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Abenzyl 10-Formyl-trideazafolic Acid (Abenzyl 10-Formyl-TDAF): An Effective Inhibitor of Glycinamide Ribonucleotide Transformylase

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Abstract—The synthesis of *N*-[7-(2-amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-6-formyl-1-oxo-heptyl]-L-glutamic acid (**2**, abenzyl 10-formyl-5,8,10-trideazafolic acid) as a potential enzyme-assembled tight binding inhibitor of glycinamide ribonucleotide transformylase (GAR Tfase) or aminoimidazole carboxamide ribonucleotide transformylase (AICAR Tfase) is reported. The inhibitor was prepared by a convergent synthesis utilizing the sequential alkylations of acetaldehyde dimethylhydrazone with **6** and **8**. The agent exhibited effective inhibition of GAR Tfase (K_i = 4.5 ± 0.3 μM) and more modest inhibition of AICAR Tfase (K_i = 42 ± 11 μM). © 1997 Elsevier Science Ltd.

Introduction

In the preceding articles, we detailed the preparation and evaluation of 10-formyl-5,8,10-trideazafolate (**3**, K_i = 0.26 μM)[1,2] and a series of structurally related agents as potential antineoplastic agents with the ability to inhibit glycinamide ribonucleotide transformylase (GAR Tfase) or aminoimidazole carboxamide ribonucleotide transformylase (AICAR Tfase), folate-dependent enzymes responsible for two independent formyl transfer reactions in the de novo synthesis of purines (Fig. 1). Here we report the extension of these studies to the preparation and evaluation of **2**, a close analogue of **3**, which proved to be predictably more stable than the sensitive aldehyde **3**. Based on the results of a preceding study of 5,10-dideazatetrahydrofolate (DDATHF, **4**),[3–11] a potent inhibitor of GAR Tfase (K_i = 0.1 μM) with efficacious antitumor properties, the replacement of the benzoyl ring with appropriate hydrocarbon spacers was found to have no detrimental effects on its enzymatic inhibitory properties and, in fact, to increase the inhibitor potency.[10–13] Thus, **2** was projected to be capable of formation of enzyme-assembled tight binding inhibitor by virtue of imine formation with GAR and consequently serve as an effective and potentially selective inhibitor of the cofactor N^{10}-formyltetrahydrofolate (**1**).[14–27] Because of its stability, **2** was anticipated to maintain or surpass the properties of **3** and permit us to address the questions of enzyme-assembled imine formation with such inhibitors as well as the origin of the modest in vitro cytotoxic activity established for **3**.[1] The aldehyde **2** proved to be an effective inhibitor of GAR Tfase (K_i = 4.5 ± 0.3 μM) albeit less potent than **3** (K_i = 0.26 ± 0.05 μM) and a more modest inhibitor of AICAR Tfase (K_i = 42 ± 11 μM).

Chemistry

The aldehyde **2** was prepared by a route analogous to that detailed for **3**.[1] Alkylation of the lithium anion of acetaldehyde dimethylhydrazone[28,29] with **6**[30] cleanly provided **7** (69%, Scheme 1). Deprotonation of the aldehyde dimethylhydrazone **7** with LDA (THF, 0 °C) followed by alkylation with the ortho ester **8**[31] (2 equiv, THF–HMPA, 0–25 °C, 12 h, 39%) cleanly provided **9** in which smooth hydrolysis of the crude ortho ester to the corresponding methyl ester routinely occurred on chromatographic purification. Treatment of **9** with LiOH (2–4 equiv, THF:CH_3OH:H_2O 3:1:1, 25 °C, 24–48 h, 63%) cleanly provided **10** which was coupled with di-*tert*-butyl L-glutamate hydrochloride (**11**)[32] upon treatment with DPPA (1.2 equiv, THF:DMF 9:1, 3 equiv Et_3N, 0 °C, 18 h, 74%). Hydrolysis of the *N,N*-dimethylhydrazone **12** was accomplished effectively and cleanly on exposure to $CuCl_2$[33] (5 equiv) in pH 7 buffered aqueous THF (0 °C, 1 h, 84%). Unlike **3**, **13** proved stable and we saw no evidence of oxidative deformylation under the reaction conditions or upon storage. Final acid-catalyzed deprotection of **13** (20% TFA–$CHCl_3$, 0–25 °C, 14 h, 84%) provided the key aldehyde **2** as a mixture of inseparable diastereomers.

