Conformationally Restricted Tricyclic Analogues of Lipophilic Pyrido[2,3-*d*]pyrimidine Antifolates [1a,b]

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The effect of conformational restriction of the C9-N10 bridge on inhibitory potency and selectivity of trimetrexate against dihydrofolate reductase, was studied. Specifically three nonclassical tricyclic 1,3-diamino-8-(3',4',5'-trimethoxybenzyl)-7,9-dihydro-pyrrolo[3,4-*c*]pyrido[2,3-*d*]pyrimidin-6(5*H*,8*H*)-one (**4**), 1,3-diamino-8-(3',4',5'-trimethoxybenzyl)-9-hydro-pyrrolo[3,4-*c*]pyrido[2,3-*d*]pyrimidin-6-(8*H*)-one (**5**) and 1,3-diamino-(*8H*)-(3',4',5'-trimethoxybenzyl)-7,9-dihydro-pyrrolo[3,4-*c*]pyrido[2,3-*d*]pyrimidine (**7**) antifolates were synthesized. The tricyclic analogues **4** and **5** were obtained *via* the regiospecific cyclocondensation of the β-keto ester **17** with 2,4,6-triaminopyrimidine. The analogue **7** was obtained *via* reduction of the lactam **4** with borane in tetrahydrofuran. Compounds **4, 5** and **7** were evaluated as inhibitors of dihydrofolate reductase from *Pneumocystis carinii, Toxoplasma gondii* and rat liver. All three compounds were more selective than trimetrexate against *Pneumocystis carinii* dihydrofolate reductase and significantly more selective than trimetrexate against *Toxoplasma gondii* dihydrofolate reductase compared with rat liver dihydrofolate reductase.

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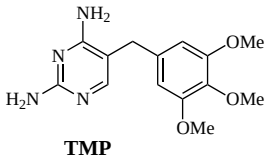
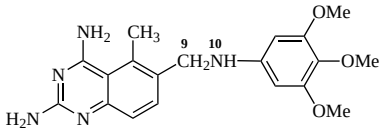
Currently available treatments for *Pneumocystis carinii* (*P. carinii*) and *Toxoplasma gondii* (*T. gondii*), which are often fatal opportunistic infections in AIDS patients, are usually a combination of agents [2-4]. The use of trimethoprim and sulfamethoxazole is considered to be the most effective combination for the treatment and prophylaxis of *P. carnii* infections [5]. *T. gondii* infections are treated by the first line combination of pyrimethamine and sulfadiazine [6]. Both these combinations utilize a selective dihydrofolate reductase inhibitor trimethoprim or pyrimethamine along with a dihydropteroate synthase inhibitor that is the sulfa drug. Though these combinations are effective, a significant number of patients suffer side effects primarily attributed to the sulfonamide drug which limits the dose and in many cases forces a discontinuation of therapy [7,8]. The dihydrofolate reductase inhibitors in these combinations are weak ineffective agents when used as monotherapy and require the sulfonamide to afford a synergistic, clinically effective combination [9,10]. In an attempt to circumvent the use of the sulfonamide in these combinations, due to its toxicity which necessitates discontinuation of treatment, considerable effort has been expended to design and synthesize pathogen selective dihydrofolate reductase inhibitors that also possess increased potencies against *P. carinii* dihydrofolate reductase and *T. gondii* dihydrofolate reductase. Such a potent and selective dihydrofolate reductase inhibitor would preclude the necessity of the sulfonamide and hence the toxicity associated with the combination and could be clinically viable monotherapy for *P. carinii* and *T. gondii* infections.

