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Design, synthesis, and biological evaluation of 10-methanesulfonyl-DDACTHF, 10-methanesulfonyl-5-DACTHF, and 10-methylthio-DDACTHF as potent inhibitors of GAR Tfase and the de novo purine biosynthetic pathway

Heng Cheng,[a,b] Youhoon Chong,[a,b] Inkyu Hwang,[a,b] Ali Tavassoli,[c] Yan Zhang,[b,d] Ian A. Wilson,[b,d] Stephen J. Benkovic[c] and Dale L. Boger[a,b,*]

[a]*Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA*
[b]*The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA*
[c]*Department of Chemistry, Pennsylvania State University, University Park, PA 16802, USA*
[d]*Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA*

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Abstract—The synthesis and evaluation of 10-methanesulfonyl-DDACTHF (**1**), 10-methanesulfonyl-5-DACTHF (**2**), and 10-methylthio-DDACTHF (**3**) as potential inhibitors of glycinamide ribonucleotide transformylase (GAR Tfase) and aminoimidazole carboxamide ribonucleotide transformylase (AICAR Tfase) are reported. The compounds 10-methanesulfonyl-DDACTHF (**1**, K_i = 0.23 μM), 10-methanesulfonyl-5-DACTHF (**2**, K_i = 0.58 μM), and 10-methylthio-DDACTHF (**3**, K_i = 0.25 μM) were found to be selective and potent inhibitors of recombinant human GAR Tfase. Of these, **3** exhibited exceptionally potent, purine sensitive growth inhibition activity (**3**, IC_{50} = 100 nM) against the CCRF–CEM cell line being 3-fold more potent than Lometrexol and 30-fold more potent than the parent, unsubstituted DDACTHF, whereas **1** and **2** exhibited more modest growth inhibition activity (**1**, IC_{50} = 1.0 μM and **2**, IC_{50} = 2.0 μM).

1. Introduction

Glycinamide ribonucleotide transformylase (GAR Tfase) and aminoimidazole carboxamide ribonucleotide transformylase (AICAR Tfase) are folate-dependent enzymes central to the de novo purine biosynthetic pathway. GAR Tfase utilizes the cofactor (6*R*)-N^{10}-formyltetrahydrofolate to transfer a formyl group to the primary amine of its substrate, glycinamide ribonucleotide (GAR, Fig. 1). This one carbon transfer incorporates 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 AICAR Tfase which also employs (6*R*)-N^{10}-formyltetrahydrofolate to transfer a formyl group to the C-5 amine of its substrate, aminoimidazole carboxamide ribonucleotide (AICAR, Fig. 1).[1] Since purines are critical components of DNA and RNA, inhibition of enzymes in the purine biosynthetic pathway had been envisioned as an effective approach for antineoplastic intervention. The disclosure that (6*R*)-5,10-dideazatetrahydrofolate (Lometrexol, (6*R*)-DDATHF, Fig. 2) 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.[2–4] Herein, we report the synthesis and evaluation of 10-methanesulfonyl-DDACTHF (**1**), 10-methanesulfonyl-5-DACTHF (**2**), and 10-methylthio-DDACTHF (**3**) as novel folate-based inhibitors of GAR Tfase, Figure 2.

2. Inhibitor design

In previous studies, we described folate-based inhibitors of GAR Tfase that incorporated electrophilic functional

* Corresponding author. Tel.: +1 858 784 7522; fax: +1 858 784 7550; e-mail: boger@scripps.edu

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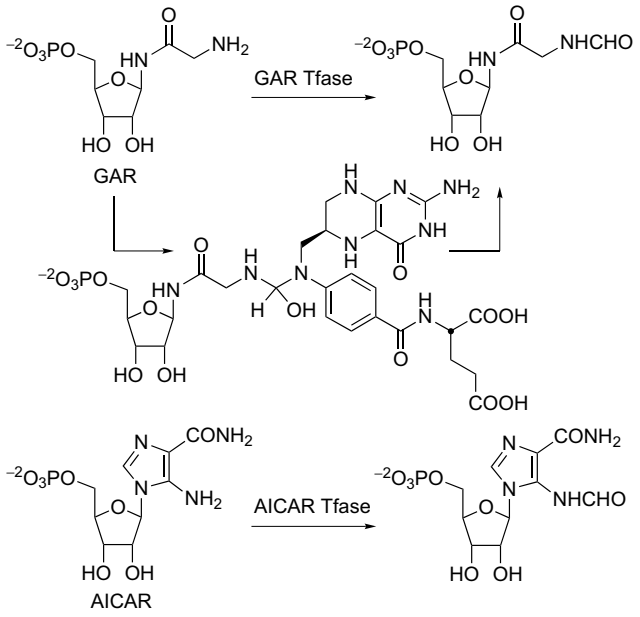


Figure 1.

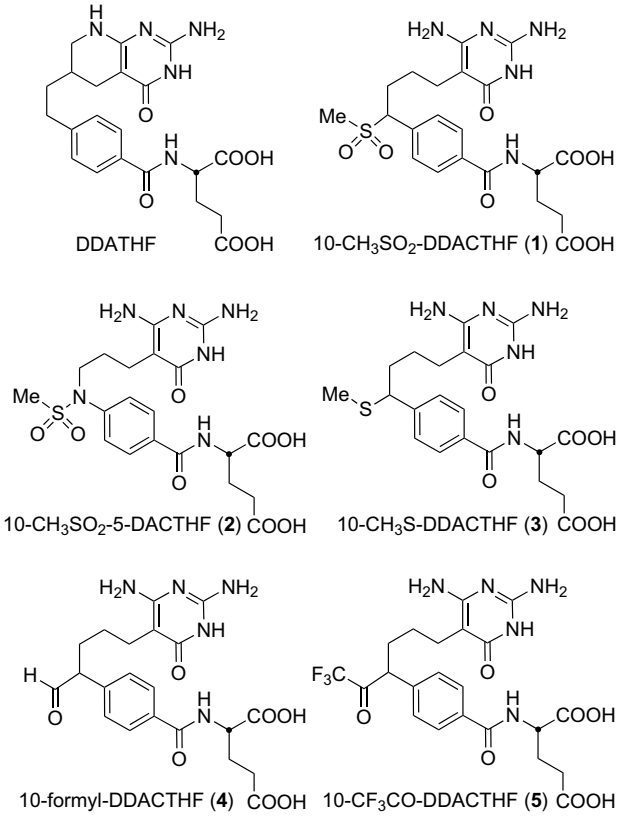


Figure 2.