For direct comparison purposes, the alcohol **15** as a mixture of diastereomers was also prepared by reduction of **13** (0.5 equiv $NaBH_4$, EtOH, 0 °C, 5 min, 59%)

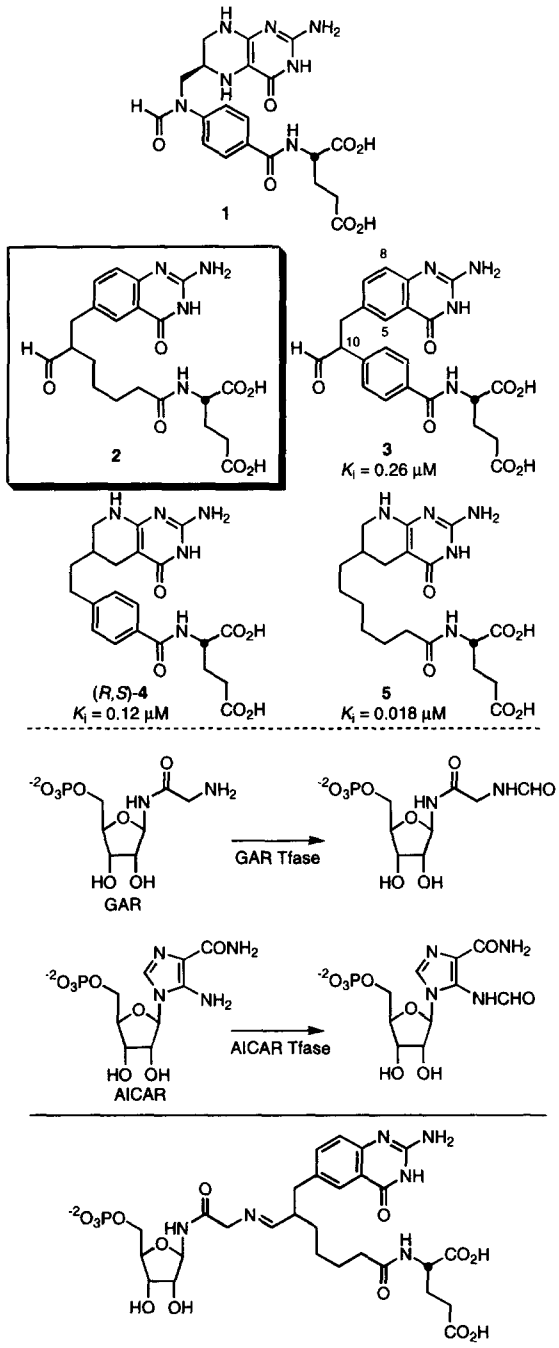
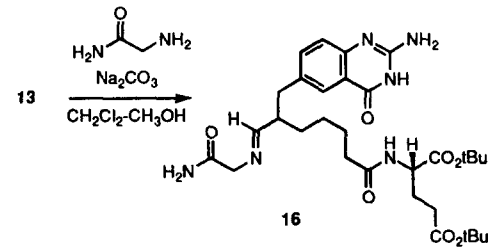


Figure 1.

followed by acid-catalyzed deprotection of **14** (20% TFA–$CHCl_3$, 0–25 °C, 10 h, 62%; Scheme 2).

Consistent with expectations, the aldehyde **2** proved much more stable than **3** and no evidence of the problematic oxidative deformylation[1] was observed. In addition, aldehyde **13** was found to readily form the corresponding imine on reaction with glycinamide (CH_3OH–CH_2Cl_2, Na_2CO_3, 4 Å MS, 8 h, 25 °C) illustrating that it would be sufficiently electrophilic for enzyme-assembled imine formation with GAR or AICAR (eq 1).