The 3,000 to 10,000 fold selectivity of trimethoprim (TMP) for bacterial dihydrofolate reductase over the mammalian enzyme has been attributed, in part, to the adoption of different conformations of the trimethoxy benzyl side chain of trimethoprim when bound to bacterial and vertebrate dihydrofolate reductase as gleaned from X-ray crystal structures of trimethoprim with dihydrofolate reductase from *Escherichia coli* and chicken liver [11]. Thus the side chain of trimethoprim adopts a "down" conformation in *E. coli* and an "up" conformation in chicken liver dihydrofolate reductase with respect to the 2,4-diaminopyrimidine moiety. X-ray crystal structure of trimethoprim and *P. carinii* dihydrofolate reductase indicate that the trimethoxybenzyl side chain of trimethoprim is oriented in the "down" conformation similar to that observed in the *E. coli* enzyme [12]. The selectivity of trimethoprim for dihydrofolate reductase from *P. carinii* compared to rat liver, though not as high as that for the *E. coli* enzyme perhaps also stems, in part, from the different side chain orientation of trimethoprim when bound to *P. carinii* dihydrofolate reductase compared to the rat liver enzyme.

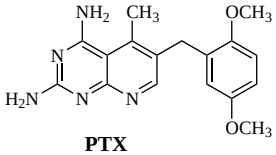
Bicyclic 2,4-diamino nonclassical dihydrofolate reductase inhibitors related to the pteridines [13], pyrido[2,3-*d*]pyrimidines [14], pyrido[3,2-*d*]pyrimidines [15], quinazolines [16], pyrrolo[2,3-*d*]pyrimidines [17], furo[2,3-*d*]pyrimidines [18], and thieno[2,3-*d*]pyrimidines [19] among others [20,21] have been synthesized as potential potent and selective inhibitors of dihydrofolate reductase from *P. carinii* and *T. gondii*. Though structure-activity/selectivity correlations for each bicyclic system have been developed no structure-activity relationship across the different heterocyclic systems is possible at this time and each heterocyclic system needs to be considered separately.



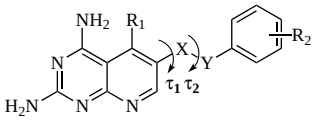
TMP



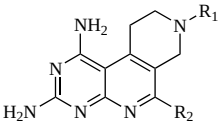
TMQ



PTX



1 $R_1 = CH_3$, X = CH_2, Y = NH or NCH_3
2 R_1 = H, X = NH or NCH_3, Y = CH_2



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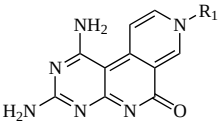
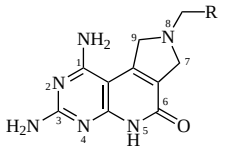


3a R_1 = 3,4,5-triOMePh

The bicyclic 2,4-diamino-6-substituted quinazoline, trimetrexate (TMQ) and the pyrido[2,3-*d*]pyrimidine piritrexim (PTX) were both found to be highly potent against dihydrofolate reductase from *P. carinii* and *T. gondii*, however these bicyclic agents were devoid of selectivity against the pathogenic enzymes [10,22]. Trimetrexate, which is approved as a second line agent against *P. carinii* infections must be used along with the folate cofactor, leucovorin, to rescue host cells [23]. Gangjee *et al.* [24] have reported a generalization in the structure-activity/selectivity of the pyrido[2,3-*d*]pyrimidine ring system which indicates that selected analogues of the 5,10-dimethyl substituted series of general structure **1** as well as selected analogues containing a 9-methyl substitution of general structure **2** [14] provide significant potency and selectivity for *P. carinii* and/or *T. gondii* dihydrofolate reductase.

Molecular modeling of 2,4-diamino-5,10-desmethyl 6-substituted pyrido[2,3-*d*]pyrimidines compared with the 5,10-dimethyl congeners using SYBYL 6.2 [25] and its SEARCH and MAXIMIN options indicated that mono and dimethyl substitution at the 5- and/or 10-positions significantly decreases the number of conformations compared to the unsubstituted analogues. Thus partial conformational restriction of the 9-10 bridge of 2,4-diamino-6-substituted pyrido[2,3-*d*]pyrimidines with methyl moieties on the 5-and 9- or 10-positions provides increased selectivity and/or potency against *P. carinii* and/or *T. gondii* dihydrofolate reductase [14,24]. It was therefore of interest to further conformationally restrict the 9-10 bridge of pyrido[2,3-*d*]pyrimidines in an attempt to study the effect on potency and selectivity against *P. carinii* and *T. gondii* dihydrofolate reductase.