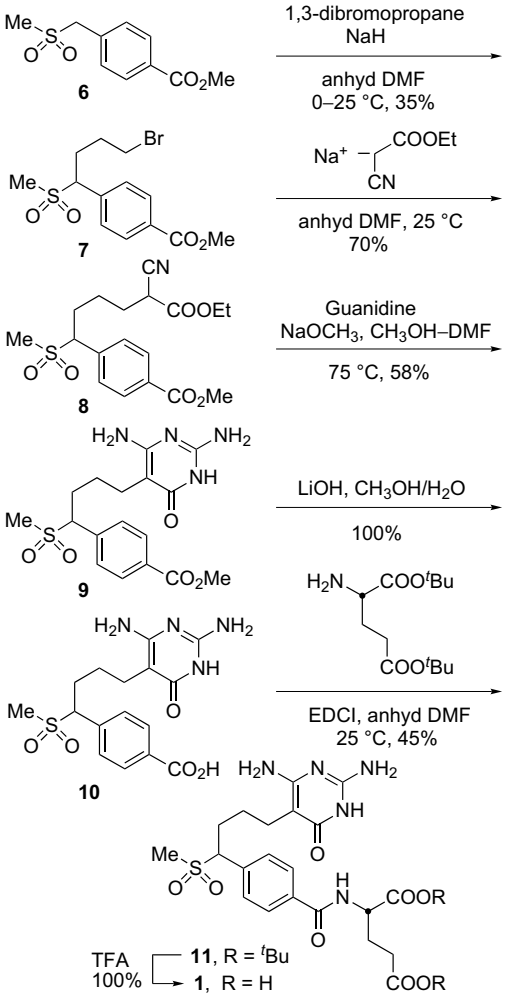
groups that could potentially interact either with active site nucleophiles or the GAR/AICAR substrate amines.[5–8] The most significant of these were 10-formyl-DDACTHF (**4**)[8] and 10-CF_3CO-DDACTHF (**5**)[9,10] (Fig. 2), bearing a nontransferable formyl or trifluoroacetyl group, which both proved to be potent GAR Tfase inhibitors. X-ray and NMR studies of the inhibitor–enzyme complexes revealed that both inhibitors bound GAR Tfase as their *gem*-diols.[10,11] The formation of the *gem*-diol mimics the formyl transfer reaction tetrahedral intermediate and provides strong stabilizing hydrogen bond interactions between the inhibitor and active site catalytic residues of the protein.

Both 10-formyl-DDACTHF (**4**) and 10-CF_3CO-DDACTHF (**5**) were shown to be selective and potent GAR Tfase inhibitors (**4**, K_i = 0.014 μM against rhGAR Tfase; **5**, K_i = 0.015 μM against rhGAR Tfase).[8–11] They were both found to be effectively transported into the cell by the reduced folate carrier and polyglutamated by FPGS, which contributes to their cytotoxic activity by enhancing intracellular accumulation (**4**, CCRF–CEM IC_{50} = 60 nM; **5**, CCRF–CEM IC_{50} = 16 nM).[8–11] 10-CF_3CO-DDACTHF (**5**) proved to be 10-fold more potent against tumor cell proliferation than Lometrexol and suitable for in vivo evaluation.[10]

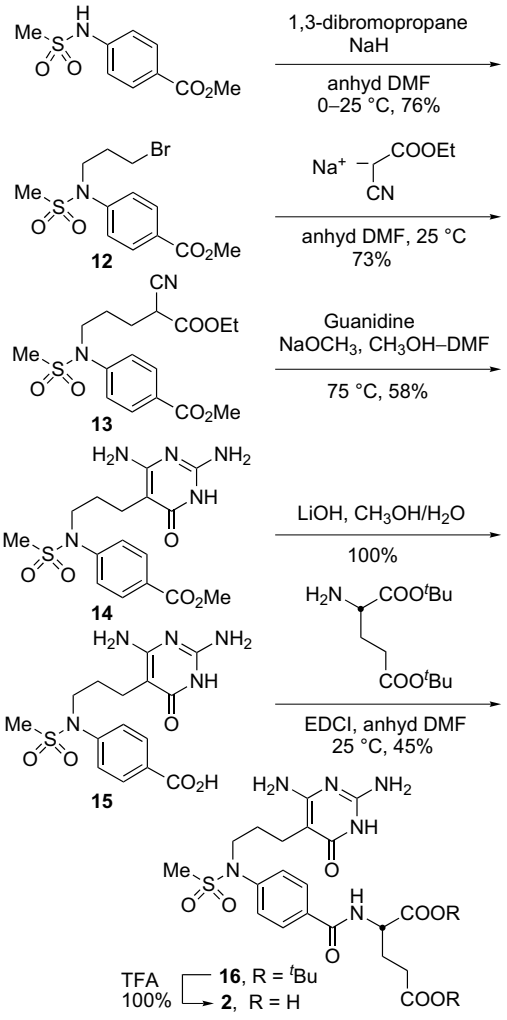
Beyond the intrinsic importance of these two inhibitors themselves, their discovery led to the recognition that such a tetrahedral intermediate mimic provides a unique design feature that conveys selectivity for GAR Tfase inhibition over all other folate-dependent enzymes that do not enlist a formyl transfer reaction. Herein we report the extension of these observations to the preparation and examination of the tetrahedral transition state inhibitors 10-methanesulfonyl-DDACTHF (**1**) and 10-methanesulfonyl-5-DACTHF (**2**), which were anticipated to be a nice complement to the corresponding aldehyde and ketones (Fig. 2). Since one potential limitation of the carbonyl-based inhibitors is their in vivo reduction, the sulfone **1** and sulfonamide **2** may well prove to be more stable and efficacious in vivo, albeit not as intrinsically potent against GAR Tfase in vitro. We also describe the synthesis and evaluation of 10-methylthio-DDACTHF (**3**), a comparison analogue of **1** where the methanesulfonyl group was replaced by a methylthio group.

3. Chemistry

The synthesis of 10-methanesulfonyl-DDACTHF (**1**) was accomplished through the alkylation of methyl 4-(methanesulfonylmethyl)benzoate[12] with 1,3-dibromopropane, as presented in Scheme 1. This was accomplished upon NaH deprotonation of methyl 4-(methanesulfonylmethyl)benzoate (DMF, 0 °C, 25 min) and subsequent treatment with excess 1,3-dibromopropane (10 equiv, DMF, 25 °C, 2.5 h, 35%) to give **7**. The preformed sodium salt of ethyl cyanoacetate (NaH, DMF, 0 °C, 30 min) was alkylated with **7** (DMF, 25 °C, 2 h, 70%) to give **8**, and its subsequent treatment with the free base of guanidine (1.2 equiv, CH_3OH–DMF, 75 °C, overnight, 58%) under basic conditions gave the desired pyrimidinone **9**. Treatment of **9** with LiOH (3 equiv, CH_3OH–H_2O 3:1, 25 °C, overnight, 100%) cleanly provided the carboxylic acid **10**, which was coupled with di-*tert*-butyl-L-glutamate hydrochloride (1.5 equiv, EDCI, $NaHCO_3$, DMF,



Scheme 1.

