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Novel non-classical C9-methyl-5-substituted-2,4-diaminopyrrolo-[2,3-*d*]pyrimidines as potential inhibitors of dihydrofolate reductase and as anti-opportunistic agents☆

Aleem Gangjee,[a,*] Jie Yang[a] and Sherry F. Queener[b]

[a]*Division of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, 600 Forbes Avenue, Duquesne University, Pittsburgh, PA 15282, USA*
[b]*Department of Pharmacology and Toxicology, School of Medicine, Indiana University, Indianapolis, IN 46202, USA*

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Abstract—Six novel C9-methyl-5-substituted-2,4-diaminopyrrolo[2,3-*d*]pyrimidines **18**–**23** were synthesized as potential inhibitors of dihydrofolate reductase (DHFR) and as anti-opportunistic agents. These compounds represent the only examples of 9-methyl substitution in the carbon–carbon bridge of 2,4-diaminopyrrolo[2,3-*d*]pyrimidines. The analogs **18**–**23** were synthesized in a concise eight-step procedure starting from the appropriate commercially available aromatic methyl ketones. The key step involved a Michael addition reaction of 2,4,6-triaminopyrimidine to the appropriate 1-nitroalkene, followed by ring closure of the nitro adducts via a Nef reaction. The compounds were evaluated as inhibitors of DHFR from *Pneumocystis carinii* (pc), *Toxoplasma gondii* (tg), *Mycobacterium avium* (ma) and rat liver (rl). The biological result indicated that some of these analogs are potent inhibitors of DHFR and some have selectivity for pathogen DHFR. Compound **23** was a two digit nanomolar inhibitor of tgDHFR with 9.6-fold selectivity for tgDHFR.

1. Introduction

Opportunistic infections caused by pathogenic organisms such as *Pneumocystis*, *Toxoplasma gondii* (tg), and *Mycobacterium avium* (ma) are the principal cause of morbidity and mortality in AIDS patients.[2] Inhibitors of dihydrofolate reductase (DHFR) from *Pneumocystis* and *Toxoplasma* are useful anti-opportunistic agents but DHFR inhibitors have yet to be clinically applied for *M. avium* infections. DHFR inhibitors that display potent activity and high selectivity for these pathogenic organisms versus human DHFR remain elusive for the treatment of opportunistic infection in AIDS patients. Infections caused by *Pneumocystis* or *Toxoplasma* are currently treated with weak but selective DHFR inhibitors such as trimethoprim (TMP **1**) and pyrimethamine (PYR **2**) (Fig. 1), and their action is augmented with sulfonamides. The combination therapy however often results in severe side effects to sulfa drugs and leads to discontinuation of treatment.[3] Trimetrexate (TMQ, **3**) and piretrexim (PTX, **4**) (Fig. 1) are potent inhibitors of both pathogenic and human DHFR which results in high toxicity to host cells. Hence **3** and **4** are used in combination with leucovorin (N^5-formyltetrahydrofolate) which is necessary to rescue the host cells.[4] Thus the development of agents that display both high potency and selectivity for pathogenic DHFR remains a desirable goal for the treatment of opportunistic infections in AIDS patients.

Gangjee et al.[5–10] reported two-atom bridged classical and non-classical, 6–5 bicyclic analogs, as potential DHFR inhibitors (Fig. 2). The structure–activity relationship (SAR) studies revealed that a N9- or C9-methyl group in the two-atom bridged side chain of the 6–5 system significantly contributes to the DHFR inhibitory activity and selectivity. Thus, in the furo[2,3-*d*]pyrimidine analogs (**5**–**10**) (Fig. 2), the N9- or C9-methyl compounds were more potent than the corresponding N9- or C9-desmethyl compounds. Molecular modeling using Sybyl 6.7[10] suggested that the 9-methyl moiety in furo[2,3-*d*]pyrimidines interacts with Val115 in human DHFR which may account for their increased potency against human DHFR. In addition, molecular modeling also indicated that the 9-methyl group restricts the rota-

☆ See Ref. 1.

* Corresponding author. Tel.: +1 412 396 6070; fax: +1 412 396 5593; e-mail: gangjee@duq.edu

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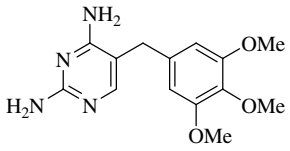
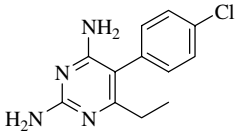
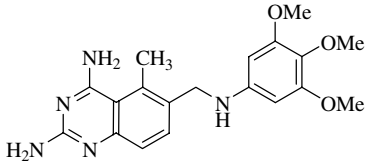


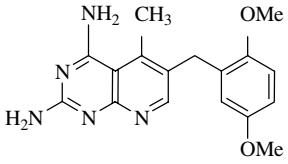
1 Trimethoprim (TMP)



2 Pyrimethamine

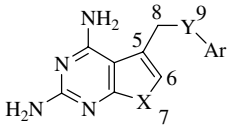


3 Trimetrexate (TMQ)

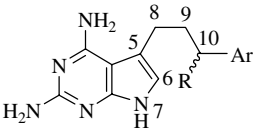


4 Piritrexim (PTX)

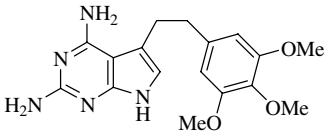
Figure 1. Known inhibitors of pathogenic DHFR.



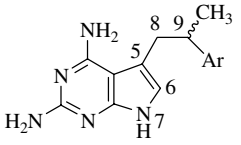
5, X = O, Y = NH, Ar = p-benzoyl-L-glutamate
6, X = O, Y = NCH_3, Ar = p-benzoyl-L-glutamate
7, X = O, Y = CH_2, Ar = p-benzoyl-L-glutamate
8, X = O, Y = $CHCH_3$, Ar = p-benzoyl-L-glutamate
9, X = O, Y = NH, Ar = 2-Naphthyl
10, X = O, Y = NCH_3, Ar = 2-Naphthyl
11, X = NH, Y = NH, Ar = p-benzoyl-L-glutamate
12, X = NH, Y = NCH_3, Ar = p-benzoyl-L-glutamate
13, X = NH, Y = NH, Ar = 1-Naphthyl
14, X = NH, Y = NCH_3, Ar = 1-Naphthyl



Ar = p-benzoyl-L-glutamate
15, R = H,
16, R = CH_3,



17



Ar
18 Ph
19 2-Naphthyl
20 2-Fluorenyl
21 4-biPh
22 2,5-diOMePh
23 3,4,5-triOMePh

Figure 2. Two atom bridged furo- and pyrrolo[2,3-*d*] pyrimidines.

tion of the C8–C9 bond (compounds **7** and **8**) and leads to a decreased number of accessible low energy conformations conducive for binding to human DHFR.[10] Thus the classical 9-methyl substituted compound **8** was twice as potent against human DHFR and displayed 10-fold greater activity in cell culture as compared to the corresponding desmethyl analog **7**.[10]

In the two-carbon atom bridged pyrrolo[2,3-*d*]pyrimidine series **11**–**14** (Fig. 2), N9-methylation of the classical compound **11** maintained the potency but increased the selectivity for tgDHFR. Similarly, the non-classical analog **14** was more potent than the corresponding desmethyl compound **13**.[5,6]

Classical anti-folates such as **5**–**8**, **11**, **12**, **15** and **16** (Fig. 2) have a polar L-glutamate side chain, and hence utilize carrier-mediated active transport mechanisms for uptake into cells. However, those pathogenic organisms that lack active transport mechanisms are not susceptible to classical anti-folates. Non-classical anti-folates without the polar L-glutamate side chain such as TMP, TMQ and PTX, are lipophilic and have been used to target these pathogenic microorganisms.