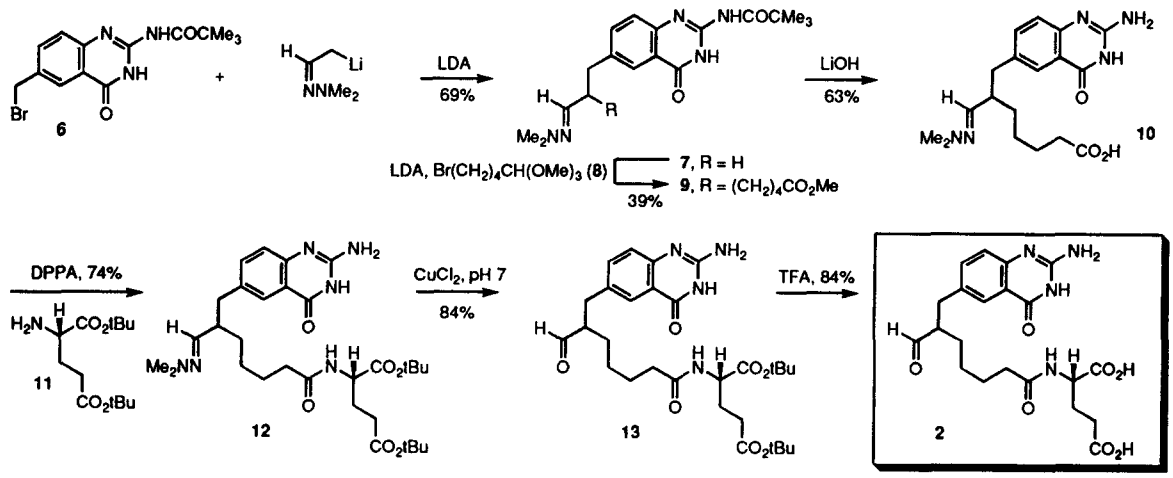
(1)

Enzyme inhibition

The inhibitor potency of aldehyde **2** and corresponding alcohol **15** were examined and the results are summarized in Table 1. The time-dependence of inhibition was also examined and the results are summarized in Tables 2 and 3. The aldehyde **2** proved to be 13 times more potent than the corresponding alcohol **15** at inhibiting GAR Tfase while both **2** and **15** were only modestly effective against AICAR Tfase. Neither exhibited evidence of time-dependent inhibition or evidence of enzyme-assembled formation of a stable, tight binding inhibitor by virtue of imine formation with the substrates GAR or AICAR. The aldehyde **2** was also 20 times less potent than **3** against GAR Tfase and five to six times less potent against AICAR Tfase. Similarly, **15** was less potent than the corresponding alcohol of **3** against GAR (20 times) and AICAR (2 times) Tfase, respectively. Thus, in both instances, the incorporation of the benzene ring of the benzoyl subunit of **1** into the inhibitors enhanced the potency against both GAR and AICAR Tfase significantly. This reduced potency was observed with a set of inhibitors that possesses greater conformational flexibility in the formyl transfer region of the structure and would suggest that the inhibitors **3** and related structures are not being substantially or adversely affected by the sp^3 nature of C-10 substitution for N-10. However, the lack of formation of a stable, enzyme-assembled imine formation with GAR or AICAR would suggest that the aldehyde of **2** and **3** may not be positioned properly within the formyl transfer region even with inhibitors that might be inherently flexible. Alternatively, it is possible that the 10-fold increase in K_i for **2** versus **15** is derived from either such an imine adduct with the substrate GAR which is rapidly but reversibly forming or from formation of a readily reversible hemiacetal type adduct with an enzyme active site residue that is providing the further binding stabilization.

Cytotoxic activity

The agents were examined for cytotoxic activity both in the presence (+) or absence (−) of added hypoxanthine against both L1210 and CCRF-CEM cell lines cultured in a medium in which the purines were removed from the FBS supplement by dialysis (Table 4). Both **2** and **15** were inactive against CCRF-CEM (>220 μM) and **15** was also inactive against L1210 (>220 μM). Only **2** exhibited detectable activity with an IC_{50} of 100 μM against L1210 and this was observed whether hypoxanthine was present or absent in the culture medium



Scheme 1.

which was otherwise free of purines. Thus, both **2** and **15** proved to be substantially less potent than **3** and its corresponding alcohol, and both do not appear to inhibit cell growth by a mechanism consistent with selective GAR or AICAR Tfase inhibition.