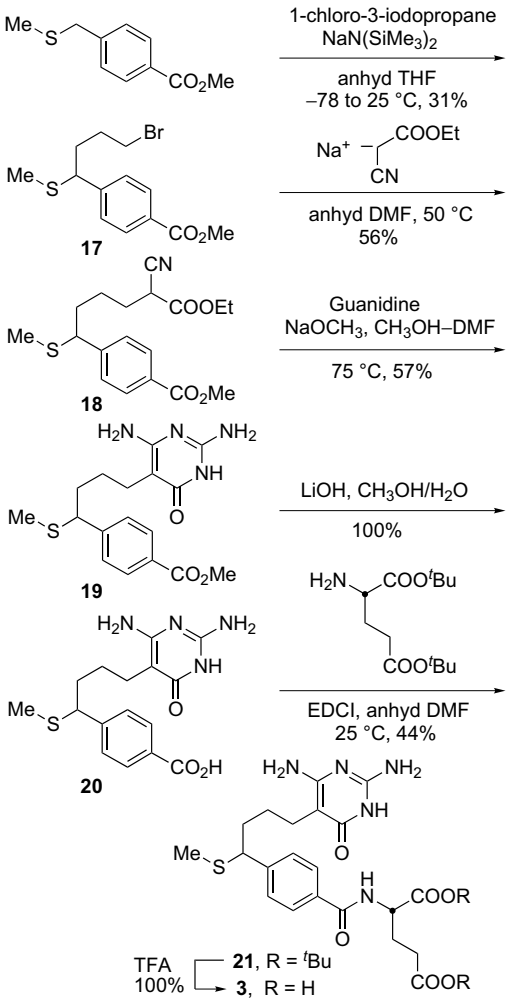


Scheme 2.

25 °C, overnight, 45%) to provide **11** as a 1:1 mixture of the inseparable diastereomers. Deprotection of **11** was accomplished by treatment with trifluoroacetic acid (TFA/$CHCl_3$, 25 °C, overnight, 100%) to provide 10-methanesulfonyl-DDACTHF (**1**).

The synthesis of 10-methanesulfonyl-5-DACTHF (**2**) was accomplished through the alkylation of methyl 4-(methanesulfonylamino)benzoate[13] with 1,3-dibromopropane, as presented in Scheme 2.[4c] This was accomplished upon NaH deprotonation of methyl 4-(methanesulfonylamino)benzoate (DMF, 0 °C, 25 min) and subsequent treatment with excess 1,3-dibromopropane (10 equiv, DMF, 25 °C, 2.5 h, 76%) to give **12**. The preformed sodium salt of ethyl cyanoacetate (NaH, DMF, 0 °C, 30 min) was alkylated with **12** (DMF, 25 °C, 2 h, 73%) to give **13**, and its treatment with the free base of guanidine (1.2 equiv, CH_3OH–DMF, 75 °C, overnight, 58%) under basic conditions gave the desired pyrimidinone **14**. Treatment of **14** with LiOH (3 equiv, CH_3OH–H_2O 3:1, 25 °C, overnight, 100%) cleanly provided the carboxylic acid **15**, which was coupled with di-*tert*-butyl-L-glutamate hydrochloride (1.5 equiv, EDCI, $NaHCO_3$, DMF, 25 °C, overnight, 45%) to provide **16**. Deprotection of **16** was accomplished by treatment with trifluoroacetic acid (TFA/$CHCl_3$, 25 °C, overnight, 100%) to provide 10-methanesulfonyl-5-DACTHF (**2**).

The synthesis of 10-methylthio-DDACTHF (**3**) is presented in Scheme 3. This was accomplished by sodium bis(trimethylsilyl)amide deprotonation of methyl 4-(methylthiomethyl)benzoate[12] (THF, −78 °C) and subsequent treatment with excess 1-chloro-3-iodopropane (10 equiv, THF, 25 °C, 45 min, 31%) to give **17**. The use of NaH for deprotonation failed to provide the desired product **17** and the use of excess 1,3-dibromopropane or 1,3-diiodopropane to alkylate the sodium bis(trimethylsilyl)amide-derived anion provided competitive elimination products. The preformed sodium salt of ethyl cyanoacetate (NaH, DMF, 0 °C, 30 min) was alkylated with **17** (DMF, 50 °C, 9 h, 56%) to give **18**, which was treated with the free base of guanidine (1.2 equiv, CH_3OH–DMF, 75 °C, overnight, 57%) under basic conditions to afford the desired pyrimidinone **19**. Treatment of **19** with LiOH (3 equiv, CH_3OH–H_2O 3:1, 25 °C, overnight, 100%) cleanly provided the carboxylic acid **20**, which was coupled with di-*tert*-butyl-L-glutamate hydrochloride (1.5 equiv, EDCI, $NaHCO_3$, DMF, 25 °C, overnight, 44%) to



Scheme 3.

provide **21** as a 1:1 mixture of the inseparable diastereomers. Deprotection of **21** was accomplished by treatment with trifluoroacetic acid (TFA/$CHCl_3$, 25 °C, overnight, 100%) to provide 10-methylthio-DDACTHF (**3**).

4. GAR Tfase and AICAR Tfase inhibition

Compounds **1**, **2**, and **3** were tested for inhibition of *E. coli* GAR Tfase, recombinant human GAR Tfase, and recombinant human AICAR Tfase, and the results are presented in Table 1. All of the compounds exhibited selective and potent inhibition of recombinant human GAR Tfase, while all three failed to inhibit *E. coli* GAR Tfase (K_i > 100 μM). The analogous selective inhibition of human versus *E. coli* GAR Tfase was similarly observed previously with **4** and **5**, but contrasts the near equipotent and less active inhibition of the two enzymes by the parent unsubstituted DDACTHF. Compound **1** (K_i = 0.23 μM) was 7-fold more potent than the parent unsubstituted DDACTHF, 4-fold less potent than the reported activity of Lometrexol, and only 15-fold less potent than **5**, the most potent rhGAR Tfase inhibitor disclosed to date.[10] From this one can infer that C-10 sulfone of **1** increases enzyme active site binding consistent with its predicted ability to mimic the formyl transfer tetrahedral intermediate, but that it does so less effectively than the corresponding trifluoromethyl ketone (bound as *gem*-diol). Substitution of the C-10 carbon of compound **1** by a nitrogen results in a 2-fold decrease in its potency against rhGAR Tfase (**2**, K_i = 0.58 μM). Compound **3** bearing a C-10 methylthio group was a surprisingly potent inhibitor of rhGAR Tfase (**3**, K_i = 0.25 μM) being essentially equipotent with **1** bearing the 10-methanesulfonyl group. While inhibitor **3** does not contain an apparent mimic of the formyl transfer reaction intermediate, it does incorporate a potential hydrogen bond acceptor and presents a soft hydrophobic substituent for active site binding, both of which presumably contribute to its 7-fold increase in K_i relative to the unsubstituted, parent DDACTHF. Compounds **1**, **2**, and **3** were inactive against rhAICAR Tfase (K_i > 100 μM).

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

Compound	*E. coli* GAR Tfase[a]	rhGAR Tfase[b]	rhAICAR Tfase[c]
1	>100	0.23	>100
2	>100	0.58	>100
3	>100	0.25	>100
4	6[d]	0.014[d]	1[d]
5	1.9[e]	0.015[e]	65[e]
DDACTHF	5	1.7	ND[f]
Lometrexol	0.1[e]	0.06[g]	1[e]

[a] *E. coli* GAR Tfase.
[b] Recombinant human GAR Tfase.
[c] Recombinant human AICAR Tfase.
[d] Ref. 8.
[e] Ref. 9.
[f] Not determined.
[g] Ref. 14.