In the two-atom bridged 6–6 bicyclic system, a methyl group in the side chain of the bridge was reported to contribute to the DHFR inhibitory activity.[11] Miwa et al.[12] reported the classical pyrrolo[2,3-*d*]pyrimidine anti-folates **15** and **16** as DHFR inhibitors. These compounds possess a three-carbon atom bridge. The C10-methylated compound **16** was similar in activity to the desmethylated compound **15**. Rosowsky et al.[13] reported the two-carbon atom bridged non-classical pyrrolo[2,3-*d*]pyrimidine analog **17** as a potent inhibitor of DHFR. It was therefore of interest to introduce a methyl group at the C9-position of the bridge in pyrrolo[2,3-*d*]pyrimidines to determine the effect on inhibitory activity and selectivity against pathogenic DHFR. Thus compounds **18**–**23** (Fig. 2), were synthesized as potential inhibitors of DHFR and as potential anti-opportunistic agents. X-ray crystal structure of similar furo[2,3-*d*]pyrimidine analogs with pcDHFR indicated that the side chain phenyl substituent has a hydrophobic interaction with Phe69 in pcDHFR;[9] similar interactions were anticipated with tgDHFR (Phe91) and maDHFR (Val58). These hydrophobic interactions would be absent with mammalian DHFR where an Asn64 replaces a Phe or Val. Thus large hydrophobic aromatic rings, such as naphthyl, fluorenyl, biphenyl and substituted phenyls were also chosen for compounds **19**–**21** to enhance the hydrophobic interactions with the pathogenic DHFR.

2. Results and discussion

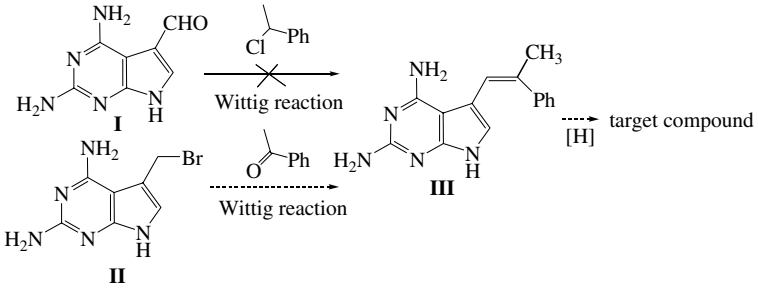
Gangjee et al. reported[7–10] the synthesis of two-carbon atom bridged furo[2,3-*d*]pyrimidines **5**–**10** by utilizing a Wittig reaction. It was anticipated that a similar Wittig reaction (Scheme 1) of compound **I** and substituted (1-chloro-ethyl)-benzene should afford intermediate **III**, which on hydrogenation would give the desired target compounds. However the Wittig reaction of **I** and (1-chloro-ethyl)-benzene under a variety of different conditions of time, base, temperature and solvent failed to afford the desired product **III**. A second synthetic strategy was devised in which compound **I** was to be converted to **II** by first reduction to the 5-hydroxymethyl analog followed by bromination. This was to be followed by a Wittig reaction with appropriately substituted ketones. However the reduction of **I** to the corresponding 5-hydroxymethyl analog could not be accomplished despite several attempts with a variety of reducing agents. Thus the proposed synthetic methodology in Scheme 1 was unsuccessful.

The failure of the Wittig reaction, as well as the conversion of **I** to its 5-hydroxymethyl analog, prompted the search for an alternate synthetic procedure. Taylor and Liu[14,15] had reported the synthesis of 2,4-diamino-pyrrolo[2,3-*d*]pyrimidines via a Nef reaction of a nitro intermediate. The key nitro intermediates were in turn obtained by a Michael addition of 5-unsubstituted-2,4,6-triaminopyrimidine and the appropriate 1-nitroalkenes. The 1-nitroalkenes were synthesized from appropriately substituted aldehydes and nitromethane by a Henry reaction (nitroaldol condensation).[16] For the synthesis of target compounds **18**–**23** the appropriately substituted aldehydes were not commercially available. The synthesis of these aldehydes was first attempted using a Heck coupling (Scheme 2),[17] however low yields (<10%) along with problems in purification of the desired product prompted the search for alternate synthetic strategies.

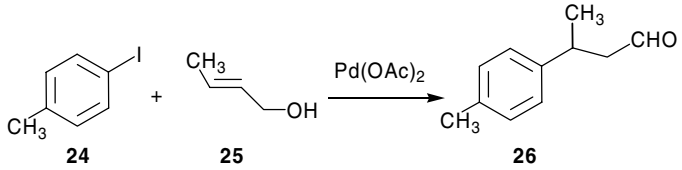
Our alternate method was to generate the α,β-unsaturated aldehyde **30** via a Wittig reaction from **27**, followed by reduction of the double bond to afford the desired aldehydes (Scheme 3). Unfortunately the initial Wittig reaction was not successful. At this stage it was decided to synthesize the esters **32**–**36**,[18] which could be reduced by $LiAlH_4$ to the primary alcohols,[19] followed by oxidation with PCC to aldehydes.[20] The total yield for this two step procedure was reasonably good (40%) for **46**, but purification of the compounds remained a problem, and the use of impure aldehyde for the subsequent step was not feasible. In view of the fact that the ester could be reduced to the corresponding aldehyde with DIBAL-H, an alterate synthetic approach was adopted in which the double bond of the α,β-unsaturated esters **32**–**36** were reduced by catalytic hydrogenation,[21] followed by reduction with DIBAL-H to afford the aldehydes **42**–**46** in very high yield (two steps >80%, Scheme 3).[22]

The Henry reaction of aldehydes **42**–**47** with nitromethane gave the expected nitro alcohol intermediates **48**–**53** (Scheme 4). This reaction was carried out under a variety of different reaction conditions using different bases (K_2CO_3, NaOEt, NaOMe, NaOH and KF), different solvents (MeOH, EtOH, THF, $CHCl_3$ and *i*-PrOH), and different temperatures (from room temperature to heating to reflux).[23–26] The optimized conditions in our hands involved using *i*-PrOH as the solvent, KF as a weak base and stirring at room temperature overnight to afford compounds **48**–**53** in >85% yield.[27] Under these conditions the product was readily purified via column chromatography.

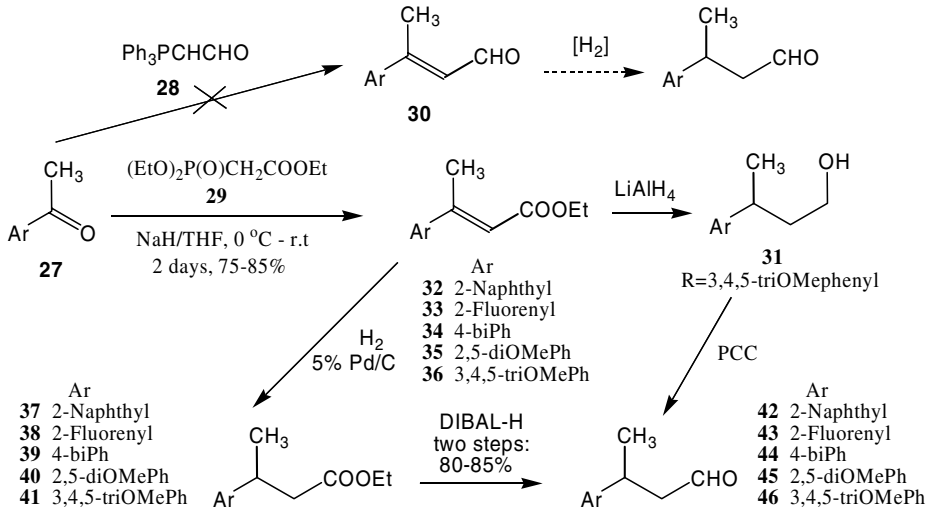
The formation of 1-nitroalkenes **54**–**59** was carried out under mild reaction conditions with MsCl and Et_3N at 0 °C and stirring for one half hour to afford **54**–**59** in >90% yield.[28,29] The Michael addition of 2,4,6-triaminopyrimidine to the 1-nitroalkenes **54**–**59** afforded the nitro adducts **60**–**65**.[30] The transformation of the nitro adduct to the carbonyl intermediate was the key step



