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Novel 2-amino-4-oxo-5-arylthio-substituted-pyrrolo[2,3-*d*]pyrimidines as nonclassical antifolate inhibitors of thymidylate synthase

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Abstract—A series of 17 novel 2-amino-4-oxo-5-[(substituted phenyl)thio]pyrrolo[2,3-*d*]pyrimidines were synthesized as potential inhibitors of thymidylate synthase (TS) and as antitumor agents. The analogues contain a variety of electron withdrawing substituents on the phenyl ring of the side chain and were evaluated as inhibitors of human TS (hTS) and *Escherichia coli* TS and of human and *E. coli* dihydrofolate reductase (DHFR). The analogues **14**, **17**, and **18** were potent inhibitors of hTS with IC_{50} values of 0.28, 0.21, and 0.22 μM, respectively, and were more potent than the clinically used ZD1694, **2** and LY231514, **3** against human TS.
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Thymidylate synthase (TS) catalyzes the reductive methylation of 2′-deoxyuridine-5′-monophosphate (dUMP) to 2′-deoxythymidine-5′-monophosphate (dTMP) utilizing 5,10-methylenetetrahydrofolate as the source of the methyl group as well as the reductant.[1] This represents the sole de novo source of dTMP and hence inhibition of TS, in the absence of salvage, leads to 'thymineless death'. Thus inhibition of TS is an attractive goal for the development of antitumor agents.[2,3]

5-Fluorouracil (5-FU), a mechanism based inhibitor of TS, is a prominent antitumor agent. The emergence of resistance as well as the insensitivity of certain tumor types to 5-FU has prompted the design of novel folate analogues as potential TS inhibitors and antitumor agents.[4] Several TS inhibitors, related to folic acid notably ZD1694[5] (Tomudex,™ Raltitrexed) and LY231514[6] (Alimta,™ Pemetrexed) (Fig. 1) have been approved in Europe and the US, respectively, as antitumor agents. The recent approval of LY231514 for the treatment of malignant pleural or peritoneal mesothelioma in combination with cisplatin and as a single-agent in the treatment of locally advanced or metastatic nonsmall cell lung cancer (NSCLC) has provided additional stimulus for the design of novel TS inhibitors as antitumor agents.

PDDF,[7] ZD1694, and LY231514 are classical antifolates. They contain a benzoyl L-glutamic acid side chain similar to natural folates. The presence of the benzoyl-L-glutamic acid side chain usually provides excellent substrate properties for the enzyme folylpoly-γ-glutamate synthetase (FPGS).[6,7] FPGS catalyzes the formation of poly-γ-glutamates, which lead to high intracellular concentrations of these antitumor agents and at the same time, increases TS inhibitory activity for certain antifolates (ZD1694 and LY231514). Although polyglutamylation of some classical antifolates is necessary for cytotoxicity, it has also been implicated as a possible cause of toxicity to host cells and in the development of resistance in tumors with low levels of FPGS. An additional problem associated with classical antifolates is their dependence on a reduced folate carrier (RFC) system to gain access into the cell. The impairment of this carrier system can also lead to drug resistance.[8]

The problems associated with classical antifolates, such as resistance due to decreased FPGS activity and/or inefficient uptake could be overcome by nonclassical lipophilic analogues, which would not be dependent on FPGS for their potency and could passively diffuse into cells.[8] AG337 (Thymitaq) (Fig. 1) is the first nonclassical TS inhibitor to reach clinical trials.[9] Gangjee et al.[10] designed the 2-amino-4-oxo-5-thiopyridyl-6-methylpyrrolo[2,3-*d*]-pyrimidine, **5** as a potent TS inhibitor (human TS IC_{50} = 0.34 μM), based on the X-ray crystal structure