5. Cytotoxic activity

Compounds **1**, **2**, and **3** were examined for cytotoxic activity both in the presence (+) and absence (−) of added hypoxanthine (purine) and thymidine (pyrimidine) against the CCRF–CEM cell line (Table 2). Each exhibited potent cytotoxic activity against the CCRF–CEM cell line when purines (hypoxanthine) were absent in the cell culture media and were inactive (IC_{50} > 100 μM) in the presence of media purines, but this activity was insensitive to the presence of thymidine. This sensitivity to the presence of purines, but not pyrimidines (thymidine), indicate that the cytotoxic activity of **1**–**3** is derived from their inhibition of an enzyme in the de novo purine biosynthetic pathway consistent with inhibition of GAR Tfase. Sulfone **1** and sulfonamide **2** were found to be two to three times more potent than the parent unsubstituted DDACTHF and 3- or 7-fold, respectively, less potent than Lometrexol. Both exhibited IC_{50} values roughly 4-fold higher than their enzymatic (GAR Tfase) K_i's. In contrast to design expectations, inhibitor **3** proved to be exceptionally potent exhibiting a CCRF–CEM IC_{50} of 100 nM. This proved to be 2- to 3-fold more potent than Lometrexol, 30- to 40-fold more potent than DDACTHF, only 6-fold less potent than the most potent and selective

Table 2. In vitro cyctotoxic activity (IC_{50}, μM)

Compound	CCRF–CEM		
	(−)T, (−)H[a]	(+)T, (−)H	(−)T, (+)H
1	1.0	0.8	>100
2	2.0	2.0	>100
3	0.1	0.08	>100
4	0.07[b]	0.06[b]	170[b]
5	0.016[c]	0.017[c]	>100[c]
DDACTHF	3.0	4.0	>100
Lometrexol	0.3	2.0	>100

Compound[a]	CCRF–CEM	CCRF–CEM/MTX	CCRF–CEM/FPGS$^-$
1	1.0	20	>100
2	2.0	20	>100
3	0.1	2.5	50
4	0.07[b]	>200[b]	>100[b]
5	0.016[c]	>100[c]	>100[c]
DDACTHF	3.0	ND (>100)[c]	ND (>100)[c]
Lometrexol	0.3	ND (>100)[c]	30 (>100)[c]

[a] (−)T, thymidine; (−)H, hypoxanthine.
[b] Ref. 8.
[c] Ref. 9.

GAR Tfase inhibitor disclosed to date (10-CF_3CO-DDACTHF, IC_{50} = 16 nM), and 2- to 3-fold *more* potent than its in vitro GAR Tfase K_i. Like Lometrexol and inhibitors **4**[8] and **5**[9], assay of **1**–**3** against CCRF–CEM cell lines deficient in the reduced folate carrier (CCRF–CEM/MTX)[15] or FPGS (CCRF–CEM/FPGS$^-$)[16] revealed that each lacked or lost activity indicating that each benefits from the reduced folate carrier transport, each is a substrate for FPGS, and that polyglutamation by FPGS contributes to their cellular activity. This may not only increase the intracellular accumulation of the inhibitors, but the corresponding polyglutamates may additionally exhibit an increased GAR Tfase binding affinity, both of which may account for the cellular potency of **3** which exceeds that of its inherent enzyme inhibitory potency. Finally, compounds **9**–**11**, **14**–**16**, and **19**–**21** were inactive (IC_{50} > 100 μM) in the CCRF–CEM assay in the presence or absence of media purines or pyrimidines.

6. Conclusions

10-Methanesulfonyl-DDACTHF (**1**) and 10-methanesulfonyl-5-DACTHF (**2**) were prepared as sulfone and sulfonamide mimics of the GAR Tfase-catalyzed formyl transfer reaction tetrahedral intermediate. Both proved to be effective GAR Tfase inhibitors (K_i = 0.23 and 0.58 μM) being 5- to 10-fold more potent than the parent, unsubstituted DDACTHF. Both exhibited purine sensitive cytotoxic activity at concentrations roughly 4-fold higher than their in vitro enzymatic activity consistent with functional inhibition GAR Tfase and de novo purine biosynthesis, and both benefit from transport into the cell by the reduced folate carrier and FPGS polyglutamation. More interestingly, 10-methylthio-DDACTHF (**3**), prepared for direct comparison alongside **1** and **2**, proved to be a surprisingly potent GAR Tfase inhibitor (K_i = 0.25 μM) and exhibited exceptionally potent, purine sensitive cytotoxic activity (CCRF–CEM IC_{50} = 100 nM). This functional cellular activity of **3** exceeded its in vitro enzymatic activity and was shown to benefit from both FPGS polyglutamation and reduced folate carrier transport into the cell. As such, its properties indicate it merits in vivo examination alongside related recently disclosed inhibitors.

7. Experimental

7.1. Methyl 4-(4-bromo-1-methanesulfonylbutyl)-benzoate (7)

A suspension of NaH (60% dispersion, 0.225 g, 5.62 mmol, 1.2 equiv) in freshly distilled DMF (3 mL) was treated with a solution of methyl 4-(methanesulfonylmethyl)benzoate (1.07 g, 4.69 mmol) in freshly distilled DMF (20 mL) at 0 °C. After the solution was stirred at 0 °C for 25 min, 1,3-dibromopropane (4.78 mL, 46.9 mmol, 10 equiv) was added to the reaction mixture. The cooling bath was removed and the reaction mixture was stirred at 25 °C for 2.5 h before being quenched by the addition of saturated aqueous NH_4Cl (50 mL). The resulting aqueous solution was extracted with EtOAc (5 × 20 mL). The combined organic layers were washed with saturated aqueous NaCl (50 mL), dried (Na_2SO_4), filtered, and concentrated. Column chromatography (SiO_2, 25–50% EtOAc–hexanes) afforded **7** (0.57 g, 35%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.04 (d, *J* = 8.2 Hz, 2H), 7.52 (d, *J* = 8.2 Hz, 2H), 4.10 (dd, *J* = 4.1, 11.1 Hz, 1H), 3.89 (s, 3H), 3.38–3.28 (m, 2H), 2.63 (s, 3H), 2.61–2.51 (m, 1H), 2.31–2.21 (m, 1H), 1.89–1.81 (m, 1H), 1.78–1.65 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.3, 137.5, 131.1, 130.4, 129.4, 68.7, 52.4, 38.8, 32.3, 29.6, 26.1; IR (neat) ν_{max} 2954, 1718, 1605, 1436, 1282, 1128, 1113 cm^{-1}; MALDI–FTMS (DHB) *m*/*z* 370.9929 (M+Na$^+$, $C_{13}H_{17}BrO_4S$ requires 370.9923).