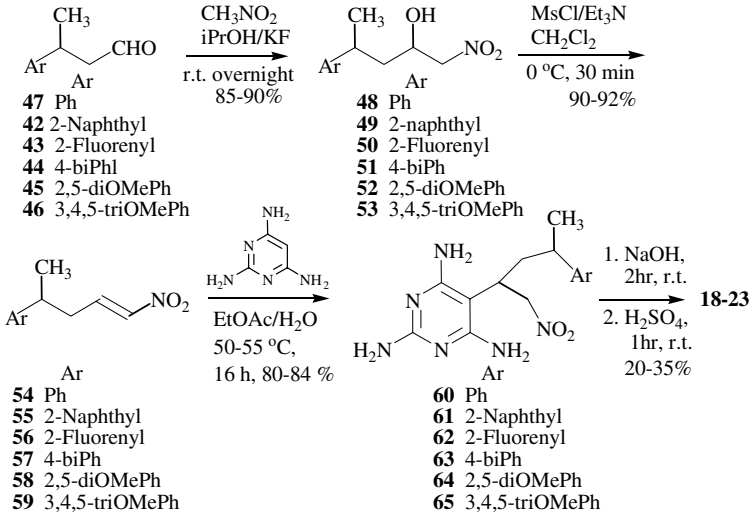
Scheme 1. Attempted synthesis of target compounds.



Scheme 2. Heck coupling for the synthesis of **26**.



Scheme 3. Synthesis of aldehydes **42–46**.



Scheme 4. Synthesis of **18–23**.

in forming the desired pyrrolo[2,3-*d*]pyrimidines. There are numerous Nef reaction conditions available for converting nitro adducts to carbonyl intermediates.[31] However the one-pot conditions reported by Taylor and Liu.[14,15] were particularly appealing. Thus the nitro adducts were first treated with aqueous NaOH solution followed by aqueous H_2SO_4 solution to afford the final products **18–23** (Scheme 4). The compounds were evaluated as inhibitors of pcDHFR, tgDHFR and maDHFR. Rat liver (rl) DHFR served as the mammalian standard.

Pneumocystis carinii DHFR (Table 1): against pcDHFR the most potent analog bore a 2-naphthyl group (**19**). Increasing the bulk of the naphthyl to a fluorenyl group (**20**) or a biphenyl (**21**) caused a drop in activity as did the 3,4,5-triOMe substitution (**23**). However, the most detrimental substitution was the 2,5-diOMe (**22**), which was about 10-fold less potent than **19**. The unsubstituted phenyl analog **18** was the least active. The compounds were not selective against pcDHFR and C9-methylation (**23**) did not improve activity compared to the desmethyl analog **17**. In fact, the addition of a 9-CH_3 to **17** caused a 3-fold decrease in activity. This result was unexpected since the 9-CH_3 was expected to interact hydrophobically or via van der Walls interaction with the close Ile123 of pcDHFR.[32] Perhaps the decrease in activity is due to an inability to accommodate both a bulky substitution at the C5-position along with a C9-CH_3.

Toxoplasma gondii DHFR (Table 1): the compounds were significantly more potent and selective against tgDHFR compared to pcDHFR. The most potent compound was again the 2-naphthyl analog **19** which had an IC_{50} value of 15 nM. Compounds **20**, **21** and **23** were almost as active, with IC_{50} values between 37 and 72 nM, indicating that bulk on the phenyl ring was highly conducive to tgDHFR inhibition. The unsubstituted phenyl analog **18** and the 2,5-dimethyl analog **22** were the least active compounds. Once again 9-CH_3 substitution as in **23** did not significantly increase potency against

Table 1. Inhibitory concentration (IC_{50} μM) and selectivity ratios against rlDHFR, pcDHFR, tgDHFR and maDHFR versus rlDHFR by compounds **18**–**23**[a]

Compound	pc	rl	rl/pc	tg	rl/tg	ma	rl/ma
17[b]	0.77	0.20	0.26	0.037	5.4	0.077	2.6
18	17.1	0.696	0.04	0.138	5	5.34	0.13
19	0.859	0.0399	0.05	0.0148	2.7	0.109	0.37
20	2.09	0.0567	0.03	0.0483	1.2	0.246	0.23
21	1.99	0.142	0.07	0.0717	2	0.0747	1.9
22	8.06	0.516	0.06	0.16	3.2	1.86	0.28
23	2.22	0.396	0.2	0.0414	9.6	0.321	1.23
TMP	12	180.0	14	2.8	65	0.30	600
PTX	0.013	0.0033	0.26	0.0043	0.76	0.00061	5.3
TMQ	0.042	0.003	0.07	0.01	0.3	0.0015	2.0

[a] All enzymes were assayed spectroscopically in a solution containing 90 micromolar dihydrofolate, 119 μM NADPH, 41 mM Na phosphate buffer, and 8.9 mM 2-mercaptoethanol at pH 7.4 and 37 C. Rat liver and Toxoplasma DHFR are assayed in the presence of 150 mM KCl. Reactions are initiated with an amount of enzyme yielding a change in OD at 340 nM of 0.035/min.
[b] The data for **17** was taken from Ref. 13.

tgDHFR compared to the desmethyl analog **17**. Selectivity, though improved from that toward pcDHFR, was not significant, with the best compound **23** being 9-fold more selective compared to rlDHFR. This was not very different from the desmethyl analog.

Mycobacterium avium DHFR (Table 1): against maDHFR the most potent analog was **23**. All analogs were more potent against the bacterial maDHFR than against pcDHFR. Comparing **17** with **23** indicates that 9-CH_3 substitution has a detrimental effect on maDHFR inhibition. There was no selectivity for maDHFR for this series of analogs. The most potent analogs were **17** and **21** indicating that neither N9-methylation nor bulk at C5 contributes significantly to activity against maDHFR.

Rat liver DHFR (Table 1): compounds **19** and **20** were potent nanomolar inhibitors of mammalian DHFR. These two analogs have bulky aromatic substitutions and were not expected to interact with rlDHFR which contain an Asn in place of Phe69 of pcDHFR. It is possible that the compounds bind to tgDHFR and to rlDHFR in different orientations that perhaps allow potent inhibitory activity for both analogs against both enzymes. Again there is no advantage of the 9-CH_3 substitution over the 9-desmethyl compound.

In summary, the phenyl unsubstituted analog **18** and the 2,5-diOMe substituted analog **22** were the least potent DHFR inhibitors tested against the four enzymes. The most potent compound against rat liver DHFR and tgDHFR was the 2-naphthyl analog **19**; compounds **17** and **19** were similar in potency against pcDHFR. The other analogs did not show any particular trend with respect to all DHFR, however differences in activities of the compounds against specific DHFR were noted. Direct comparison of the N9-CH_3 analog **23** with the corresponding N9-desmethyl analog **17** indicates that the N9-CH_3 group is detrimental to activity. Bulky substitutions on the C5-position did not afford significant increase in potency (except against maDHFR) or selectivity. Thus the SAR is different against different DHFR and the SAR of each enzyme target needs to be compared separately with the mammalian standard. In this limited series N9-methylation and C5-bulky substitutions does not afford both potent and selective inhibitors for any of the pathogenic DHFR evaluated. However, the 2 digit nanomolar potency of compounds **19** and **20** against tgDHFR and **21** against tgDHFR and maDHFR, indicates that potency can be maintained or improved with some bulky substitutions at C5 compared to the phenyl analog **18**.