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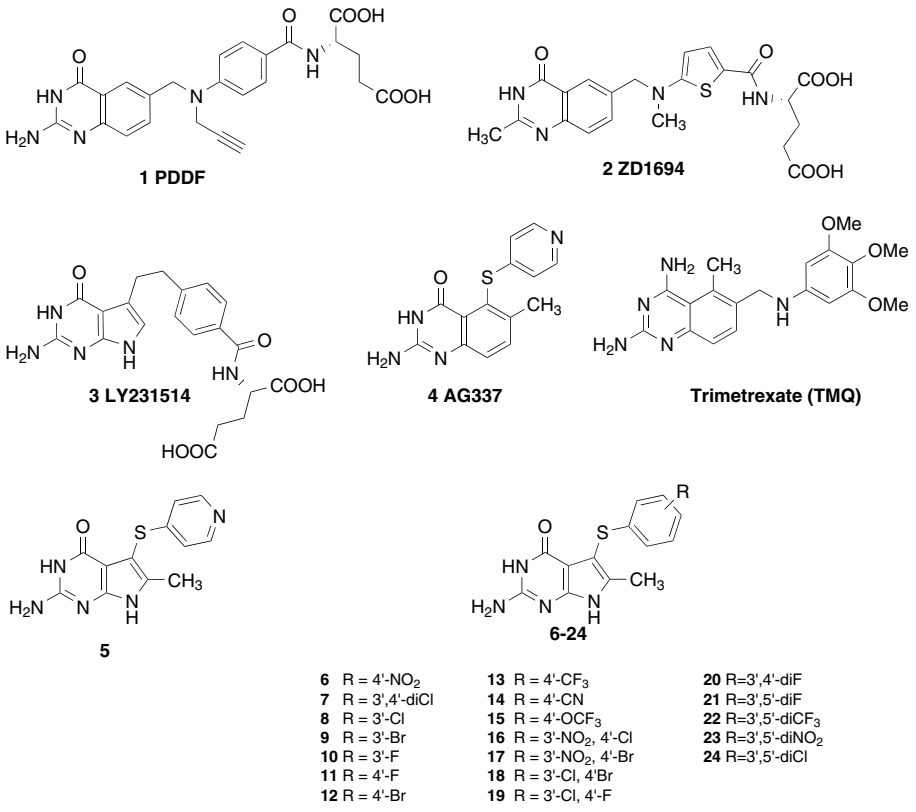


Figure 1.

of TS and AG337.[8] Molecular modeling of compound **5** in human TS indicated that the 6-methyl group makes an important hydrophobic contact with a Tryptophan (Trp109) of human TS and it also sterically restricts the rotation of the 5-position side chain so that it adopts the most favorable conformation for binding to human TS. SAR studies on **5** afforded **6** and **7** (Fig. 1), which were somewhat more potent inhibitors of human TS with IC_{50} values of 0.15 and 0.13 μM, respectively.[11] Compounds **6** and **7**, with electron withdrawing nitro- or chloro- substituents in the 3′- and/or 4′-positions of the side chain phenyl ring, were found to be much more potent than the unsubstituted phenyl (IC_{50} = 30 μM) or an analogue containing 3′,4′-dimethoxy, electron donating, substituents (IC_{50} = 2.4 μM) against human TS. The 3′,4′-dichloro analogue, **7**, was 8-fold more potent than the 4′-chloro analogue (IC_{50} = 1.0 μM) against human TS. Thus the introduction of an additional chlorine atom at the 3′-position resulted in increased activity against human TS. The increased activity could be attributed to the presence of additional bulk at the 3′-position and/or reinforcement of the direction and magnitude of the dipole in the molecule.[8] The nature of the electron withdrawing group present at the 4′-position also influences the inhibitory activity against human TS. Thus the 4′-nitro analogue, **6**, was about 7-fold more potent than the 4′-chloro analogue (IC_{50} = 1.0 μM) against human TS. Compounds **6** and **7** were also more potent than the classical analogues PDDF, ZD1694, and LY231514 against human TS. In addition, potent nonclassical TS inhibitors reported in the literature possess electron withdrawing groups in the phenyl ring in place of the benzoyl-L-glutamate of classical antifolates.[8,11] In contrast, *para* substituted electron donating groups on the phenyl ring have been reported to be detrimental to TS inhibitory activity.[8,12,13]

In previous studies Gangjee et al.[10,11] have shown that TS inhibitors demonstrate differences in inhibitory potency between TS from different sources. We reasoned that lipophilic nonclassical inhibitors of TS may also provide for selective inhibition of bacterial TS. Thus the target nonclassical TS inhibitors, **8**–**24** (Fig. 1), in addition to their antitumor effects, were also of interest as potential selective antibacterial agents.