7.2. Methyl 4-(5-cyano-5-ethoxycarbonyl-1-methanesulfonylpentyl)benzoate (8)

A suspension of NaH (60% dispersion, 78.7 mg, 1.97 mmol, 1.2 equiv) in freshly distilled DMF (1.5 mL) was treated with ethyl cyanoacetate (0.227 mL, 2.13 mmol, 1.3 equiv) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, forming the sodium salt as a clear solution. A solution of **7** (0.57 g, 1.64 mmol) in freshly distilled DMF (1.5 mL) was added at 0 °C and the reaction mixture was stirred at 25 °C for 2 h before being quenched by the addition of saturated aqueous NH_4Cl (30 mL). The resulting aqueous solution was extracted with EtOAc (5 × 10 mL). The combined organic layers were washed with saturated aqueous NaCl (30 mL), dried (Na_2SO_4), filtered, and concentrated. Column chromatography (SiO_2, 33–67% EtOAc–hexanes) afforded **8** (0.44 g, 70%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.08 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.4 Hz, 2H), 4.21 (q, *J* = 7.4 Hz, 2H), 4.08 (dd, *J* = 4.1, 11.4 Hz, 1H), 3.92 (s, 3H), 3.46–3.42 (m, 1H), 2.61 (s, 3H), 2.48–2.42

(m, 1H), 2.20–2.12 (m, 1H), 2.01–1.91 (m, 2H), 1.52–1.47 (m, 1H), 1.41–1.37 (m, 1H), 1.24 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 166.7, 166.0, 138.0, 131.7, 130.9, 129.7, 116.4, 69.7, 63.4, 52.8, 39.2, 37.5, 29.5, 26.9, 24.4, 14.4; IR (neat) ν_{max} 2933, 1738, 1718, 1436, 1282, 1190, 1133, 1108, 1021 cm^{-1}; MALDI–FTMS (DHB) *m/z* 404.1142 (M+Na$^+$, $C_{18}H_{23}NO_6S$ requires 404.1138).

7.3. Methyl 4-[4-(2,4-diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-1-methanesulfonylbutyl]benzoate (9)

A solution of NaOMe prepared by dissolving sodium (40.5 mg, 1.76 mmol, 2.1 equiv) in anhydrous MeOH (1.06 mL) was treated with guanidine hydrochloride (96.2 mg, 1.01 mmol, 1.2 equiv) at ambient temperature. After the reaction mixture was stirred at ambient temperature for 30 min, compound **8** (0.32 g, 0.84 mmol) in freshly distilled DMF (1.06 mL) was added. The reaction mixture was allowed to warm to 75 °C and stirred for 16 h. The reaction was quenched by the addition of acetic acid (53.3 μL, 0.92 mmol, 1.1 equiv), and column chromatography (SiO_2, 6–13% MeOH–$CHCl_3$) afforded **9** (0.19 g, 58%) as a light yellow solid: ^{1}H NMR (CD_3OD, 500 MHz): δ 7.81 (d, $J = 8.1$ Hz, 2H), 7.36 (d, $J = 8.1$ Hz, 2H), 4.29 (dd, $J = 3.3$, 11.4 Hz, 1H), 3.68 (s, 3H), 2.54 (s, 3H), 2.15–2.00 (m, 3H), 1.95–1.91 (m, 1H), 1.15–1.09 (m, 2H); ^{13}C NMR (CD_3OD, 100 MHz): δ 168.2, 165.3, 164.5, 155.1, 139.9, 131.9, 131.5, 131.0, 89.2, 70.0, 52.9, 39.2, 28.1, 24.2, 22.8; IR (neat) ν_{max} 2911, 2351, 1712, 1644, 1610, 1432, 1281, 1118 cm^{-1}; MALDI–FTMS (DHB) *m/z* 417.1184 (M+Na$^+$, $C_{17}H_{22}N_4O_5S$ requires 417.1203).

7.4. 4-[4-(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-1-methanesulfonylbutyl]benzoic acid (10)

A solution of **9** (0.117 g, 0.30 mmol) in MeOH (4.1 mL) was treated with LiOH monohydrate (37.4 mg, 0.89 mmol) in water (1.3 mL), and the reaction solution was stirred at ambient temperature for 24 h. The reaction solution was diluted with water (10 mL), washed with EtOAc (2 × 10 mL), acidified to pH 4 by addition of 1 N aqueous HCl, and concentrated. Removal of traces of water by treatment of the residue with benzene (3 × 5 mL) provided **10** (0.113 g, 100%) as a white solid: ^{1}H NMR (D_2O, 400 MHz): δ 7.92 (d, $J = 8.2$ Hz, 2H), 7.44 (d, $J = 8.2$ Hz, 2H), 4.48 (dd, $J = 3.2$, 11.4 Hz, 1H), 2.84 (s, 3H), 2.26–2.21 (m, 1H), 2.19–2.14 (m, 2H), 2.03–1.98 (m, 1H), 1.37–1.30 (m, 1H), 1.20–1.14 (m, 1H); MALDI–FTMS (DHB) *m/z* 381.1220 (M+H$^+$, $C_{16}H_{20}N_4O_5S$ requires 381.1227).

7.5. Di-*tert*-butyl-*N*-{4-[4-(2,4-diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-1-methanesulfonylbutyl]benzoyl}-L-glutamate (11)

A solution of **10** (0.108 g, 0.28 mmol), di-*tert*-butyl-L-glutamate hydrochloride (0.126 g, 0.43 mmol, 1.5 equiv) and $NaHCO_3$ (71.6 mg, 0.85 mmol, 3 equiv) in DMF (2.7 mL) was treated with EDCI (0.163 g, 0.85 mmol, 3 equiv) at 0 °C. The reaction mixture was stirred at ambient temperature overnight before the addition of $CHCl_3$ (10 mL). The resulting solution was washed with saturated aqueous $NaHCO_3$ (2 × 10 mL), dried (Na_2SO_4), filtered, and concentrated. Column chromatography (SiO_2, 5–17% MeOH–$CHCl_3$) afforded **11** (78 mg, 45%) as a light yellow solid: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.81 (d, $J = 7.6$ Hz, 2H), 7.48 (d, $J = 7.6$ Hz, 2H), 4.67–4.63 (m, 1H), 4.42–4.34 (m, 0.5H), 4.22 (t, 0.5H, $J = 5.9$ Hz), 2.63 (s, 3H), 2.48–2.36 (m, 4H), 2.29–2.19 (m, 2H), 2.13–2.03 (m, 2H), 1.49 (s, 9H), 1.42 (s, 9H), 1.39–1.28 (m, 2H); IR (neat) ν_{max} 3342, 2910, 1731, 1637, 1618, 1453, 1362, 1286, 1147 cm^{-1}; MALDI–FTMS (DHB) *m/z* 622.2912 (M+H$^+$, $C_{29}H_{43}N_5O_8S$ requires 622.2900).