3. Experimental

Melting points were determined on a Mel-Temp II melting point apparatus with FLUKE 51 K/J electronic thermometer and were uncorrected. Nuclear magnetic resonance spectra for proton (^1H) were recorded on a Bruker WH-300 (300 MHz) spectrometer. The chemical shift values were expressed in ppm (parts per million) relative to tetramethylsilane as internal standard; s = singlet, d = double, t = triplet, q = quartet, m = multiplet, br s = broad singlet. The relative integrals of peak areas agreed with those expected for the assigned structures. High-resolution mass spectra (HRMS), using electron impact (EI), were recorded on a VG AUTOSPEC (Fisons Instruments) micromass (EBE Geometry) double focusing mass spectrometer. Thin-layer chromatography (TLC) was performed on POLYGRAM Sil G/UV254 silica gel plates with fluorescent indicator, and the spots were visualized under 254 and 366 nm illumination. Proportions of solvents used for TLC were by volume. Column chromatography was performed on 230–400 mesh silica gel purchased from Aldrich Chemical Co., Milwaukee, WI. All evaporations were carried out in vacuo with a rotary evaporator. Analytical samples were dried in vacuo (0.2 mmHg) in an Abderhalden drying apparatus over P_2O_5 at 75–110 °C. Elemental analysis was performed by Altlantic Microlabs, Norcross, GA. Element compositions are within ±0.4% of calculated values. Fractional moles of water or organic solvents frequently found in some analytical samples could not be prevented despite 24–48 h of drying in vacuo and were confirmed where possible by their presence in the ^1H NMR spectra. All solvents and chemicals were

purchased from Aldrich Chemical Co. and Fisher Scientific and were used as received.

3.1. General procedure for the synthesis of *E*-ethyl 3-(substituted-phenyl)but-2-enoate (32–36)

To a suspension of NaH (290 mg, 11.9 mmol) in 30 mL of dry THF was added triethyl phosphonoacetate (2.67 g, 11.9 mmol). The mixture was stirred for 1 h at room temperature and then the appropriate commercially available arylmethylketone (11.9 mmol) in 30 mL THF was added dropwise. After stirring at room temperature for 3 days, the reaction mixture was concentrated by evaporation of the solvent and then diluted with 300 mL of water. The resulting aqueous solution was extracted with Et_2O (100 mL × 3) and the organic layers combined and dried over Na_2SO_4. The solvent was removed under reduced pressure to give 2–3 mL of a liquid residue, which was loaded on a silica gel column (15 × 150 mm) and eluted with hexane, 5% EtOAc in hexane and then 10% EtOAc in hexane. The fractions containing the product (TLC) were pooled and evaporated to afford **32**–**36**. Here only the *E*-isomer of the product was isolated and characterized. The *Z*-isomer was difficult to isolate from the mixture (*E*/*Z*), and was not characterized.

3.2. *E*-Ethyl 3-(2-naphthyl)but-2-enoate (32)

Using the general procedure described above, compound **32** was obtained from 2-acetonaphthone as a colorless oil in 75% yield. R_f 0.53 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.34 (t, 3H, CH_3), 2.69 (s, 3H, CH_3), 4.23 (q, 2H, CH_2), 6.29 (s, 1H, CH), 7.47–7.48 (m, 7H, Ar-H × 7).

3.3. *E*-Ethyl 3-(2-fluorenyl)but-2-enoate (33)

Using the general procedure described above, compound **33** was obtained from 2-acetylfluorene as an off-yellow solid in 61% yield. Mp: 87–89 °C; R_f 0.49 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.33 (t, 3H, CH_3), 2.64 (s, 3H, CH_3), 3.91 (s, 2H, CH_2), 4.23 (q, 2H, CH_2), 6.21 (s, 1H, CH), 7.30–7.81 (m, 7H, Ar-H × 7).

3.4. *E*-Ethyl 3-(4-biphenyl)but-2-enoate (34)

Using the general procedure described above, compound **34** was obtained from 4-acetyl-biphenyl as an off-white solid in 80% yield. Mp: 63–65 °C (lit. 63–64 °C); (lit.[33] 63–64° C); R_f 0.55 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.33 (t, 3H, CH_3), 2.64 (s, 3H, CH_3), 4.26 (q, 2H, CH_2), 6.21 (s, 1H, CH), 7.34–7.71 (m, 9H, Ar-H × 9).

3.5. *E*-Ethyl 3-(2,5-dimethoxyphenyl)but-2-enoate (35)

Using the general procedure described above, compound **35** was obtained from 2,5-dimethoxyacetophenone as a colorless oil in 74% yield. R_f 0.49 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.28 (t, 3H, CH_3), 2.56 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.18 (q, 2H, CH_2), 5.90 (s, 1H, CH), 6.72–6.89 (m, 3H, Ar-H × 3).

3.6. *E*-Ethyl 3-(3,4,5-trimethoxyphenyl)but-2-enoate (36)

Using the general procedure described above, compound **36** was obtained from 3,4,5-trimethoxyacetophenone as an off-white solid in 63% yield. Mp: 57-59 °C; (lit.[34] 55–57 °C); R_f 0.50 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.30 (t, 3H, CH_3), 2.55 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 3.89 (s, 6H, 2 × OCH_3), 4.20 (q, 2H, CH_2), 6.09 (s, 1H, CH), 6.75 (s, 2H, Ar-H × 2).

3.7. General procedure for synthesis of (±)-ethyl 3-(substituted-phenyl)butanoate (37-41)

Ethyl *E*-3-(substituted-phenyl)but-2-enoate (**32**–**36**) (11.7 mmol) was dissolved in 150 mL of EtOAc in a 500 mL flask, and 5% Pd/C (2.5 g) was added. The suspension was stirred under 1 atm H_2 until the disappearance of the starting material (TLC). The catalyst was filtered and the solvent evaporated under reduced pressure to afford **37**–**41**, which was used directly for the next step without further purification.

3.8. (±)-Ethyl 3-(2-naphthyl)butanoate (37)

Using the general procedure described above, compound **37** was obtained from *E*-ethyl 3-(2-naphthyl)but-2-enoate **32** as a colorless oil in 95% yield. R_f 0.65 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.17 (t, 3H, CH_3), 1.39 (d, 3H, CH_3), 2.65–2.72 (m, 2H, CH_2), 3.46–3.48 (m, 1H, CH), 4.07 (q, 2H, CH_2), 7.37–7.45 (m, 3H, Ar-H × 3), 7.79–7.81 (m, 4H, Ar-H × 4).

3.9. (±)-Ethyl 3-(2-fluorenyl)butanoate (38)

Using the general procedure described above, compound **38** was obtained from *E*-ethyl 3-(2-fluorenyl)but-2-enoate **33** as a colorless oil in 97% yield. R_f 0.71 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.18 (t, 3H, CH_3), 1.34 (d, 3H, CH_3), 2.55–2.70 (m, 2H, CH_2), 3.32–3.40 (m, 1H, CH), 3.80 (s, 2H, CH_2), 4.07 (q, 2H, CH_2), 7.22–7.65 (m, 4H, Ar-H × 4), 7.70–7.76 (m, 3H, Ar-H × 3).

3.10. (±)-Ethyl 3-(4-biphenyl)butanoate (39)

Using the general procedure described above, compound **39** was obtained from *E*-ethyl 3-(4-biphenyl)but-2-enoate **34** as a colorless oil in 98% yield. R_f 0.70 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.19 (t, 3H, CH_3), 1.35 (d, 3H, CH_3), 2.65–2.70 (m, 2H, CH_2), 3.34–3.41 (m, 1H, CH), 4.10 (q, 2H, CH_2), 7.27–7.72 (m, 9H, Ar-H × 9).

3.11. (±)-Ethyl 3-(2,5-dimethoxyphenyl)butanoate (40)

Using the general procedure described above, compound **40** was obtained from *E*-ethyl 3-(2,5-dimethoxyphenyl)but-2-enoate **35** as a colorless oil in 98% yield. R_f 0.60 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.18 (t,

3H, CH_3), 1.25 (d, 3H, CH_3), 2.43–2.70 (m, 2H, CH_2), 3.57–3.71 (m, 1H, CH), 3.72 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 4.08 (q, 2H, CH_2), 6.67–6.79 (m, 3H, Ar-H × 3).