Thus both the position and nature of the electron withdrawing groups present on the phenyl ring of the side chain influences human TS inhibitory activity.[10,11] In an attempt to determine the optimum electron withdrawing substituent(s) on the phenyl ring of the side chain for human TS inhibitory activity compounds **8**–**24** were synthesized with a variety of electron withdrawing groups including chlorine, fluorine, nitrile, bromine, nitro, trifluoromethyl, and trifluoromethoxy at the 3′ and/or 4′-positions of the phenyl side chain. The purpose of this study was to determine the optimum location and nature of the electron withdrawing group(s) on the phenyl side chain for human TS and bacterial TS inhibitory activity.

Recently, Gangjee et al.[14] suggested dual binding modes for 2-amino-4-oxo-pyrrolo[2,3-*d*]pyrimidines. These consist of the 2-amino-4-oxo mode (Fig. 2), which is the usual binding mode of antifolates for TS inhibition[19]

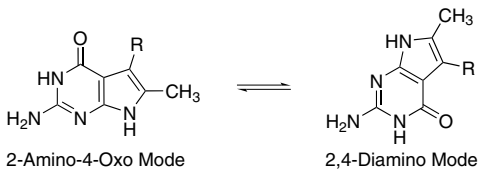
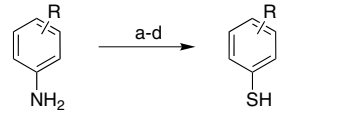


Figure 2.

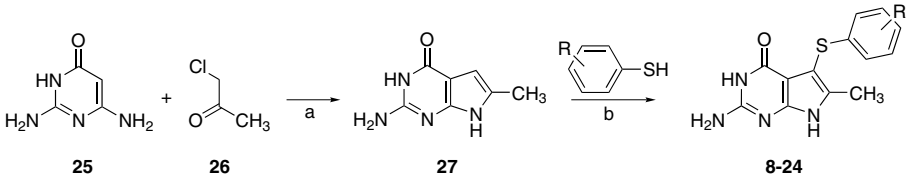
and the flipped 2,4-diamino mode, which is the usual binding mode of antifolates for DHFR inhibition. This flipped mode is obtained by a 180° rotation about the 2-NH_2–C_2 bond of the 2-amino-4-oxo mode. In this binding mode the pyrrole NH mimics the 4-amino moiety of the 2,4-diamino pyrimidine such as trimetrexate. Since compounds **8**–**24** are pyrrolo[2,3-*d*]pyrimidines and could adopt both binding modes discussed above, these compounds were also of interest as potential DHFR inhibitors.

Compounds **8**–**24** were synthesized from the key intermediate 2-amino-4-oxo-6-methylpyrrolo[2,3-*d*]pyrimidine, **27**[11] (Scheme 1), to which various aryl thiols were conveniently attached at the 5-position via a modification of an oxidative thiolation procedure reported by Gangjee et al.[10,11] The original procedure of 5-thioarylation involved a two step sequence in which the 2-amino group of compound **27** was protected with a pivaloyl group, in order to increase its solubility, followed by oxidative addition of the aryl thiols to the 5-position of the pivaloyl protected compound. This oxidative addition step also provide for concurrent deprotection of the 2-amino group.[10,11] The disadvantage of the reported method is that it involves a tedious protection and deprotection of the 2-amino group. The protection requires 48 h while the deprotection requires an additional 12–16 h. To provide a more efficient procedure the oxidative addition of the aryl thiols was attempted on **27** without 2-amino protection.[10,11] Compound **27** was obtained by the addition of chloroacetone, **26**, to a solution of 2,6-diamino-4-hydroxypyrimidine, **25**, in NaOAc and water as reported previously.[11] Following several variations of reaction time, temperature and sequence of addition of the reactants, it was determined that the addition of I_2 after, rather than before, the addition of the thiophenol affords better yields of the target compounds without the necessity of 2-amino protection compared with the previously reported method.[10,11] Thus **8**–**24** were synthesized in a one-step oxidative addition, of the appropriately substituted aryl thiols, to the 5-position of **27**. The optimized procedure involves heating a mixture of **27** with the appropriate aryl thiol in ethanol/water (2:1) at reflux followed by the addition of 2 equiv of I_2 and reflux maintained for 3–4 h. Purification via addition of NaS_2O_7, to remove the excess I_2, was an additional modification, which significantly reduces the volume of eluent ($CHCl_3$) necessary in the subsequent chromatographic purification. Evaporation of the solvent under reduced pressure gave a residue, which was purified by column chromatography to afford the target compounds **8**–**24** in 9–55% yields. The absence of the 5-aromatic proton and the presence of the appropriate protons of the phenyl side chain for **8**–**24** confirmed that substitution had occurred. This method has the advantage of decreasing the reaction steps to one and 3–4 h for completion with an average yield of 39% compared to the previous method with an overall average yield of only 25% over two steps. The aryl thiols **28**–**30** were not commercially available and were synthesized[15] from the precursor amines **31**–**33**. The method involved diazotization of the amine with $NaNO_2$ in aq HCl acid, followed by nucleophilic displacement of the diazonium moiety with ethyl xanthic acid potassium salt, and hydrolysis of the resulting xanthate ester with KOH (Scheme 2). Acidification of the xanthic acid obtained resulted in the evolution of carbon oxysulfide and the formation of an oil, which was extracted with ether. The crude oil obtained on evaporation of the ether contained the desired aryl thiols, which were used without further purification.