Conformational restriction of the 9-10 bridge beyond that obtained by methyl substitutions on the 5- and 10-positions could be achieved by tethering the 5- and 10-positions *via* a methyl or ethyl link to afford tricyclic pyrrolo or pyrido annulated 2,4-diamino pyrido-[2,3-*d*]pyrimidines. Gangjee *et al.* [26] recently reported the tricyclic pyrido annulated analogues in which the C5 and N10 were tethered *via* an ethyl link effectively restricting the 9-10 bridge. Some members of this series of conformationally restricted tricyclic analogues represented by general structure **3** were potent inhibitors of rat liver dihydrofolate reductase with inhibitory constants (IC_{50}) which were highly potent (IC_{50} 86 n*M*, for **3** R_1 = 3,4,5-OMePh, R_2 = OH) but the series lacked selectivity for *P. carinii* or *T. gondii* dihydrofolate reductase and were in fact much more inhibitory against mammalian dihydrofolate reductase.



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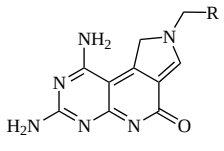

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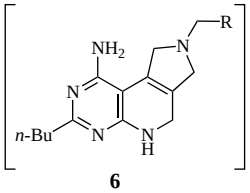

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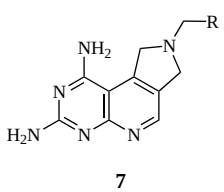

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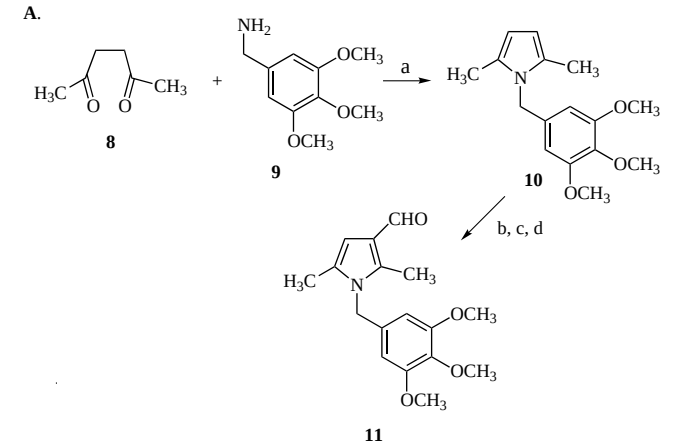
The lack of potent inhibitory effects against *P. carinii* and *T. gondii* dihydrofolate reductase of analogues **3** was surprising, particularly since the 5,10-dimethyl analogue **1** (R_1 = CH_3, X = CH_2, Y = NCH_3, R_2 = 3,4,5 triOMePh) was significantly potent against all three dihydrofolate reductases, and displayed a 9-fold selectivity for *T. gondii* dihydrofolate reductase as compared to rat liver dihydrofolate reductase (Table 1). Clearly the ethyl bridge between the 5- and 10-positions was detrimental to potency against *P. carinii* and *T. gondii* dihydrofolate reductase. The reason for this was attributed, in part, to the inappropriate orientation of the trimethoxyphenyl ring, which is predicated on the conformation of the C-ring, and was not conducive to *P. carinii* or *T. gondii* dihydrofolate reductase binding.