3.12. (±)-Ethyl 3-(3,4,5-trimethoxyphenyl)butanoate (41)

Using the general procedure described above, compound **41** was obtained from *E*-ethyl 3-(3,4,5-trimethoxyphenyl)but-2-enoate **36** as a colorless oil in 100% yield. R_f 0.60 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.18 (t, 3H, CH_3), 1.28 (d, 3H, CH_3), 2.47–2.63 (m, 2H, CH_2), 3.18–3.23 (m, 1H, CH), 3.81 (s, 3H, OCH_3), 3.85 (s, 6H, 2 × OCH_3), 4.08 (q, 2H, CH_2), 6.43 (s, 2H, Ar-H × 2).

3.13. General procedure for synthesis of (±)-3-(substituted-phenyl)butylaldehyde (42–46)

To a cooled (−78 °C) solution of (±)-ethyl 3-(substituted-phenyl)butanoate (**37-41**) (10 mmol) in 15 mL of dry CH_2Cl_2 was added dropwise a solution of DIBAL-H (10 mL, 10 mmol, 1.0 M in hexane solution). After the reaction mixture was stirred at −78 °C for 30 min, methanol (6 mL) was added dropwise and then the reaction mixture was allowed to warm to room temperature. To this was added a solution of saturated NH_4Cl (10 mL) and the mixture was extracted with $CHCl_3$ (25 mL × 3). The organic layers were combined and washed with 1 N HCl (5 mL) and brine (5 mL) and dried ($MgSO_4$). The solvent was removed under reduced pressure to give 2–3 mL of an oil, which was transferred to a silica gel column (15 × 150 mm), and eluted with 5% EtOAc in hexane and 10% EtOAc in hexane. Fractions containing the product (TLC) were pooled and evaporated to afford **42**–**46**.

3.14. (±)-3-(2-Naphthyl)butylaldehyde (42)

Using the general procedure described above, compound **42** was obtained from (±)-ethyl 3-(2-naphthyl)butanoate **37** as a colorless oil in 85% yield. R_f 0.60 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.42 (d, 3H, CH_3), 2.65–2.95 (m, 2H, CH_2), 3.45–3.70 (m, 1H, CH), 7.35–7.82 (m, 7H, Ar-H × 7), 9.75 (s, 1H, CHO).

3.15. (±)-3-(2-Fluorenyl)butylaldehyde (43)

Using the general procedure described above, compound **43** was obtained from (±)-ethyl 3-(2-fluorenyl)butanoate **38** as a colorless oil in 80% yield. R_f 0.62 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.39 (d, 3H, CH_3), 2.65–2.95 (m, 2H, CH_2), 3.30–3.60 (m, 1H, CH), 3.88 (s, 2H, CH_2), 7.23–7.76 (m, 7H, Ar-H × 7), 9.74 (s, 1H, CHO).

3.16. (±)-3-(4-Biphenyl)butylaldehyde (44)

Using the general procedure described above, compound **44** was obtained from (±)-ethyl 3-(4-biphenyl)butanoate **39** as a colorless oil in 75% yield. R_f 0.61 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.35 (d, 3H, CH_3), 2.55–2.90 (m, 2H, CH_2), 3.37–3.50 (m, 1H, CH), 7.23–7.58 (m, 9H, Ar-H × 9), 9.23 (s, 1H, CHO).

3.17. (±)-3-(2,5-Dimethoxyphenyl)butrylaldehyde (45)

Using the general procedure described above, compound **45** was obtained from (±)-ethyl 3-(2,5-dimethoxyphenyl)butanoate **40** as a colorless oil in 85% yield. R_f 0.55 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.29 (d, 3H, CH_3), 2.54–2.74 (m, 2H, CH_2), 3.60–3.65 (m, 1H, CH), 3.83 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.69–6.80 (m, 3H, Ar-H × 3).

3.18. (±)-3-(3,4,5-Trimethoxyphenyl)butrylaldehyde (46)

Using the general procedure described above, compound **46** was obtained from (±)-ethyl 3-(3,4,5-trimethoxyphenyl)butanoate **41** as a colorless oil in 81% yield. R_f 0.38 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.28 (d, 3H, CH_3), 2.65–2.72 (m, 2H, CH_2), 3.28–3.33 (m, 1H, CH), 3.82 (s, 3H, OCH_3), 3.85 (s, 6H, 2 × OCH_3), 6.42 (s, 2H, Ar-H × 2), 9.71 (s, 1H, CHO).

3.19. General procedure for the synthesis of 1-nitro-4-(substituted-phenyl)-2-pentanol (48–53)

To a solution of (±)-3-(substituted-phenyl)-butrylaldehyde (**42**–**47**) (10 mmol) in 10 mL of *iso*-propanol was added KF (29 mg, 0.5 mmol) and nitromethane (2.5 mL, 40 mmol). The reaction mixture was stirred at room temperature overnight, and the solid KF was filtered and the solvent and excess nitromethane were evaporated under reduced pressure. The residue was dissolved in ethyl acetate (50 mL) and washed with water (2×10 mL), and dried ($MgSO_4$). After evaporating the solvent, the residue was dissolved in 2 mL of $CHCl_3$ and was loaded on a 15 × 150 mm silica gel column and eluted with hexane, hexane/EtOAc/10:1 and hexane/EtOAc/7:1. Fractions containing the product (TLC) were pooled and evaporated to afford **48**–**53**.

3.20. 1-Nitro-4-phenyl-2-pentanol (48)

Using the general procedure described above, compound **48** was obtained from (±)-3-phenyl-butrylaldehyde **47** as a colorless oil in 70% yield. R_f 0.43 ($CHCl_3$). ^{1}H NMR (DMSO-d_6): δ 1.22 (d, 3H, CH_3), 1.50–1.90 (m, 2H, CH_2), 2.75–3.10 (m, 1H, CH), 3.65–4.15 (m, 1H, CH), 4.25–4.80 (m, 2H, CH_2), 5.36–5.43 (m, 1H, OH), 7.19–7.32 (m, 5H, Ar-H × 5).

3.21. 1-Nitro-4-(2-naphthyl)-2-pentanol (49)

Using the general procedure described above, compound **49** was obtained from (±)-3-(2-naphthyl)-butrylaldehyde **42** as a colorless oil in 80% yield. R_f 0.40 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.38 (d, 3H, CH_3), 1.65–2.20 (m, 2H, CH_2), 2.54 (d, 1H, OH), 3.05–3.25 (m, 1H, CH), 3.90–4.15 (m, 1H, CH), 4.20–4.60 (m, 2H, CH_2), 7.34–7.83 (m, 7H, Ar-H × 7).

3.22. 1-Nitro-4-(2-fluorenyl)-2-pentanol (50)

Using the general procedure described above, compound **50** was obtained from (±)-3-(2-fluorenyl)-butrylaldehyde **43** as a colorless oil in 67% yield. R_f 0.45

(hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.34 (d, 3H, CH_3), 1.60–1.95 (m, 2H, CH_2), 2.52 (d, 1H, OH), 3.00–3.30 (m, 1H, CH), 3.89 (s, 2H, CH_2), 3.90–4.10 (m, 1H, CH), 4.15–4.50 (m, 2 H, CH_2), 7.20–7.95 (m, 7H, Ar-H × 7).

3.23. 1-Nitro-4-(4-biphenyl)-2-pentanol (51)

Using the general procedure described above, compound **51** was obtained from (±)-3-(4-biphenyl)-butrylaldehyde **44** as a colorless oil in 72% yield. R_f 0.51 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.35 (d, 3H, CH_3), 1.60–2.00 (m, 2H, CH_2), 2.53 (d, 1H, OH), 2.95–3.20 (m, 1H, CH), 4.00–4.20 (m, 1H, CH), 4.20–4.50 (m, 2H, CH_2), 7.25–7.74 (m, 9H, Ar-H × 9).