Compounds **8**–**24**, along with LY231514 and ZD1694 were evaluated[16,17] as inhibitors of human TS and *Escherichia coli* TS (Table 1). All of the compounds were moderate to potent inhibitors of human TS except **22**, which was inactive at the concentration tested. Compounds **14**, **17**, and **18** were the most potent inhibitors of human TS and were more potent than the classical, clinically used analogues ZD1694 and LY231514. They were also comparable in potency to the previously synthesized compounds, **6** and **7**.[11] In general, all compounds (except **22** and **23**) were more potent than LY231514 against human TS. Compounds **14**, **17**, and **18** were approximately 34-, 43-, and 45-fold more potent



Scheme 2. Reagents and conditions: (a) $NaNO_2$, concd HCl, 0 °C; (b) ethyl xanthic acid potassium salt, 60 °C; (c) KOH, reflux; (d) concd HCl.



Scheme 1. Reagents and conditions: (a) NaOAc/H_2O, 100 °C; (b) I_2, EtOH/H_2O (2:1), 100–110 °C.

Table 1. Inhibitory concentration (IC_{50}s in M) against Isolated TS and DHFR[a]

Compd	TS		DHFR	
	Human[b] $\times 10^{-6}$	*E. coli*[b] $\times 10^{-5}$	*E. coli*[c] $\times 10^{-5}$	Human[d] $\times 10^{-5}$
5	0.34[e]			
6	0.15[f]			
7	0.13[f]			
8	1.4		>3.3(0)[g]	>3.2(0)
9	1.2	>2.4(17)	>2.8(22)	
10	2.8	>2.8(9)	>3.3(0)	
11	1.4		>3.3(0)	>3.2(0)
12	0.46		>2.8(0)	>2.8(20)
13	0.8		>2.9(23)	>2.9(0)
14	0.28		>3.4(0)	>3.4(33)
15	2.3	>2.3(18)	>2.8(18)	
16	0.46	2.3	>2.8(6)	
17	0.21	1.1	>2.5(18)	
18	0.22		>2.6(41)	>2.6(0)
19	0.83		>3.1(12)	>3.1(12)
20	0.9		>3.2(10)	>3.2(14)
21	2.7		>3.2(0)	>3.2(10)
22	>21		>2.5(39)	>2.5(13)
23	23	>2.3(10)	>2.7(0)	
24	2.4	>2.4(26)	>2.9(15)	
ZD1694[h]	0.38	5.7		
LY231514[i]	9.5	76	230	6.6
MTX			6.6×10^{-4}	2.2×10^{-3}

[a] The percent inhibition was determined at a minimum of four inhibitor concentration within 20% of the 50% point. The standard deviations for determination of IC_{50} values were within ± 10% of the given value.
[b] Enzymes kindly supplied by Dr. F. Maley, New York State Dept. Health, Albany, NY.
[c] Kindly provided by Dr. R. L. Blakley, St. Jude Children's Hospital, Memphis, TN.
[d] Kindly provided by Dr. J. H. Freisheim, Medical college of Ohio, Toledo, OH.
[e] The data has been taken from Ref. 11.
[f] The data has been taken from Ref. 12.
[g] Numbers in parentheses indicate % inhibition at the given concentration.
[h] Kindly provided by Dr. Ann Jackman, Institute of Cancer Research, Sutton, Surrey, UK.
[i] Kindly provided by Dr. Chuan Shih, Eli Lilly & Co.