7.6. *N*-{4-[4-(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-1-methanesulfonylbutyl]benzoyl}-L-glutamic acid (1)

A solution of **11** (3.8 mg, 6.11 μmol) in $CHCl_3$ (0.2 mL) was treated with trifluoroacetic acid (1 mL) at 0 °C. The reaction solution was allowed to warm to 25 °C, and stirred overnight. The solution was concentrated, and triturated with Et_2O (3 × 1 mL) to give **1**–CF_3COOH (3.8 mg, 100%) as a white solid: ^{1}H NMR (D_2O, 500 MHz): δ 7.63 (d, $J = 7.7$ Hz, 2H), 7.32 (d, $J = 7.7$ Hz, 2H), 4.44–4.36 (m, 1H), 4.23–4.19 (m, 1H), 2.49 (s, 3H), 2.26–2.20 (m, 2H), 2.13–2.03 (m, 4H), 1.91–1.82 (m, 2H), 1.16–1.05 (m, 2H); MALDI–FTMS (DHB) *m/z* 510.1637 (M+H$^+$, $C_{21}H_{27}N_5O_8S$ requires 510.1648).

7.7. Methyl 4-[(3-bromopropyl)methanesulfonylamino]benzoate (12)

A suspension of NaH (60% dispersion, 0.175 g, 4.36 mmol, 1 equiv) in freshly distilled DMF (3 mL) was treated with a solution of methyl 4-(methanesulfonylamino)benzoate (1 g, 4.36 mmol) in freshly distilled DMF (20 mL) at 0 °C. After the solution was stirred at 0 °C for 25 min, 1,3-dibromopropane (4.47 mL, 43.6 mmol, 10 equiv) was added to the reaction mixture. The cooling bath was removed and the reaction mixture was stirred at 25 °C for 2.5 h before being quenched by the addition of saturated aqueous NH_4Cl (50 mL). The resulting aqueous solution was extracted with EtOAc (5 × 20 mL). The combined organic layers were washed with saturated aqueous NaCl (50 mL), dried (Na_2SO_4), filtered, and concentrated. Chromatography (SiO_2, 25–50% EtOAc–hexanes) afforded **12** (1.16 g, 76%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.07 (d, $J = 8.8$ Hz, 2H), 7.43 (d, $J = 8.8$ Hz, 2H), 3.91 (s, 3H), 3.88 (t, $J = 7.0$ Hz, 2H), 3.40 (t, $J = 6.2$ Hz, 2H), 2.89 (s, 3H), 2.08–2.02 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.5, 143.7, 131.4, 130.0, 127.5, 52.8, 49.3, 37.6, 32.1, 30.1; IR (neat) ν_{max} 2952, 1719, 1602, 1499, 1431, 1341, 1283, 1152, 1111 cm^{-1}; MALDI–FTMS (DHB) *m/z* 371.9885 (M+Na$^+$, $C_{12}H_{16}BrNO_4S$ requires 371.9876).

7.8. Methyl 4-[(4-cyano-4-ethoxycarbonylbutyl)methanesulfonylamino]benzoate (13)

A suspension of NaH (60% dispersion, 59.0 mg, 1.47 mmol, 1.2 equiv) in freshly distilled DMF

(1.2 mL) was treated with ethyl cyanoacetate (0.17 mL, 1.6 mmol, 1.3 equiv) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, forming the sodium salt as a clear solution. A solution of **12** (0.43 g, 1.23 mmol) in freshly distilled DMF (1.2 mL) was added at 0 °C and the reaction mixture was stirred at 25 °C for 2 h before being quenched by the addition of saturated aqueous NH_4Cl (30 mL). The resulting aqueous solution was extracted with EtOAc (5 × 10 mL). The combined organic layers were washed with saturated aqueous NaCl (30 mL), dried (Na_2SO_4), filtered, and concentrated. Flash chromatography (SiO_2, 33–67% EtOAc–hexanes) afforded **13** (0.34 g, 73%) as a colorless oil: ^1H NMR ($CDCl_3$, 400 MHz): δ 8.00 (d, J = 7.9 Hz, 2H), 7.38 (d, J = 7.9 Hz, 2H), 4.13 (q, J = 6.8 Hz, 2H), 3.84 (s, 3H), 3.74 (t, J = 5.6 Hz, 2H), 3.53 (t, J = 6.5 Hz, 1H), 2.82 (s, 3H), 2.00–1.91 (m, 2H), 1.62–1.57 (m, 2H), 1.19 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.0, 165.7, 142.8, 130.9, 129.6, 127.8, 116.3, 62.8, 52.3, 48.9, 37.0, 36.7, 26.3, 25.2, 13.9; IR (neat) ν_{max} 2943, 1742, 1719, 1602, 1337, 1278, 1152 cm^{-1}; MALDI–FTMS (DHB) m/z 405.1095 ($M+Na^+$, $C_{17}H_{22}N_2O_6S$ requires 405.1091).

7.9. Methyl 4-[[3-(2,4-diamino-6-oxo-1,6-dihydropyrimidin-5-yl)propyl]methanesulfonylamino]benzoate (14)

A solution of NaOMe prepared by dissolving sodium (43.1 mg, 1.87 mmol, 2.1 equiv) in anhydrous MeOH (1.13 mL) was treated with guanidine hydrochloride (102 mg, 1.07 mmol, 1.2 equiv) at ambient temperature. After the reaction mixture was stirred at ambient temperature for 30 min, **13** (0.341 g, 0.892 mmol) in freshly distilled DMF (1.13 mL) was added. The reaction mixture was allowed to warm to 75 °C and stirred for 16 h. The reaction was quenched by the addition of acetic acid (56.7 μL, 0.98 mmol, 1.1 equiv), and column chromatography (SiO_2, 8–25% MeOH–$CHCl_3$) afforded **14** (0.204 g, 58%) as a light yellow solid: ^1H NMR (CD_3OD, 500 MHz): δ 7.95 (d, J = 8.5 Hz, 2H), 7.51 (d, J = 8.5 Hz, 2H), 3.84 (s, 3H), 3.66 (t, J = 7.4 Hz, 2H), 3.01 (s, 3H), 2.12 (t, J = 7.3 Hz, 2H), 1.40–1.34 (m, 2H); IR (neat) ν_{max} 2920, 1716, 1706, 1652, 1590, 1431, 1320, 1278 cm^{-1}; MALDI–FTMS (DHB) m/z 396.1330 ($M+H^+$, $C_{16}H_{21}N_5O_5S$ requires 396.1336).

7.10. 4-[[3-(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)propyl]methanesulfonylamino]benzoic acid (15)

A solution of **14** (0.1 g, 0.25 mmol) in MeOH (3.5 mL) was treated with LiOH monohydrate (31.9 mg, 0.76 mmol) in water (1.14 mL), and the reaction solution was stirred at ambient temperature for 24 h. The reaction solution was diluted with water (10 mL), washed with EtOAc (2 × 10 mL), acidified to pH 4 by addition of 1 N aqueous HCl, and concentrated. Removal of traces of water by treatment of the residue with benzene (3 × 5 mL) provided **15** (96.4 mg, 100%) as a white solid: ^1H NMR (D_2O, 400 MHz): δ 7.68 (d, J = 7.0 Hz, 2H), 7.18 (d, J = 7.0 Hz, 2H), 3.47 (t, J = 7.4 Hz, 2H), 2.84 (s, 3H), 2.00 (t, J = 7.3 Hz, 2H), 1.41–1.36 (m, 2H); MALDI–FTMS (DHB) m/z 382.1167 ($M+H^+$, $C_{15}H_{19}N_5O_5S$ requires 382.1180).