3.24. 1-Nitro-4-(2,5-dimethoxyphenyl)-2-pentanol (52)

Using the general procedure described above, compound **52** was obtained from (±)-3-(2,5-dimethoxyphenyl)-butrylaldehyde **45** as a colorless oil in 75% yield. R_f 0.37 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.29 (d, 3H, CH_3), 1.40–1.90 (m, 2H, CH_2), 2.54 (d, 1H, OH), 3.32–3.51 (m, 1H, CH), 3.78 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.85–4.05 (m, 1H, CH), 4.15–4.40 (m, 2H, CH_2), 6.72–6.86 (m, 3H, Ar-H × 3).

3.25. 1-Nitro-4-(3,4,5-trimethoxyphenyl)-2-pentanol (53)

Using the general procedure described above, compound **53** was obtained from (±)-3-(3,4,5-trimethoxyphenyl)-butrylaldehyde **46** as a colorless oil in 78% yield. R_f 0.45 ($CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.43 (d, 3H, CH_3), 1.60–1.88 (m, 2H, CH_2), 2.56 (d, 1H, OH), 2.89–3.02 (m, 1H, CH), 3.83 (s, 3H, OCH_3), 3.86 (s, 6H, 2 × OCH_3), 3.94–4.04 (m, 1H, CH), 4.29–4.43 (m, 2H, CH_2), 6.41 (s, 2H, Ar-H × 2).

3.26. General procedure for the synthesis of (±)-1-nitro-4-(substituted-phenyl)-1-pentene (54–59)

To a solution of 1-nitro-4-(substituted-phenyl)-2-pentanol (**48–53**) (10 mmol) in 15 mL of dry CH_2Cl_2 at 0 °C, was added methanesulfonyl chloride (1.18 g, 0.8 mL, 10 mmol), followed by Et_3N (2.2 g, 2.8 mL, 20 mmol). The mixture was stirred at 0 °C for 20 min, and then poured into 30 mL of water and the aqueous mixture extracted with CH_2Cl_2 (50 mL × 3). The organic layers were combined and dried ($MgSO_4$). After evaporating the solvent, the residue was dissolved in 1 mL of CH_2Cl_2 and was placed on a 15 × 150 mm silica gel column and eluted with hexane, hexane/EtOAc/50:1. Fractions containing the product (TLC) were pooled and evaporated to afford **54–59**.

3.27. (±)-1-Nitro-4-phenyl-l-pentene (54)

Using the general procedure described above, compound **54** was obtained from (±)-1-nitro-4-phenyl-2-pentanol **48** as a light yellow oil in 90% yield. R_f 0.63 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.35 (d, 3H, CH_3), 2.48–2.62 (m, 2H, CH_2), 2.91–3.00 (m, 1H, CH), 6.88 (d, 1H, CH), 7.11–7.35 (m, 6H, CH and Ar-H × 5).

3.28. (±)-1-Nitro-4-(2-naphthyl)-l-pentene (55)

Using the general procedure described above, compound **55** was obtained from (±)-1-nitro-4-(2-naphthyl)-2-pentanol **49** as a light yellow oil in 91% yield. R_f 0.73 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.41 (d, 3H, CH_3), 2.50–2.80 (m, 2H, CH_2), 3.00–3.30 (m, 1H, CH), 6.87 (d, 1H, CH), 7.15–7.90 (m, 8H, CH and Ar-H × 7).

3.29. (±)-1-Nitro-4-(2-fluorenyl)-l-pentene (56)

Using the general procedure described above, compound **56** was obtained from (±)-1-nitro-4-(2-fluorenyl)-2-pentanol **50** as a light yellow oil in 90% yield. R_f 0.69 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.30 (d, 3H, CH_3), 2.33–2.60 (m, 2H, CH_2), 2.75–3.00 (m, 1H, CH), 3.79 (s, 2H, CH_2), 6.78 (d, 1H, CH), 7.00–7.90 (m, 8H, CH and Ar-H × 7).

3.30. (±)-1-Nitro-4-(4-biphenyl)-l-pentene (57)

Using the general procedure described above, compound **57** was obtained from (±)-1-nitro-4-(4-biphenyl)-2-pentanol **51** as a light yellow oil in 92% yield. R_f 0.69 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.38 (d, 3H, CH_3), 2.49–2.65 (m, 2H, CH_2), 2.98–3.15 (m, 1H, CH), 6.91 (d, 1H, CH), 7.14–7.70 (m, 10H, CH and Ar-H × 9).

3.31. (±)-1-Nitro-4-(2,5-dimethoxyphenyl)-l-pentene (58)

Using the general procedure described above, compound **58** was obtained from (±)-1-nitro-4-(2,5-dimethoxyphenyl)-2-pentanol **52** as a light yellow oil in 90% yield. R_f 0.60 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.26 (d, 3H, CH_3), 2.46–2.25 (m, 2H, CH_2), 3.30–3.50 (m, 1H, CH), 3.76 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 6.70–6.80 (m, 3H, Ar-H × 3), 6.87 (d, 1H, CH), 7.16–7.25 (m, 1H, CH).

3.32. (±)-1-Nitro-4-(3,4,5-trimethoxyphenyl)-l-pentene (59)

Using the general procedure described above, compound **59** was obtained from (±)-1-nitro-4-(3,4,5-trimethoxyphenyl)-2-pentanol **53** as a light yellow oil in 91% yield. R_f 0.40 (hexane/EtOAc/3:1). ^{1}H NMR ($CDCl_3$): δ 1.32 (d, 3H, CH_3), 2.46–2.52 (m, 2H, CH_2), 3.30–3.14 (m, 1H, CH), 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.41 (s, 2H, Ar-H × 2), 6.91 (d, 1H, CH), 7.10–7.19 (m, 1H, CH).

3.33. General procedure for the synthesis of 5-[1-(nitromethyl)-3-substituted arylbutyl]pyrimidine-2,4,6-triamine (60–65)

A mixture of (±)-1-nitro-4-(substituted-aryl)-pentene (**54–59**) (5 mmol) and (5 mmol) of 2,4,6-triaminopyrimidine in 20 mL water and 20 mL ethyl acetate was stirred

at 50–55 °C for 24 h. The solid dissolved on heating. The reaction mixture was poured into 200 mL ethyl acetate, washed with water (2×40 mL), dried (Na_2SO_4) and purified by silica gel column chromatography with 5% CH_3OH in $CHCl_3$. Fractions containing the product (TLC) were pooled and evaporated to afford **60**–**65**.

3.34. 5-[(1-Nitromethyl)-3-phenylbutyl]pyrimidine-2,4,6-triamine (60)

Using the general procedure described above, compound **60** was obtained from (±)-1-nitro-4-phenyl-1-pentene **54** as a yellow solid in 82% yield. Mp: 70–73 °C; R_f 0.30 ($CHCl_3$:CH_3OH/5:1). ^1H NMR (DMSO-d_6): δ 1.13–1.20 (m, 3H, CH_3), 1.60–2.30 (m, 2H, CH_2), 2.50–2.65 (m, 1H, CH), 2.97–3.54 (m, 1H, CH), 4.65–4.80 (m, 2H, CH_2), 5.05–5.35 (m, 4H, 2 × NH_2), 5.40–5.65 (m, 2H, NH_2), 7.10–7.90 (m, 5H, Ar-H × 5). Anal. Calcd for $C_{15}H_{20}N_6O_2 \cdot 0.2CH_3OH$: C, 56.56; H, 6.50; N, 26.04. Found: C, 56.73; H, 6.40; N, 25.87.