Experimental

Methyl (6*R)-6-(Dimethylhydrazono)methyl-7-(2-trimethylacetimido-3,4-dihydro-4-oxo-quinazolin-6-yl)-heptanoate (9).** A solution of freshly prepared LDA (1.6 mL of 0.3 M, 0.5 mmol) at 0 °C in THF under N_2 was treated with **7**[28,29] (38.8 mg, 0.1 mmol) in THF (282 μL) and the resulting solution was stirred at 0 °C for 1 h. A solution of **8**[31] (54.5 mg, 0.2 mmol) dissolved in a minimal amount of HMPA was added and the mixture was stirred at 0 °C for 2 h before the mixture was warmed to 25 °C and stirred for an additional 24 h under N_2. The reaction mixture was quenched by the dropwise addition of a saturated solution of NH_4Cl (5 mL) and 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. Chromatography (SiO_2, 20 × 4 cm, 50% hexane–EtOAc) afforded **9** (20.1 mg, 39%) as a yellow oil: ^{1}H NMR (CD_3OD, 400 MHz) δ 7.88 (1H, d, J = 1.8 Hz, H-5), 7.56 (1H, dd, J = 2.0, 8.4 Hz, H-7), 7.44 (1H, d, J = 8.3 Hz, H-8), 6.59 (1H, d, J = 7.3 Hz, HC=N), 3.63 (3H, s, OCH_3), 2.88 (1H, dd, J = 9.2, 15.6 Hz, C*H*H), 2.80 (1H, dd, J = 8.2, 13.5 Hz, CH*H*), 2.60 (6H, s, $N(CH_3)_2$), 2.51 (1H, m, CH), 2.28 (2H, t, J = 7.4 Hz, $CH_2CO_2CH_3$), 1.53 (4H, m, CH_2CH_2), 1.35 (2H, m, CH_2), 1.31 (9H, s, $COC(CH_3)_3$); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.5, 160.4, 145.9, 139.6, 138.4, 137.7, 128.2, 127.93, 127.91, 127.3, 121.1, 52.6, 46.3, 44.3, 41.4, 35.3, 34.6, 28.4, 27.7, 26.6; IR (neat) ν_{max} 3210, 2861, 1728, 1666 cm^{-1}; FABHRMS (NBA) *m/z* 458.2753 (M^+ + H, $C_{24}H_{35}N_5O_3$ requires 458.2767).

(6*R)-6-(Dimethylhydrazono)methyl-7-(2-trimethylacetimido-3,4-dihydro-4-oxo-quinazolin-6-yl)-heptanoic acid (10).** A solution of **9** (15.3 mg, 0.03 mmol) in THF:H_2O:MeOH (3:1:1, 84 μL) was treated with

Table 1. GAR and AICAR Tfase inhibition, K_i (μM)

Agent	GAR Tfase[a]	AICAR Tfase[b]
3	0.26 ± 0.05 μM	7.6 ± 1.5 μM
2	4.5 ± 0.3 μM	42 ± 11 μM
15	60 ± 20 μM	47 ± 14 μM

[a]*pur*N GAR Tfase.
[b]Avian AICAR Tfase.

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

	Enzyme activity (%)				
Agent	t = 3 min	15 min	90 min	3 h	6 h
None	100	100	—	96	92
3	3	3	5	7	9
2	50	55	—	—	62
15	79	100	—	—	93

[a]*pur*N GAR Tfase (2 nM), 10 μM inhibitor.