7.11. Di-*tert*-butyl-*N*-{4-[[3-(2,4-diamino-6-oxo-1,6-dihydropyrimidin-5-yl)propyl]methanesulfonylamino]benzoyl}-L-glutamate (16)

A solution of **15** (96.4 mg, 0.25 mmol), di-*tert*-butyl-L-glutamate hydrochloride (0.112 g, 0.38 mmol, 1.5 equiv) and $NaHCO_3$ (63.8 mg, 0.76 mmol, 3 equiv) in DMF (2.4 mL) was treated with EDCI (0.146 g, 0.76 mmol, 3 equiv) at 0 °C. The reaction mixture was stirred at ambient temperature overnight before the addition of $CHCl_3$ (10 mL). The resulting solution was washed with saturated aqueous $NaHCO_3$ (2 × 10 mL), dried (Na_2SO_4), filtered, and concentrated. PCTLC (SiO_2, 1 mm plate, 14% MeOH–$CHCl_3$) afforded **16** (70.8 mg, 45%) as a light yellow solid: ^1H NMR ($CDCl_3$, 400 MHz): δ 7.63 (d, J = 7.9 Hz, 2H), 7.27 (d, J = 7.9 Hz, 2H), 4.25 (dd, J = 4.7, 8.8 Hz, 1H), 3.52 (t, J = 7.6 Hz, 2H), 2.71 (s, 3H), 2.15 (t, J = 7.0 Hz, 2H), 2.07 (t, J = 6.5 Hz, 2H), 1.97–1.92 (m, 0.5H), 1.82–1.74 (m, 1H), 1.56–1.52 (m, 0.5H), 1.39–1.34 (m, 2H), 1.24 (s, 9H), 1.19 (s, 9H); IR (neat) ν_{max} 3342, 3173, 2983, 2930, 1727, 1616, 1600, 1489, 1447, 1368, 1331, 1146 cm^{-1}; MALDI–FTMS (DHB) m/z 645.2662 ($M+Na^+$, $C_{28}H_{42}N_6O_8S$ requires 645.2677).

7.12. *N*-{4-[[3-(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)propyl]methanesulfonylamino]benzoyl}-L-glutamic acid (2)

A solution of **16** (10 mg, 16.1 μmol) in $CHCl_3$ (0.2 mL) was treated with trifluoroacetic acid (1 mL) at 0 °C. The reaction solution was allowed to warm to 25 °C, and stirred overnight. The solution was concentrated, and triturated with Et_2O (3 × 1 mL) to give **2**–CF_3COOH (10.0 mg, 100%) as a white solid: ^1H NMR (D_2O, 400 MHz): δ 7.67 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 8.2 Hz, 2H), 4.50–4.45 (m, 1H), 3.56 (t, J = 7.6 Hz, 2H), 2.69 (s, 3H), 2.40 (t, J = 6.7 Hz, 2H), 2.19–2.13 (m, 1H), 1.99–1.95 (m, 1H), 1.88–1.82 (m, 1H), 1.50–1.47 (m, 2H), 0.98–0.94 (m, 1H); MALDI–FTMS (DHB) m/z 511.1594 ($M+H^+$, $C_{20}H_{26}N_6O_8S$ requires 511.1606).

7.13. Methyl 4-(4-chloro-1-methylthiobutyl)benzoate (17)

After a solution of sodium bis(trimethylsilyl)amide (1.0 M in THF, 1.07 mL, 1.07 mmol, 1.05 equiv) was treated with a solution of methyl 4-(methylthiomethyl)benzoate (0.2 g, 1.02 mmol) in freshly distilled THF (3 mL) at −78 °C, 1-chloro-3-iodopropane (1.1 mL, 10.2 mmol, 10 equiv) was added quickly to the reaction mixture. The cooling bath was removed, and the reaction mixture was stirred at 25 °C for 45 min before being quenched by addition of saturated aqueous NH_4Cl (10 mL). The resulting aqueous solution was extracted with EtOAc (4 × 5 mL). The combined organic layers were washed with saturated aqueous NaCl (15 mL), dried (Na_2SO_4), filtered, and concentrated. Column chromatography (SiO_2, 5–17%

EtOAc–hexanes) afforded **17** (86 mg, 31%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.00 (d, J = 8.1 Hz, 2H), 7.36 (d, J = 8.1 Hz, 2H), 3.91 (s, 3H), 3.71 (t, J = 7.7 Hz, 1H), 3.51 (t, J = 6.6 Hz, 2H), 2.10–1.96 (m, 2H), 1.86 (s, 3H), 1.89–1.83 (m, 1H), 1.75–1.69 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.8, 147.4, 129.9, 129.1, 127.8, 52.1, 50.5, 44.5, 32.9, 30.5, 14.2; IR (neat) ν_{max} 2955, 1719, 1430, 1280, 1189, 1111 cm^{-1}; ESI–MS (NBA) m/z 295.1 (M+Na$^+$, $C_{13}H_{17}ClO_2S$ requires 295.1).

7.14. Methyl 4-(5-cyano-5-ethoxycarbonyl-1-methylthiopentyl)benzoate (18)

A suspension of NaH (60% dispersion, 73.9 mg, 1.85 mmol, 1.2 equiv) in freshly distilled DMF (1.5 mL) was treated with ethyl cyanoacetate (0.214 mL, 2.01 mmol, 1.3 equiv) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, forming the sodium salt as a clear solution. A solution of **17** (0.42 g, 1.54 mmol) in freshly distilled DMF (1.5 mL) was added at 0 °C and the reaction mixture was allowed to warm to 50 °C, and stirred for 9 h before being quenched by addition of saturated aqueous NH_4Cl (30 mL). The resulting aqueous solution was extracted with EtOAc (5 × 5 mL). The combined organic layers were washed with saturated aqueous NaCl (20 mL), dried (Na_2SO_4), filtered, and concentrated. Column chromatography (SiO_2, 6–33% EtOAc–hexanes) afforded **18** (0.30 g, 56%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.00 (d, J = 7.9 Hz, 2H), 7.36 (d, J = 7.9 Hz, 2H), 4.23 (q, J = 7.4 Hz, 2H), 3.92 (s, 3H), 3.70 (t, J = 7.0 Hz, 1H), 3.43 (dd, J = 7.4, 14.1 Hz, 1H), 2.02–1.91 (m, 4H), 1.85 (s, 3H), 1.65–1.45 (m, 2H), 1.28 (t, J = 7.3 Hz, 3H); IR (neat) ν_{max} 2919, 1725, 1705, 1611, 1430, 1274, 1177, 1105 cm^{-1}; MALDI–FTMS (DHB) m/z 372.1243 (M+Na$^+$, $C_{18}H_{23}NO_4S$ requires 372.1240).