In an attempt to further restrict the 9-10 bond, tricyclic analogues **4-7** in which the 5- and 10-positions were linked *via* a methylene rather than an ethyl link were designed. This afforded a 5-membered C-ring in which the 9-10 bridge was now constrained with less flexibility than in analogues **3**. Molecular modeling [25] of analogues **4-7** indicated that the triOMePh side chain was oriented in different conformations to that observed for analogues **3** and were in close proximity to that observed for trimetrexate bound to *P. carinii* dihydrofolate reductase [12]. Molecular modeling further indicated that a methylene spacer between the tricyclic pyrrolo annulated system and the 3,4,5-trimethoxyphenyl side chain allowed for flexibility in the side chain and that the trimethoxybenzyl analogues **4-7** provided a better overlap with dihydrofolate reductase bound trimetrexate. Thus the trimethoxybenzyl moiety was included as the side chain in preference to the trimethoxyphenyl. Though compound **7** was the primary target, we were very much interested in the partially reduced analogues **4** and **6** as well as the essentially planar analogue **5**. The varying levels of unsaturation in rings B and C of **4-7** provide subtle variations in the tricyclic ring conformation which provides increased potency and/or selectivity as we have previously reported for the pyrido annulated analogues [26]. In fact, in the tricyclic series **3**, the most selective

Scheme I



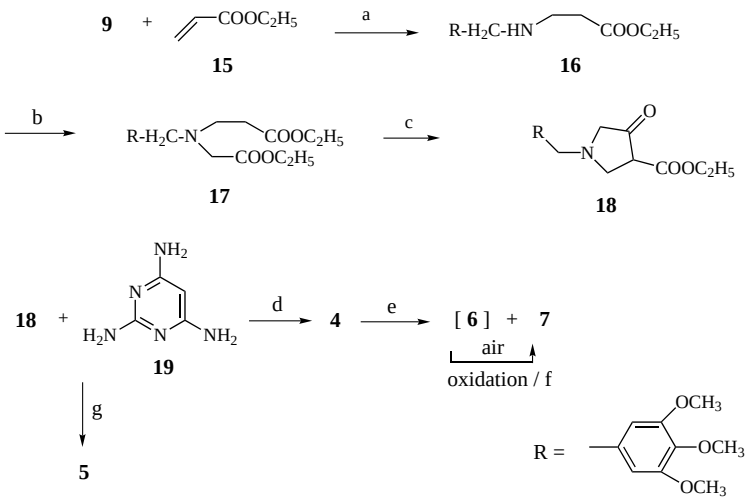
a: Toluene/reflux; b: DMF/ $POCl_3$/15 °C; c: **10** in DMF/30minutes/ 0 °C; d: 90 °C/2 hours

e: EtOH/Glacial AcOH/90 °C/48 hours; f: Fischer indole conditions.

analogue was the essentially planar compound **3a** thus supporting the synthesis and evaluation of compound **5** as a target analogue.

Gangjee *et al.* [28] reported a novel strategy for the synthesis of the tricyclic pyrido[2,3-*d*]pyrimidines *via* a Fischer indole type cyclization of a 2-amino-4-oxo afforded the 3-formyl pyrrole, **11**, as a tan solid in 91% yield. The hydrazone **13** was synthesized by condensing an equimolar quantity of the hydrazine **12** and aldehyde **11** in a mixture of ethanol and glacial acetic acid. Attempted Fischer indole cyclization of **13** under a variety of conditions, however, failed to yield the desired product.

Scheme 2



a: THF/ 60 °C/3 days; b: $BrCH_2CO_2C_2H_5$/DMF/60 °C; c: NaOEt/ Toluene; d: Glacial AcOH/120 °C/12 hours; e: BH_3/THF; f: MeOH/reflux; g: Dowtherm-A/240 °C/2 hours.