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

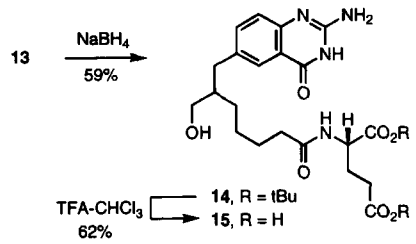
	Enzyme activity (%)				
Agent	t = 3 min	15 min	90 min	3 h	6 h
None	100	—	—	100	100
3	58	—	—	—	46
2	80	—	—	—	86
15	79	—	—	—	90

[a]Avian AICAR Tfase (100 nM), 10 μM inhibitor.

Table 4. Cytotoxic activity (μM)[a]

Agent	L1210[b]	CCRF-CEM[b]
(6*R*)-DDATHF	>225, 0.07	>225, 0.05
Methotrexate	0.05, 0.05	0.06, 0.07
2	100, 100	>220, >220
15	>220, >220	>220, >220

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



Scheme 2.

aqueous 1 N LiOH (67 μL, 0.07 mmol) and the solution was stirred at 25 °C for 24 h. Additional aqueous 1 N LiOH (67 μL, 0.07 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 × 10 mL) before being acidified with the addition of 10% aqueous HCl (1 mL, pH 4). The precipitated product was collected by filtration to give **9**·HCl (7.6 mg, 63%) as a white solid: mp > 250 °C; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.78 (1H, d, *J* = 1.7 Hz, H-5), 7.44 (1H, dd, *J* = 1.9, 8.4 Hz, H-7), 7.19 (1H, d, *J* = 8.4 Hz, H-8), 6.60 (1H, d, *J* = 7.3 Hz, HC=N), 2.79 (2H, m, C*H*$_2$CH), 2.50 (1H, m, CH), 2.60 (6H, s, N(CH$_3$)$_2$), 2.21 (2H, t, *J* = 7.4 Hz, C*H*$_2$CO$_2$H), 1.60–1.27 (6H, m, CH$_2$CH$_2$CH$_2$); ^{13}C NMR (CD_3OD, 100 MHz) δ 178.8, 176.9, 162.9, 154.1, 141.2, 135.6, 133.9, 126.7, 122.9, 117.8, 44.6, 43.1, 39.7, 37.1, 33.4, 27.4, 26.5, 23.8; IR (neat) ν_{max} 3320, 3172, 2927, 1716, 1703, 1560 cm^{-1}; FABHRMS (NBA) *m/z* 360.2031 (M$^+$ + H, $C_{18}H_{35}N_5O_3$ requires 360.2036).

Di-*tert*-butyl (6*RS*)-*N*-[7-(2-amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-6-(dimethylhydrazono)methyl-1-oxo-1-heptyl]-L-glutamate (12). A slurry of **10** (3 mg, 0.008 mmol) and **11** (3 mg, 0.01 mmol) in 10% DMF–THF (800 μL) cooled to 0 °C was treated with Et_3N (3.6 μL, 0.025 mmol) followed by diphenylphosphoryl azide (DPPA, 2.5 μL, 0.01 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 **12** (3.7 mg, 74%) as a yellow solid: mp 135–137 °C; ^{1}H NMR (DMF-d_7, 400 MHz) δ 7.78 (1H, d, *J* = 1.7 Hz, H-5), 7.45 (1H, dd, *J* = 2.0, 8.4 Hz, H-7), 7.18 (1H, d, *J* = 7.7 Hz, H-8), 6.60 (1H, d, *J* = 7.3 HC=N), 4.26 (1H, m, NHC*H*CO$_2$C(CH$_3$)$_3$), 2.79 (2H, m, CH$_2$), 2.61 (6H, s, N(CH$_3$)$_2$), 2.50 (1H, m, CH), 2.29 (2H, t, *J* = 7.5 Hz, CH$_2$CO), 2.20 (2H, t, *J* = 7.3 Hz, CH$_2$C*H*$_2$CO$_2$C(CH$_3$)$_3$), 2.04 (1H, m, C*H*HCH$_2$CO$_2$C(CH$_3$)$_3$), 1.81 (1H, m, CH*H*CH$_2$CO$_2$C(CH$_3$)$_3$), 1.61 (2H, m, CH$_2$), 1.51 (2H, m, CH$_2$), 1.44 (9H, s, CO$_2$C(CH$_3$)$_3$), 1.43 (9H, s, CO$_2$C(CH$_3$)$_3$), 1.31 (2H, m, CH$_2$); ^{13}C NMR (DMF-d_7, 100 MHz) δ 173.6, 170.9, 169.9, 143.0, 134.5, 133.8, 125.3, 125.1, 119.26, 119.24, 80.1, 79.2, 51.0, 43.0, 41.0, 38.9, 37.9, 34.0, 31.3, 29.9, 28.1, 25.7, 25.6, 25.2, 24.4, 24.3, 22.7, 16.6; IR (neat) ν_{max} 3314, 3172, 2979, 1728 cm^{-1}; FABHRMS (NBA) *m/z* 601.3711 (M$^+$ + H, $C_{31}H_{48}N_6O_6$ requires 601.3714).

