetrahedron* **1992**, *48*, 19. Bigham, E. C.; Hodson, S. J.; Mallory, W. R.; Wilson, D.; Duch, D. S.; Smith, G. K.; Robert, F. *J. Med. Chem.* **1992**, *35*, 1399. Taylor, E. C.; Kuhnt, D.; Shih, C.; Rinzel, S. M.; Grindey, G. B.; Barredo, J.; Jannatipour, M.; Moran, R. G. *J. Med. Chem.* **1992**, *35*, 4450. Piper, J. R.; Johnson, C. A.; Otter, G. M.; Sirotnak, F. M. *J. Med. Chem.* **1992**, *35*, 3002. Shih, C.; Gossett, L. S.; Worzalla, J. F.; Rinzel, S. M.; Grindey, G. B.; Harrington, P. M., Taylor, E. C. *J. Med. Chem.* **1992**, *35*, 1109. Shih, C.; Grindey, G. B.; Taylor, E. C.; Harrington, P. M. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 339. Shih, C.; Hu, Y.; Gossett, L. S.; Habeck, L. L.; Mendelsohn, L. G.; Grindey, G. B. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2657. Taylor, E. C. In *Chemistry and Biology of Pteridines and Folates*; Ayling, J. E.; Nair, M. G.; Baugh, C. M., Eds.; Plenum: New York, 1993; pp 387–408. Durucasu, I. *Heterocycles* **1993**, *35*, 1527. Habeck, L. L.; Leitner, T. A.; Shackelford, K. A.; Gossett, L. S.; Schultz, R. M.; Andis, S. L.; Shih, C.; Grindey, G. B.; Mendelsohn, L. G. *Cancer Res.* **1994**, *54*, 1021. Taylor, E. C.; Yoon, C.; Hamby, J. M. *J. Org. Chem.* **1994**, *59*, 7092. Taylor, E. C.; Yoon, C. *J. Org. Chem.* **1994**, *59*, 7096. Pizzorno, G.; Moroson, B. A.; Cashmore, A. R.; Russello, O.; Mayer, J. R.; Galivan, J.; Bunni, M. A.; Priest, D. G.; Beardsley, G. P. *Cancer Res.* **1995**, *55*, 566. Piper, J. R.; Ramamurthy, B.; Johnson, C. A.; Otter, G. M.; Sirotnak, F. M. *J. Med. Chem.* **1996**, *39*, 614. Taylor, E. C.; Young, W. B.; Spanka, C. *J. Org. Chem.* **1996**, *61*, 1261. Gossett, L. S.; Habeck, L. L.; Gates, S. B.; Andis, S. L.; Worzalla, J. F.; Schultz, R. M.; Mendelsohn, L. G.; Kohler, W.; Ratnam, M.; Grindey, G. B.; Shih, C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 473. Taylor, E. C.; Dowling, J. E. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 453. Taylor, E. C.; Zhou, P.; Jennings, L. D.; Mas, Z.; Hu, B.; Jun, J.-G. *Tetrahedron Lett.* **1997**, *33*, 521.

24. Flaks, J. G.; Erwin, M. J.; Buchanon, J. M. *J. Biol. Chem.* **1957**, *229*, 603. Flaks, J. G.; Warren, L.; Buchanon, J. M. *J. Biol. Chem.* **1957**, *228*, 215. Warren, L.; Flaks, J. G.; Buchanon, J. M. *J. Biol. Chem.* **1957**, *229*, 627.

25. Smith, G. K.; Mueller, W. T.; Benkovic, P. A.; Slieker, L. J.; DeBrosse, C. W.; Benkovic, S. J. In *Chemistry and Biology of Pteridins*; Blair, J. A., Ed.; Walter de Gruyter: Berlin, 1983; pp 247–250.

26. Baggott, J. E.; Krumdieck, C. L. *Biochemistry* **1979**, *18*, 1036.

27. Rayl, E. A.; Moroson, B. A.; Beardsley, G. P. *J. Biol. Chem.* **1996**, *271*, 2225. Ni, L.; Guan, K.; Zalkin, H.; Dixon, J. E. *Gene* **1991**, *106*, 197. Chopra, A. K.; Peterson, J. W.; Prasad, R. *Biochim. Biophys. Acta* **1991**, *1090*, 351. Szabados, E.; Hindmarsh, E. J.; Phillips, L.; Duggelby, R. G.;

Christopherson, R. I. *Biochemistry* **1994**, *33*, 14237. Mueller, W. T.; Benkovic, S. J. *Biochemistry* **1981**, *20*, 737. Aiba, A.; Mizobuchi, K. *J. Biol. Chem.* **1989**, *264*, 21239. Ebbole, D. J.; Zalkin, H. *J. Biol. Chem.* **1987**, *262*, 8274.