3.35. 5-[3-(2-Naphthyl)-1-(nitromethyl)butyl]pyrimidine-2,4,6-triamine (61)

Using the general procedure described above, compound **61** was obtained from (±)-1-nitro-4-(2-naphthyl)-1-pentene **55** as a yellow solid in 81% yield. Mp: 75–77 °C; R_f 0.50 ($CHCl_3$:CH_3OH/5:1). ^1H NMR (DMSO-d_6): δ 1.23–1.30 (m, 3H, CH_3), 1.65–2.30 (m, 2H, CH_2), 2.60–2.90 (m, 1H, CH), 3.55–3.75 (m, 1H, CH), 4.65-4.95 (m, 2H, CH_2), 5.09–5.53 (m, 6H, 3 × NH_2), 7.36–7.83 (m, 7H, Ar-H × 7). Anal. Calcd for $C_{19}H_{22}N_6O_2$: C, 62.28; H, 6.05; N, 22.94. Found: C, 62.44; H, 6.03; N, 22.60.

3.36. 5-[3-(2-Fluorenyl)-1-(nitromethyl)butyl]pyrimidine-2,4,6-triamine (62)

Using the general procedure described above, compound **62** was obtained from (±)-1-nitro-4-(2-fluorenyl)-1-pentene **56** as a yellow solid in 75% yield. Mp: 78–80 °C; R_f 0.53 ($CHCl_3$:CH_3OH/5:1). ^1H NMR (DMSO-d_6): δ 1.19–1.26 (m, 3H, CH_3), 1.15–2.25 (m, 2H, CH_2), 2.50–2.80 (m, 1H, CH), 3.90–3.65 (m, 1H, CH), 3.86 (s, 2H, CH_2), 4.65–4.90 (m, 2H, CH_2), 5.03–5.60 (m, 6H, 3 × NH_2), 7.18–7.86 (m, 7H, Ar-H × 7). Anal. Calcd for $C_{22}H_{24}N_6O_2$: C, 65.33; H, 5.98; N, 20.78. Found: C, 65.14; H, 5.99; N, 20.66.

3.37. 5-[3-(4-Biphenyl)-1-(nitromethyl)butyl]pyrimidine-2,4,6-triamine (63)

Using the general procedure described above, compound **63** was obtained from (±)-1-nitro-4-(4-biphenyl)-1-pentene **57** as a yellow solid in 78% yield. Mp: 75–76 °C; R_f 0.45 ($CHCl_3$:CH_3OH/5:1). ^1H NMR (DMSO-d_6): δ 1.22 (d, 3H, CH_3), 1.65-2.25 (m, 2H, CH_2), 2.30–2.55 (m, 1H, CH), 3.70–3.95 (m, 1H, CH), 4.65–4.90 (m, 2H, CH_2), 5.30–5.51 (m, 6H, 3 × NH_2), 7.20–7.70 (m, 9H, Ar-H × 9). Anal. Calcd for $C_{21}H_{24}N_6O_2$: C, 64.27; H, 6.16; N, 21.41. Found: C, 64.22; H, 6.17; N, 21.48.

3.38. 5-[3-(2,5-Dimethoxyphenyl)-1-(nitromethyl)butyl]pyrimidine-2,4,6-triamine (64)

Using the general procedure described above, compound **64** was obtained from (±)-1-nitro-4-(2,5-dimethoxyphenyl)-1-pentene **58** as a yellow solid in 80% yield. Mp: 80–83 °C; R_f 0.41 ($CHCl_3$:CH_3OH/5:1). ^1H NMR (DMSO-d_6): δ 1.05–1.16 (m, 3H, CH_3), 1.65–2.15 (m, 2H, CH_2), 2.85–3.10 (m, 1H, CH), 3.40–3.60 (m, 1 H, CH), 3.66 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3). 4.65–4.90 (m, 2H, CH_2), 5.20–5.70 (m, 6H, 3 × NH_2), 6.72–6.85 (m, 3H, Ar-H × 3). Anal. Calcd for $C_{17}H_{24}N_6O_4 \cdot 0.4H_2O$: C, 53.23; H, 6.52; N, 21.91. Found: C, 52.89; H, 6.38; N, 21.70.

3.39. 5-[3-(3,4,5-Trimethoxyphenyl)1-(nitromethyl)butyl]pyrimidine-2,4,6-triamine (65)

Using the general procedure described above, compound **65** was obtained from (±)-1-nitro-4-(3,4,5-trimethoxyphenyl)-1-pentene **59** as a yellow solid in 79% yield. Mp: 84–87 °C; R_f 0.41 ($CHCl_3$:CH_3OH/5:1). ^1H NMR (DMSO-d_6): δ 1.19 (d, 3H, CH_3), 1.62–2.20 (m, 2H, CH_2), 2.85–3.10 (m, 1H, CH), 3.40–3.60 (m, 1H, CH), 3.74 (s, 3H, OCH_3), 3.76 (s, 6H, 2 × OCH_3), 4.69–4.78 (m, 2H, CH_2), 5.10–5.60 (m, 6H, 3 × NH_2), 6.44 (s, 2H, Ar-H × 2). Anal. Calcd for $C_{15}H_{20}N_6O_2 \cdot 0.6H_2O$: C, 51.81; H, 6.57; N, 20.14. Found: C, 52.06; H, 6.43; N, 19.75.

3.40. General procedure for the synthesis of (±)-2,4-diamino-5-[2-methyl-2-(substituted-pheny)]ethyl-pyrrolo[2,3-*d*]pyrimidine (18–23)

To a solution of NaOH (0.24 g, 6.0 mmol) in 3.0 mL of water was added (1.0 mmol) of (±)-1-nitro-2-(2,4,6-triaminopyrimidin-5-yl)-4-(substituted-phenyl)-pentane (**60**–**65**) and the mixture was stirred at room temperature for 2 h. This mixture was added dropwise to a solution of 98% H_2SO_4 (0.55 mL, 0.98 g, 10.0 mmol) in 4.0 mL of water at 0 °C. After stirring for an additional 3 h, the pH of the mixture was adjusted to 7 with 2 N NaOH at 0 °C. The mixture was stirred at room temperature for another 1 h and then acidified with acetic acid (0.5 mL). The precipitated solid was collected by filtration, washed with water, followed by ethyl acetate, and dried in vacuum to afford an off-white solid. This solid was dissolved in 5 mL of methanol and 100 mg of silica gel was added and the solvent was removed to form a plug, which was loaded on a silica gel column (15 × 150 mm), and eluted with 4% methanol in chloroform. Fractions containing the product (TLC) were pooled and evaporated to afford **18**–**23**.

3.41. (±)-2,4-Diamino-5-(2-methyl-2-phenyl)ethyl-pyrrolo[2,3-*d*]pyrimidine (18)

Using the general procedure described above, compound **18** was obtained from (±)-1-nitro-2-(2,4,6-triaminopyrimidin-5-yl)-4-phenyl-pentane **60** as an off-white solid in 49% yield. Mp: >250 °C; R_f 0.65 ($CHCl_3$:CH_3OH/5:1). ^1H NMR (DMSO-d_6): δ 1.18 (d,

3H, CH_3), 2.89–2.98 (m, 3H, CH and CH_2), 5.42 (s, 2H, NH_2), 5.98 (s, 2H, NH_2), 6.23 (s, 1H, CH), 7.14–7.23 (m, 5H, Ar-H × 5), 10.35 (s, 1H, NH). Anal. Calcd for $C_{15}H_{17}N_5$: C, 67.39; H, 6.41; N, 26.20. Found: C, 67.25; H, 6.41; N, 26.08.