Di-*tert*-butyl (6*RS*)-*N*-[7-(2-amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-6-formyl-1-oxo-1-heptyl]-L-glutamate (13). A solution of **12** (5.9 mg, 0.009 mmol) in THF (150 μL) and pH 7 aqueous phosphate buffer (49 μL) cooled to 0 °C was treated with a solution of $CuCl_2$ (8.4 mg, 0.05 mmol) in H_2O (33 μL). The reaction mixture was stirred at 0 °C for 1 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 $CHCl_3$ (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 **13** (4.6 mg, 84%) as a yellow oil; ^{1}H NMR (DMF-d_7, 400 MHz) δ 11.31 (1H, br s, NH), 9.69 (1H, d, *J* = 2.5 Hz, CHO), 7.79 (1H, d, *J* = 2.0 Hz, H-5), 7.46 (1H, dd, *J* = 2.1, 8.2 Hz, H-7), 7.17 (1H, d, *J* = 8.3 Hz, H-8), 6.60 (1H, br s, NH), 4.28 (1H, m, NHC*H*CO$_2$C(CH$_3$)$_3$), 3.03 (1H, dd, *J* = 7.7, 13.9 Hz, C*H*H), 2.80 (1H, dd, *J* = 7.6, 14.0 Hz, CH*H*), 2.66 (1H, m, CH), 2.34 (2H, t, *J* = 8.0 Hz, CH$_2$CO), 2.19 (2H, t, *J* = 7.2 Hz, CH$_2$C*H*$_2$CO$_2$C(CH$_3$)$_3$), 2.01 (1H, m, C*H*HCH$_2$CO$_2$C(CH$_3$)$_3$), 1.83 (1H, m, CH*H*CH$_2$CO$_2$C(CH$_3$)$_3$), 1.65 (2H, m, CH$_2$), 1.56 (2H, t, *J* = 7.2 Hz, CHC*H*$_2$CH$_2$), 1.25 (2H, m, CH$_2$), 1.41 (9H, s, CO$_2$C(CH$_3$)$_3$), 1.40 (9H, s, CO$_2$C(CH$_3$)$_3$); ^{13}C NMR (DMF-d_7, 100 MHz) δ 205.5, 173.1, 172.3, 170.0, 162.9, 152.9, 135.8, 133.5, 126.4, 124.5, 118.0, 81.2, 80.4, 53.9, 52.7, 31.9, 28.7, 28.0, 27.4, 27.0, 26.2; IR (neat) ν_{max} 3318, 2961, 2723, 1726 1650 cm^{-1}; FABHRMS (NBA) *m/z* 559.3109 (M$^+$ + H, $C_{29}H_{42}N_4O_7$ requires 559.3132).