28. Li, S. W.; Nair, M. G. *Med. Chem. Res.* **1991**, *1*, 353.

29. Boger, D. L.; Haynes, N.-E., Warren, M. S.; Gooljarsingh, L. T.; Ramcharan, J.; Kitos, P. A.; Benkovic, S. J. *Bioorg. Med. Chem.* **1997**, *5*, 1831. Boger, D. L.; Haynes, N.-E.; Warren, M. S.; Ramcharan, J.; Kitos, P. A.; Benkovic, S. J. *Bioorg. Med. Chem.* **1997**, *5*, 1839. Boger, D. L.; Haynes, N.-E.; Warren, M. S.; Ramcharan, J.; Marolewski, A. E.; Kitos, P. A.; Benkovic, S. J. *Bioorg. Med. Chem.* **1997**, *5*, 1847.

30. For related studies with 5-DACTHF, see: Bigham, E. C.; Mallory, W. R.; Hodson, S. J.; Duch, D. S.; Ferone, R.; Smith, G. K. *Heterocycles* **1993**, *35*, 1289.

31. Inglese, J.; Blatchly, R. A.; Benkovic, S. J. *J. Med. Chem.* **1989**, *32*, 937. Inglese, J.; Benkovic, S. J. *Tetrahedron* **1991**, *47*, 2351.

32. Stura, E. A.; Johnson, D. L.; Inglese, J.; Smith, J. M.; Benkovic, S. J.; Wilson, I. A. *J. Biol. Chem.* **1989**, *264*, 9703. Chen, P.; Schulze-Gahmen, U.; Stura, E. A.; Inglese, J.; Johnson, D. L.; Marolewski, A.; Benkovic, S. J.; Wilson, I. A. *J. Mol. Biol.* **1992**, *227*, 283. Almassy, R. J.; Janson, C. A.; Kan, C.-C.; Hostomska, Z. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6114. Klein, C.; Chen, P.; Arevalo, J. H.; Stura, E. A.; Marolewski, A.; Warren, M. S.; Benkovic, S. J.; Wilson, I. A. *J. Mol. Biol.* **1995**, *249*, 153.

33. Smith, G. K.; Mueller, W. T.; Benkovic, P. A.; Benkovic, S. J. *Biochemistry* **1981**, *20*, 1241.

34. Corey, E. J.; Enders, D. *Tetrahedron Lett.* **1976**, 3. Corey, E. J.; Enders, D. *Chem. Ber.* **1978**, *111*, 1337, 1362.

35. Nair, M. G.; Murthy, B. R.; Patil, S. D.; Kisliuk, R. L.; Thorndike, J.; Gaumont, Y.; Ferone, R.; Duch, D. S.; Edelstein, M. P. *J. Med. Chem.* **1989**, *32*, 1277.

36. Acharya, S. P.; Hynes, J. B. *J. Heterocycl. Chem.* **1975**, *12*, 1283.

37. No attempt was made to separate the readily equilibrated mixture of aldehyde diastereomers.

38. Corey, E. J.; Knapp, S. *Tetrahedron Lett.* **1976**, 3667.

39. Commercially available from Bachem Bioscience.

40. Avramoff, M.; Sprinzak, Y. *J. Am. Chem. Soc.* **1963**, *85*, 1655. Wellman, J.; Park, J.; Hershberger, J. F. *Chem. Phys. Lett.* **1991**, *178*, 405. Wallace, T. J.; Pobiner, H.; Schriesheim, A. *J. Org. Chem.* **1965**, *30*, 3768.

41. Warren, M. S.; Mattia, K. M.; Marolewski, A. E.; Benkovic, S. J. *Pure Appl. Chem.* **1996**, *68*, 2029.

42. Segel, I. H. *Biochemical Calculations*, 2nd edn; Wiley: New York, 1976.

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