than LY231514, respectively. Compounds **12** and **16** were equipotent with ZD1694, while **19** and **20** were approximately one-half as potent as ZD1694 against human TS. In general compounds bearing an electron withdrawing group at both the 3′- and 4′-positions (**16**–**20**) were the most potent inhibitors of human TS. Monosubstitution of an electron withdrawing group at either the 3′- or 4′-position (**8**–**15**) was found to be more favorable for human TS inhibition than having substitution at both the 3′- and 5′-positions (**21**–**24**). The nature of the halogen substituent at the 3′-position has little influence on human TS inhibition, (compare compounds **8**, **9**, and **10**) However in the 4′-position the nitrile (**14**) is the most favorable substituent and is equipotent with 4′-nitro analogue **6**.[11] Thus large electron withdrawing groups at the 4′-position afford potent human TS inhibition. The trend at the 4′-position substitution for human TS inhibitory activity is as follows: nitro > nitrile > bromine > trifluoromethyl > fluorine > trifluoromethoxy. This indicates that size along with strong electron withdrawing properties at the 4′-position is conducive to potent human TS inhibition. In addition, a strong electron withdrawing group at the 4′-position is better than at the 3′-position (compare **10** and **11**; and **12** and **9**). In the 3′,5′-disubstituted series electron withdrawing chlorine and fluorine are more favorable than nitro or trifluoromethyl substituents for human TS inhibition. The replacement of a chlorine (**16**) with a bromine (**17**) results in a 2-fold increase in activity against human TS, again suggesting that size along with an electron withdrawing effect at the 4′-position is conducive for potent TS inhibition. The same trend was observed in **18** and **19** where the replacement of an electron withdrawing fluorine (**19**) with bromine (**18**) results in a 4-fold increase in human TS inhibitory activity. In the 3′,4′-disubstituted compounds the nature of the electron withdrawing group present at the 4′-position dictates the activity against human TS. Thus **17** and **18** with bromine at the 4′-position but different electron withdrawing groups at the 3′-position have similar potency, however compounds **16** and **17** have the same electron withdrawing nitro group at the 3′-position but differ in the 4′-halogen substitution and have different activities. Compound, **17** (4′-Br) is approximately 2-fold more potent than **16** (4′-Cl). A similar trend was observed for compounds **18** and **19** where a 4′-bromine compared to fluorine imparts a 4-fold increase in TS inhibitory activity. Compounds **19** and **20** which have fluorine at the 4′-position but different electron withdrawing groups at the 3′-position are similar in activity to **17** and **18**. The position of the electron withdrawing substituents on the phenyl ring also plays an important role in human TS inhibition. The 3′,5′-difluoro substituted **21** was approximately 3-fold less active than the 3′,4′-