(6*RS*)-*N*-[7-(2-Amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-6-formyl-1-oxo-1-heptyl]-L-glutamic acid (2). A solution of **13** (9.4 mg, 0.01 mmol) in $CHCl_3$ (1,3 mL) cooled to 0 °C was treated with 337 μL of trifluoroacetic acid and the solution was stirred at 0 °C for 2 h and at 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 **2**·CF_3CO_2H (8.5 mg, 84%) as a white solid: mp >300 °C; ^{1}H NMR (DMF-d_7, 400 MHz) δ 9.69 (1H, d, *J* = 2.2 Hz, CHO), 9.44 (2H, br s, NH_2), 7.89 (1H, s, H-5), 7.72 (1H, d, *J* = 7.9 Hz, H-7), 7.35 (1H, d, *J* = 8.0 Hz, H-8), 4.41 (1H, m, NHC*H*CO$_2$H), 3.10 (1H, dd, *J* = 7.8, 13.9 Hz, C*H*H), 2.87 (1H, dd, *J* = 6.4, 13.9 Hz, CH*H*), 2.72 (1H, m, CH), 2.40 (2H, t, *J* = 7.9 Hz, CH$_2$CO), 2.21 (2H, t, *J* = 7.2 Hz, CH$_2$C*H*$_2$CO$_2$H), 2.10 (1H, m, C*H*HCH$_2$CO$_2$H), 1.90 (1H, m, CH*H*CH$_2$CO$_2$H), 1.66 (2H, m, CH$_2$), 1.56 (2H, t, *J* = 7.2 Hz, CHC*H*$_2$CH$_2$), 1.36 (2H, m, CH$_2$); ^{13}C NMR (DMF-d_7, 100 MHz) δ 205.3, 174.4, 173.1, 138.4, 137.2, 127.5, 122.7, 119.9, 118.8, 81.2, 80.4, 53.7, 52.9, 28.7, 27.9, 27.5, 26.9, 26.2; IR (KBr) ν_{max} 3466, 2932, 2849, 2766, 1684 cm^{-1}; Ionspray MS (NBA) *m/z* 445 (M − H$^+$, $C_{21}H_{26}N_4O_7$ requires 445).

Di-*tert*-butyl (6*RS*)-*N*-[7-(2-amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-6-hydroxymethyl-1-oxo-1-heptyl]-L-glutamate (14). A solution of **13** (6.8 mg, 0.01 mmol)

in EtOH (122 μL) at 0 °C was treated with $NaBH_4$ (0.2 mg, 0.007 mmol) and the solution was stirred at 0 °C for 5 min before being quenched by the dropwise addition of H_2O. This mixture was poured into H_2O (1 mL). The product was extracted into $CHCl_3$ (5 × 3 mL), dried (Na_2SO_4), filtered, and then concentrated under reduced pressure. PCTLC (SiO_2, 0.5 mm plate, 10% CH_3OH–$CHCl_3$) afforded **14** (4.0 mg, 59%) as a clear oil: ^1H NMR (DMF-d_7, 400 MHz) δ 10.96 (1H, br s, NH), 7.77 (1H, d, J = 1.9 Hz, H-5), 7.42 (1H, dd, J = 2.2, 8.4 Hz, H-7), 7.15 (1H, d, J = 8.3 Hz, H-8), 6.49 (2H, br s, NH_2), 4.46 (1H, t, J = 5.4 Hz, OH), 4.28 (1H, m, NHC*H*$CO_2C(CH_3)_3$), 3.39 (2H, t, J = 4.9 Hz, C*H*$_2$OH), 3.27 (1H, m, CH), 2.67 (1H, m, C*H*H), 2.57 (1H, dd, J = 6.3, 13.7 Hz, CH*H*), 2.34 (2H, t, J = 7.8 Hz, CH_2CO), 2.19 (2H, t, J = 7.2 Hz, $CH_2CH_2CO_2C(CH_3)_3$), 1.71 (2H, m, CH_2), 1.53 (2H, m, CH_2), 1.41 (9H, s, $CO_2C(CH_3)_3$), 1.40 (9H, s, $CO_2C(CH_3)_3$), 1.26 (2H, m, CH_2); ^{13}C NMR (DMF-d_7, 100 MHz) δ 173.2, 172.3, 172.1, 162.8, 152.6, 136.1, 135.4, 126.5, 125.0, 117.9, 81.1, 80.4, 70.8, 63.4, 52.7, 43.4, 37.2, 36.1, 31.9, 28.0, 27.4, 26.7, 25.6; IR (neat) ν_{max} 2975, 2927, 1728, 1646, cm^{-1}; FABHRMS (NBA) m/z 561.3299 (M^+ + H, $C_{29}H_{44}N_4O_7$ requires 561.3288).