7.15. Methyl 4-[4-(2,4-diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-1-methylthiobutyl]benzoate (19)

A solution of NaOMe prepared by dissolving sodium (23.8 mg, 1.03 mmol, 2.1 equiv) in anhydrous MeOH (0.62 mL) was treated with guanidine hydrochloride (56.4 mg, 0.59 mmol, 1.2 equiv) at ambient temperature. After the reaction mixture was stirred at ambient temperature for 30 min, **18** (0.172 g, 0.49 mmol) in freshly distilled DMF (0.62 mL) was added. The reaction mixture was allowed to warm to 75 °C and stirred for 16 h. The reaction was quenched by addition of acetic acid (31.3 μL, 0.54 mmol, 1.1 equiv), and column chromatography (SiO_2, 8–25% MeOH–$CHCl_3$) afforded **19** (0.102 g, 57%) as a light yellow solid: ^{1}H NMR (CD_3OD, 500 MHz): δ 7.95 (d, J = 8.1 Hz, 2H), 7.33 (d, J = 8.1 Hz, 2H), 3.88 (s, 3H), 3.72 (t, J = 7.4 Hz, 1H), 2.23–2.19 (m, 2H), 1.91–1.81 (m, 2H), 1.80 (s, 3H), 1.51–1.45 (m, 1H), 1.42–1.36 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.9, 164.4, 161.9, 153.3, 148.0, 129.8, 128.8, 127.9, 89.6, 52.1, 50.9, 36.5, 35.3, 25.9, 14.2; IR (neat) ν_{max} 2909, 1707, 1632, 1601, 1428, 1277 cm^{-1}; MALDI–FTMS (DHB) m/z 363.1490 (M+H$^+$, $C_{17}H_{22}N_4O_3S$ requires 363.1485).

7.16. 4-[4-(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-1-methylthiobutyl]benzoic acid (20)

A solution of **19** (29.6 mg, 81.7 μmol) in MeOH (1.13 mL) was treated with LiOH monohydrate (10.3 mg, 0.245 mmol, 3 equiv) in water (0.38 mL), and the reaction solution was stirred at ambient temperature for 24 h. The reaction solution was diluted with water (10 mL), washed with EtOAc (2 × 10 mL), acidified to pH 4 by addition of 1 N aqueous HCl, and concentrated. Removal of traces of water by treatment of the residue with benzene (3 × 5 mL) provided **20** (28.4 mg, 100%) as a white solid: ^{1}H NMR (D_2O, 500 MHz): δ 7.70 (d, J = 8.1 Hz, 2H), 7.21 (d, J = 8.1 Hz, 2H), 3.68 (dd, J = 6.3, 9.2 Hz, 1H), 2.12 (t, J = 7.0 Hz, 2H), 1.94–1.83 (m, 2H), 1.77 (s, 3H), 1.38–1.32 (m, 1H), 1.24–1.21 (m, 1H); MALDI–FTMS (DHB) m/z 349.1334 (M+H$^+$, $C_{16}H_{20}N_4O_3S$ requires 349.1329).

7.17. Di-*tert*-butyl-*N*-{4-[4-(2,4-diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-1-methylthiobutyl]benzoyl}-L-glutamate (21)

A solution of **20** (25.5 mg, 73.4 μmol), di-*tert*-butyl-L-glutamate hydrochloride (32.6 mg, 0.11 mmol, 1.5 equiv) and $NaHCO_3$ (18.5 mg, 0.22 mmol, 3 equiv) in DMF (0.71 mL) was treated with EDCI (42.2 mg, 0.22 mmol, 3 equiv) at 0 °C. The reaction mixture was stirred at ambient temperature overnight before the addition of $CHCl_3$ (10 mL). The resulting solution was washed with saturated aqueous $NaHCO_3$ (2 × 10 mL), dried (Na_2SO_4), filtered, and concentrated. PCTLC (SiO_2, 1 mm plate, 14% MeOH–$CHCl_3$) afforded **21** (19.0 mg, 44%) as a light yellow solid: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.73 (d, J = 7.0 Hz, 2H), 7.33 (d, J = 7.0 Hz, 2H), 4.70–4.66 (m, 1H), 3.74 (t, J = 6.6 Hz, 1H), 2.46–2.40 (m, 1H), 2.37–2.30 (m, 1H), 2.27–2.20 (m, 2H), 2.08–2.03 (m, 1H), 1.96–1.90 (m, 1H), 1.87–1.83 (m, 1H), 1.81 (s, 3H), 1.77–1.69 (m, 1H), 1.50 (s, 9H), 1.41 (s, 9H), 1.26–1.24 (m, 2H); IR (neat) ν_{max} 3329, 2922, 1722, 1624, 1601, 1360, 1142 cm^{-1}; MALDI–FTMS (DHB) m/z 590.3000 (M+H$^+$, $C_{29}H_{43}N_5O_6S$ requires 590.3007).

7.18. *N*-{4-[4-(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-1-methylthiobutyl]benzoyl}-L-glutamic acid (3)

A solution of **21** (3.3 mg, 5.60 μmol) in $CHCl_3$ (0.2 mL) was treated with trifluoroacetic acid (1 mL) at 0 °C. The reaction solution was allowed to warm to 25 °C, and stirred overnight. The solution was concentrated, and triturated with Et_2O (3 × 1 mL) to give **3**–CF_3COOH (3.3 mg, 100%) as a white solid: ^{1}H NMR (D_2O, 500 MHz): δ 7.59 (d, J = 6.2 Hz, 2H), 7.26 (d, J = 6.2 Hz, 2H), 4.47–4.44 (m, 1H), 3.68–3.65 (m, 1 H), 2.41 (t, J = 6.2 Hz, 2H), 2.19–2.15 (m, 1H), 2.08 (t, J = 7.4 Hz, 2H), 2.00–1.97 (m, 1H), 1.83–1.79 (m, 1H), 1.72 (s, 3H), 1.69–1.65 (m, 1H), 1.38–1.32 (m, 1H), 1.19–1.11 (m, 1H); MALDI–FTMS (DHB) m/z 478.1746 (M+H$^+$, $C_{21}H_{27}N_5O_6S$ requires 478.1755).

7.19. GAR and AICAR Tfase assay

The K_i values for the folate analogues were measured as previously described.[7] For the GAR Tfase inhibition assay, each compound was dissolved in DMSO and then diluted in assay buffer and the concentration of DMSO did not affect enzyme activity. Thus, all assays were conducted by mixing 10 μM of 10-formyl-5,8-dideazafolic acid (fDDF), 20 μM of inhibitor in total volume of 1 mL buffer (0.1 M HEPES, pH 7.5) at 26 °C, and the reaction initiated by the addition of 76 nM *E. coli* or rhGAR Tfase. 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. If the inhibitor was found to be active, a series of $1/v_i$ versus 1/[GAR] at different, fixed concentrations of I (e.g., 1, 2, 4, 8, 12, 16, 20, 32 μM) were generated in order to determine K_i using the Michaelis–Menton equation for competitive inhibition. AICAR Tfase inhibition studies was conducted in the absence of 5 μM β-mercaptoethanol and screened with 10 nM enzyme, 25 μM inhibitor and 22.5 μM of cofactor. The results for the inhibition assays are summarized in Table 1.

7.20. Cytotoxic assay

The cytotoxic activity of the compounds was measured using the CCRF–CEM human leukemia cell lines as described previously (72 h assay).[10]

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