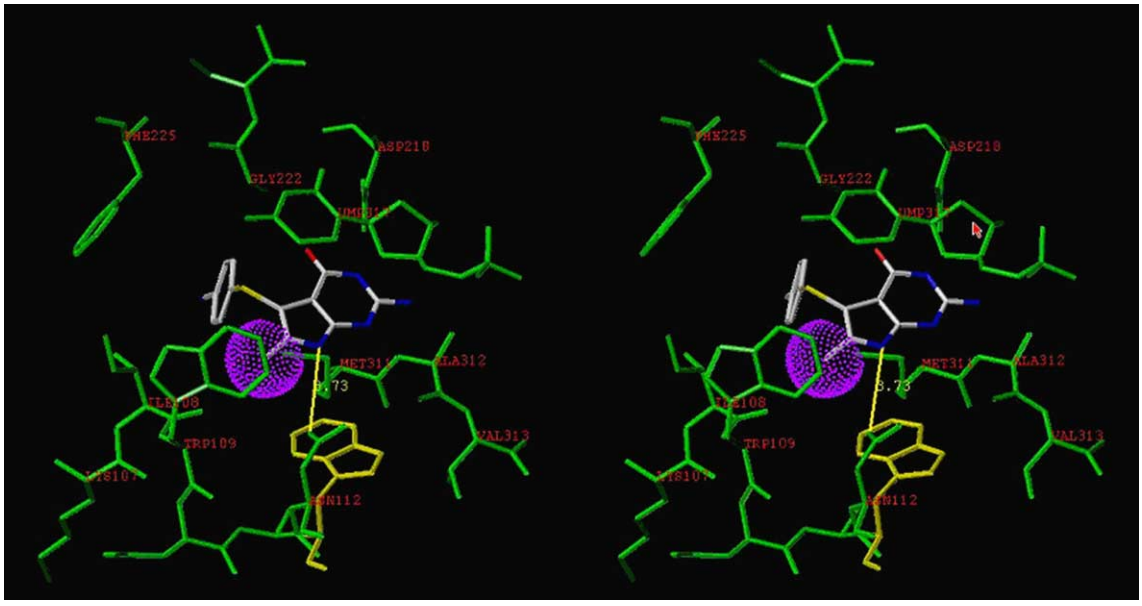


Figure 3. Stereoview showing the interaction of the 6-methyl moiety (van der walls dot surface in violet) of compound **14** with Trp 109 in human TS (PDB2KCE)[20] and the hydrogen bonding between 7NH and δ^1-O of Asn 112. The Trp83 of *E. coli* TS in yellow.

difluoro **20** against human TS. Similarly the 3′,5′-dichloro **24** was approximately 18-fold less active than the previously synthesized 3′,4′-dichloro **7** suggesting that the 3′,4′-disubstitution is more conducive to human TS inhibitory activity. Selected analogues were tested against human DHFR and were found to be poorly active (Table 1) suggesting that perhaps these compounds do not flip to adopt the '2,4-diamino' binding mode where the pyrrole NH mimics the 4-amino moiety of DHFR inhibitors. All of the compounds tested were also inactive against *E. coli* DHFR (Table 1).

Compounds **16** and **17** were moderately potent inhibitors of *E. coli* TS. All of the analogues tested were more potent against human TS than *E. coli* TS indicating a difference in the architecture of human TS and *E. coli* TS. Molecular modeling using SYBYL 6.91[18] and superimposition of the 6-5 pyrrolo[2,3-*d*]pyrimidine of compounds **8**–**24** on the 6-5 ring system of ZD1694 in the crystal structure of ZD1694 in human TS (PDB 2KCE)[19] suggested that the 7NH of compounds **8**–**24** can hydrogen bond with the δ^1-O of Asn 112 in human TS.[19] This interaction is not possible in *E. coli* TS in which Asn 112 is replaced by Trp 83. Stroud and coworkers[20] have shown these interactions in the crystal structure of LY231514 in human TS and *E. coli* TS and this could account for the increased activity of **8**–**24** against human TS compared to *E. coli* TS. The interaction of the 6-methyl moiety of compounds **8**–**24** with Trp 109, which enhances TS inhibitory activity, is illustrated by van der Waals dot surface shown in Figure 3. The lipophilic analogue, **17**, was selected for evaluation by the National Cancer Institute in its preclinical in vitro tumor screening program and was found to be moderately cytotoxic with a growth inhibitory concentration $GI_{50} = 4.23$ μM against Molt-4 leukemia cancer cells, 6.18 μM against HT29 colon cancer cells, and 6.55 and 3.28 μM against MDA-MB-231 and MDA-MB-435 breast cancer cell lines, respectively.

In summary, the SAR for 5-phenylsubstituted-2-amino-4-oxopyrrolo[2,3-*d*]pyrimidines against human TS indicates that the nature and position of the substituent(s) on the phenyl ring is important. In general, electron withdrawing 3′,4′-disubstituted compounds (**7**, **17**, and **18**) have the highest potency. Disubstitution, at both the 3′- and 5′-positions (**22** and **23**) is detrimental to potent TS inhibition. In addition, the nature of the electron withdrawing group at the 4′-position is important while the nature of the electron withdrawing group at the 3′-position is less important. The analogues synthesized in this series demonstrates that strong electron withdrawing groups in the 3′- and 4′-positions of the phenyl side chain can provide nonclassical antifolates that are better human TS inhibitors than the clinically used classical compounds ZD1694 and LY231514.

Acknowledgements

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Supplementary data

Details of the synthesis and characterization of all new compounds is provided. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.03.029.

References and notes

1. Carreras, C. W.; Santi, D. V. *Annu. Rev. Biochem.* **1995**, *64*, 721.
2. Douglas, K. T. *Med. Res. Rev.* **1987**, *7*, 441.