(6*RS*)-*N*-[7-(2-Amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-6-hydroxymethyl-1-oxo-1-heptyl]-L-glutamic acid (15). A solution of **14** (4.0 mg, 0.007 mmol) in $CHCl_3$ (570 μL) cooled to 0 °C was treated with 142 μL of trifluoroacetic acid. The solution stirred at 0 °C for 1 h and at 25 °C for 9 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 (3.0 mg, 62%) as a white solid: mp > 300 °C; ^1H NMR (DMF-d_7, 400 MHz) δ 8.93 (1H, br s, NH), 7.87 (1H, d, J = 2.0 Hz, H-5), 7.66 (1H, d, J = 8.0 Hz, H-7), 7.35 (1H, d, J = 8.4 Hz, H-8), 4.42 (1H, m, NHC*H*CO_2H), 4.34 (2H, d, J = 3.4 Hz, C*H*$_2$OH), 3.28 (1H, m, CH), 2.77 (2H, m, C*H*$_2$CH), 2.43 (2H, m, CH_2CO), 2.22 (2H, t, J = 7.1 Hz, $CH_2CH_2CO_2H$), 2.12 (1H, m, C*H*HCH_2CO_2H), 1.91 (1H, m, CH*H*CH_2CO_2H), 1.55 (2H, m, CH_2), 1.42 (4H, m, CH_2CH_2); IR (neat) ν_{max} 3334, 2922, 1710, 1629 cm^{-1}; ^{13}C NMR (DMF-d_7, 100 MHz) δ 174.4, 174.2, 173.2, 152.9, 136.8, 127.1, 79.7, 70.7, 52.1, 39.5, 37.0, 35.9, 27.5, 27.3, 26.7, 26.3; Ionspray MS (NBA–NaI) m/z 449 (M^+ + H, $C_{21}H_{28}N_4O_7$ requires 449).

Di-*tert*-butyl (6*RS*)-*N*-[7-(2-amino-3,4-dihydro-4-oxo-quinazonlin-6-yl)-6-{(*N*-amidomethyl)iminomethylene}-1-oxo-heptyl]-L-glutamate (16). A slurry of **13** (1.2 mg, 0.002 mmol), glycinamide (0.2 mg, 0.002 mmol) and Na_2CO_3 (0.5 mg, 0.004 mmol) in 25% CH_3OH–CH_2Cl_2 (0.02 mL) was stirred at 25 °C for 8 h. The remaining solids were removed by filtration and the filtrate was concentrated under reduced pressure to afford **16** (0.6 mg, 45%) as a yellow oil: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.77 (1H, s, H-5), 7.70 (1H, d, J = 4.4 Hz, CHN), 7.44 (1H, d, J = 8.2 Hz, H-8), 7.13 (1H, d, J = 8.3 Hz, H-7), 6.54 (2H, br s, NH_2), 4.38 (1H, m, CH), 3.91 (2H, s, CH_2), 2.82 (2H, m, CH_2), 2.45 (1H, m, CH), 2.33 (2H, t, J = 6.1 Hz, CH_2), 2.19 (2H, t, J = 7.6 Hz, CH_2), 2.00 (3H, m, CH_2 and CH), 1.84 (1H, m, CH), 1.54 (2H, m, CH_2), 1.41 (9H, s, $CO_2C(CH_3)_3$), 1.40 (9H, s, $CO_2C(CH_3)_3$); IR (neat) ν_{max} 3334, 2921, 1724, 1666 cm^{-1}; FABHRMS (NBA–CsI) m/z 747.2461 (M^+ + Cs, $C_{31}H_{46}N_6O_7$ requires 747.2482).

GAR and AICAR Tfase inhibition

The evaluation of **2** and **15** was conducted as detailed in the accompanying article.[1]

Cytotoxicity testing

The cytotoxic activity of **2** and **15** was determined following protocols we have described in full detail.[1]

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