3.42. (±)-2,4-Diamino-5-[2-methyl-2-(2-naphthyl)]ethyl-pyrrolo[2,3-*d*]pyrimidine (19)

Using the general procedure described above, compound **19** was obtained from (±)-1-nitro-2-(2,4,6-triaminopyrimidin-5-yl)-4-(2-naphthyl)-pentane **61** as an off-white solid in 53% yield. Mp: >200 °C; R_f 0.64 ($CHCl_3$:CH_3OH/5:1). ^1H NMR (DMSO-d_6): δ 1.31 (d, 3H, CH_3), 2.95–3.20 (m, 3H, CH and CH_2), 5.35 (s, 2H, NH_2), 5.96 (s, 2H, NH_2), 6.23 (s, 1H, CH), 7.46–7.92 (m, 7H, Ar-H × 7), 10.27 (s, 1H, NH). Anal. Calcd for $C_{15}H_{17}N_5 \cdot 0.6H_2O$: C, 69.53; H, 6.20; N, 21.34. Found: C, 69.61; H, 6.25; N, 21.02.

3.43. (±)-2,4-Diamino-5-[2-methyl-2-(2-fluorenyl)]ethyl-pyrrolo[2,3-*d*]pyrimidine (20)

Using the general procedure described above, compound **20** was obtained from (±)-1-nitro-2-(2,4,6-triaminopyrimidin-5-yl)-4-(2-fluorenyl)-pentane **62** as an off-white solid in 48% yield. Mp: >200 °C; R_f 0.68 ($CHCl_3$:CH_3OH/5:1). ^1H NMR (DMSO-d_6): δ 1.27 (d, 3H, CH_3), 2.85–3.15 (m, 3H, CH and CH_2), 3.87 (s, 2H, CH_2), 5.37 (s, 2H, NH_2), 5.96 (s, 2H, NH_2), 6.28 (s, 1H, CH), 7.15–7.95 (m, 7H, Ar-H × 7), 10.32 (s, 1H, NH). Anal. Calcd for $C_{22}H_{21}N_5 \cdot 0.8H_2O$: C, 71.44; H, 6.16; N, 18.94. Found: C, 71.28; H, 6.10; N, 18.63.

3.44. (±)-2,4-Diamino-5-[2-methyl-2-(4-biphenyl)]ethyl-pyrrolo[2,3-*d*]pyrimidine (21)

Using the general procedure described above, compound **21** was obtained from (±)-1-nitro-2-(2,4,6-triaminopyrimidin-5-yl)-4-(4-biphenyl)-pentane **63** as an off-white solid in 45% yield. Mp: >230 °C; R_f 0.66 ($CHCl_3$:CH_3OH/5:1). ^1H NMR (DMSO-d_6): δ 1.22 (d, 3H, CH_3), 2.85–3.15 (m, 3H, CH and CH_2), 5.42 (s, 2H, NH_2), 5.99 (s, 2H, NH_2), 6.29 (s, 1H, CH), 7.33–7.64 (m, 9H, Ar-H × 9), 10.36 (s, 1H, NH). Anal. Calcd for $C_{21}H_{21}N_5 \cdot 1.2H_2O$: C, 69.09; H, 6.46; N, 19.18. Found: C, 69.33; H, 6.07; N, 18.83.

3.45. (±)-2,4-Diamino-5-[2-methyl-2-(2,5-dimethoxyphenyl)]ethyl-pyrrolo[2,3-*d*]pyrimidine (22)

Using the general procedure described above, compound **22** was obtained from (±)-1-nitro-2-(2,4,6-triaminopyrimidin-5-yl)-4-(2,5-dimethoxyphenyl)-pentane **64** as an off-white solid in 50% yield. Mp: >250 °C; R_f 0.57 ($CHCl_3$:CH_3OH/5:1). ^1H NMR (DMSO-d_6): δ 1.08 (d, 3H, CH_3), 2.36–3.30 (m, 3H, CH and CH_2), 3.70 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 5.38 (s, 2H, NH_2), 6.20 (s, 2H, NH_2), 6.39 (s, 1H, CH), 6.72–6.90 (m, 3H, Ar-H × 3), 10.39 (s, 1H, NH). Anal. Calcd for $C_{17}H_{21}N_5O_2$: C, 62.37; H, 6.47; N, 21.39. Found: C, 62.21; H, 6.59; N, 21.30.

3.46. (±)-2,4-Diamino-5-[2-methyl-2-(3,4,5-triOMepheny)]ethyl-pyrrolo[2,3-*d*]pyrimidine(23)

Using the general procedure described above, compound **23** was obtained from (±)-1-nitro-2-(2,4,6-triaminopyrimidin-5-yl)-4-(3,4,5-trimethoxyphenyl)-pentane **65** as an off-white solid in 49% yield. Mp: >250 °C; R_f 0.53 ($CHCl_3$:CH_3OH/5:1). ^1H NMR (DMSO-d_6): δ 1.18 (d, 3H, CH_3), 2.84–2.94 (m, 3H, CH and CH_2), 3.60 (s, 3H, OCH_3), 3.65 (s, 6H, 2 × OCH_3), 5.36 (s, 2H, NH_2), 5.92 (s, 2H, NH_2), 6.31 (s, 1H, CH), 6.51 (s, 2H, Ar-H × 2), 10.34 (s, 1H, NH). Anal. Calcd $C_{18}H_{23}N_5O_3 \cdot 0.1H_2O$: C, 60.19; H, 6.51; N, 19.50. Found: C, 59.86; H, 6.62; N, 19.24.

3.47. Sources of DHFR enzymes

Dihydrofolate reductase from *P. carinii* was produced as the recombinant enzyme expressed in *Escherichia coli*.[35] The sequence of the protein was identical to that predicted for the previously reported gene sequence.[36]

Dihydrofolate reductase from *T. gondii* was isolated directly from the RH strain of *T. gondii* grown in culture on mutant Chinese hamster ovary cells lacking dihydrofolate reductase (CHO/dhfr$^-$, American Type Culture Collection 3952 CL).[36] The organisms are introduced into a confluent monolayer of the cells and harvested when they have lysed the mammalian cells. The 100,000*g* supernate is stored in liquid nitrogen.

Mycobacterium avium used in these studies was a clinical isolate from Indiana University School of Medicine, Department of Pathology; it was identified as a serovar 4. The strain was maintained on Lowenstein-Jensen slants (Baxter Scientific) grown at room temperature. To produce enzyme, the organism was grown in Middlebrook 7H-9 liquid medium at 37 °C to an OD_{660} of 0.5–0.7, which took several weeks. At harvest, the bacteria were sedimented by centrifugation, sonicated, and the 100,000*g* supernate was stored under liquid nitrogen until assay. These supernates contained both dihydrofolate reductase and dihydroopteroate synthetase activity.

Rat liver dihydrofolate reductase was prepared from livers of adult female Sprague–Dawley rats. The 100,000*g* supernate prepared from crude homogenates was partially purified by ammonium sulfate precipitation; the 50–90% precipitate was redissolved and stored in liquid nitrogen.

3.48. DHFR assay

The spectrophotometric assay for dihydrofolate reductase was modified to optimize for temperature, substrate concentration, and cofactor concentration for each enzyme form assayed. The standard assay contained sodium phosphate-buffer, pH 7.4 (40.7 mM), 2-mercaptoethanol (8.9 mM), NADPH (0.117 mM), dihydrofolic acid (0.092 mM), and sufficient enzyme to produce a change in OD_{340} of 0.035/min. KCL (150 mM) was added to the reaction for the *T. gondii*, *M. avium*, and rat liver enzymes. All assays were performed at 37 °C. The

reaction was followed for 3 min with continuous recording. Activity under these conditions was linear with enzyme concentration over at least a 4-fold range. Background activity in the absence of dihydrofolic acid was near zero and was subtracted from all rates.

3.49. Determination of IC_{50} values

Dihydrofolate reductase was assayed in the presence of a series of concentrations of inhibitor to produce a range of rates from 1% to 90% of the uninhibited rate. At least three concentrations were required for calculation; most curves contained five or more concentrations. Semilogarithmic plots of the data yielded normal sigmoidal curves for most inhibitors. The concentration yielding 50% inhibition (IC_{50}) was calculated using Prism 4.0 (GraphPad).

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