3. Berman, E. M.; Werbel, L. M. *J. Med. Chem.* **1991**, *34*, 479.
4. Gangjee, A.; Elzein, E.; Kothare, M.; Vasudevan, A. *Curr. Pharm. Des.* **1996**, *2*, 263.
5. Jackman, A. L.; Taylor, G. A.; Gibson, W.; Kimbell, R.; Brown, M.; Calvert, A. H.; Judson, I. R.; Hughes, L. R. *Cancer Res.* **1991**, *51*, 5579.
6. Taylor, E. C.; Kuhnt, D.; Shih, C.; Rinzel, S. M.; Grindey, G. B.; Barredo, J.; Jannatipour, M.; Moran, R. A. *J. Med. Chem.* **1992**, *35*, 4450.
7. Jones, T. R.; Calvert, A. H.; Jackman, A. L.; Brown, S. J.; Jones, M.; Harrap, K. R. *Eur. J. Cancer* **1981**, *17*, 11.
8. Webber, S. E.; Bleckman, T. M.; Attard, J.; Deal, J. G.; Katherdekar, V.; Welsh, K. M.; Webber, S.; Janson, C. A.; Matthews, D. A.; Smith, W. W.; Freer, S. T.; Jordan, S. R.; Bacquet, R. J.; Howland, E. F.; Booth, C. J. L.; Ward, R. W.; Hermann, S. M.; White, J.; Morse, C. A.; Hilliard, J. A.; Bartlett, C. A. *J. Med. Chem.* **1993**, *36*, 733.
9. Creaven, P. J.; Pendyala, L.; Meropol, N. J.; Clendeninn, N. J.; Wu, E. Y.; Loewen, G. M.; Proefrock, A.; Johnston, A.; Dixon, M. *Cancer Chemother. Pharmacol.* **1998**, *41*, 167.
10. Gangjee, A.; Devraj, R.; McGuire, J. J.; Kisliuk, R. L. *J. Med. Chem.* **1995**, *38*, 4495.
11. Gangjee, A.; Mavandadi, F.; Kisliuk, R. L.; McGuire, J. J.; Queener, S. F. *J. Med. Chem.* **1996**, *39*, 4563.
12. McNamara, D. J.; Berman, E. M.; Fry, D. W.; Werbel, L. M. *J. Med. Chem.* **1990**, *33*, 2045.
13. Appelt, K.; Bacquet, R. J.; Bartlett, C. A.; Booth, C. L. J.; Freer, S. T.; Fuhry, M. A.; Gehring, M. R.; Herrman, S. M.; Howland, E. F.; Janson, C. A.; Jones, T. R.; Kan, C. C.; Kathardekar, V.; Lewis, K. K.; Marzoni, G. P.; Matthews, D. A.; Mohr, C.; Moomaw, E. W.; Morse, C. A.; Oatley, S. J.; Ogden, R. C.; Reddy, M. R.; Reich, S. H.; Schoettlin, W. S.; Smith, W. W.; Varney, M. D.; Villafranca, E. J.; Ward, R. W.; Webber, S.; Webber, S. E.; Welsh, K. M.; White, J. *J. Med. Chem.* **1991**, *34*, 1925.
14. Gangjee, A.; Jain, H.; McGuire, J. J.; Chu, E.; Kisliuk, R. L. *Abstract of Papers*, 227th National Meeting of the American Chemical Society, Anaheim, CA; American Chemical Society: Washington, DC, 1995; Abstract 076.
15. Gangjee, A.; Dubash, N. P.; Queener, S. F. *J. Heterocycl. Chem.* **2000**, *37*, 935.
16. Kisliuk, R. L.; Strumpf, D.; Gaumont, Y.; Leary, R. P.; Plante, L. *J. Med. Chem.* **1977**, *20*, 1531.
17. Wahba, A. J.; Friedkin, M. *J. Biol. Chem.* **1962**, *237*, 3794.
18. Tripos Associates, Inc., 1699; S. Hanely Road, Suite 303, St. Louis, MO 63144, USA.
19. Rutenber, E. E.; Stroud, R. M. *Structure* **1996**, *4*, 1317.
20. Sayre, P. H.; Finer-Moore, J. S.; Fritz, T. A.; Biermann, D.; Gates, S. B.; MacKellar, W. C.; Patel, V. F.; Stroud, R. M. *J. Mol. Biol.* **2001**, *313*, 813.