hydrazone. A similar strategy was initially attempted for the synthesis of tricyclic analogues **4-7** as shown in Scheme I. The synthesis of 2,4-diamino-6-hydrazinopyrimidine (**12**) involved the reaction of 2,4-diamino-6-chloropyrimidine with excess hydrazine in butanol. The product precipitated from the reaction mixture as a white, methanol insoluble solid and was isolated as a powder in 45% yield. The insolubility of **12** in deuterated chloroform and its instability in deuterated dimethylsulfoxide made it difficult to obtain a ^1H nmr spectrum. For model studies, the 2,5-dimethyl pyrrole-3-carboxaldehyde, **11**, was selected to avoid any potential α-polymerization. The *N*-substituted pyrrole-3-carboxaldehyde, **11**, was obtained using the Paal-Knorr synthesis (Scheme 1). The pyrrole **10** was synthesized first by refluxing equimolar qauntities of acetonyl acetone (**8**) and 3,4,5-trimethoxybenzylamine (**9**), in a flask fitted with a Dean-Stark trap, until separation of water ceased (4 hours). Upon cooling, a tan solid was obtained in 70% yield. The *N*-trimethoxybenzyl-2,5-dimethylpyrrole (**10**) was formylated *via* a Vilsmeier Haack formylation using dimethylformamide and phosphorus oxychloride. A solution of **10** in dimethylformamide was slowly added to a freshly prepared mixture of dimethylformamide and phosphorus oxychloride at 0 °C. The reaction mixture was then heated briefly (2 hours) at 90 °C and after work up

An alternate methodology, which could afford the desired tricyclic analogues, was the condensation of 2,4,6-triaminopyrimidine with a suitable biselectrophile. Gangjee *et al.* [27] had reported the synthesis of similar compounds using this method and hence this was a logical choice. Syntheses of the target compounds **4-7** were accomplished *via* the regiospecific cyclocondensation of the β-keto ester **18** (as the biselectrophile) with 2,4,6-triaminopyrimidine (Scheme 2). Hurlbert *et al.* [29] as well as reports from Gangjee *et al.* [30,31] and DeGraw *et al.* [32] have confirmed that β-keto esters condense with appropriate 6-aminopyrimidines to afford regiospecifically angular, 5,6-disubstituted pyrido[2,3-*d*]pyrimidines rather than the linear, 6,7-disubstituted isomers. Compound **16** was synthesized by the reaction of 3,4,5-trimethoxybenzylamine **9** with ethyl acrylate (**15**) followed by alkylation with ethylbromoacetate to afford **17** which on Dieckmann cyclization gave the desired β-ketoester **18**. Though diphenyl ether had been previously employed as the solvent [27], it was of interest to explore different solvent systems since the extent of unsaturation in the product could be influenced by the solvent in the cyclocondensation reaction [26,30,31]. Cyclocondensation of **18** with 2,4,6-triaminopyrimidine (**19**) in glacial acetic acid at 120 °C for 12 hours afforded exclusively the

angular lactam **4** in 55% yield, while similar cyclocondensation in Dowtherm-A at 240 °C for 2 hours gave the dehydrogenated angular lactam **5** as the sole product in 40% yield. ^{1}H nmr of **4** showed an exchangeable (D_2O) lactam NH at δ 8.98 and three sets of methylene protons at δ 3.17, 3.78 and 4.43, as was previously reported for similar tricyclic compounds [30-32]. In contrast the ^{1}H nmr of **5** indicated a nonexchangeable olefinic proton at δ 8.82 as we have observed earlier, and only two sets of methylene protons at δ 4.38 and 4.65. The lactam **4** was reduced using borane in tetrahydrofuran to afford a mixture of **6** and **7** in a 1:1 ratio as indicated by the ^{1}H nmr and the mass spectrum of the mixture. All attempts to separate the mixture resulted in the oxidation of **6** to **7**. Thus only **7** was isolated as the pure reduced lactam. The oxidation also occurs when the mixture is allowed to stand at room temperature for an extended period. Conversion of the mixture to pure **7** was also accomplished by reflux in methanol. Compound **7** has been claimed in a patent [33] *via* a different synthetic route [34], however, no spectral or biological data was reported.

The compounds **4-5** and **7** were evaluated as inhibitors of dihydrofolate reductase from *P. carinii* [35], *T. gondii* [36] and rat liver. Selectivity ratios (IC_{50} rat liver dihydrofolate reductase/IC_{50} *P. carinii* dihydrofolate reductase or *T. gondii* dihydrofolate reductase) were determined using rat liver dihydrofolate reductase as the mammalian source. These IC_{50} values along with selectivity ratios are shown in Table I. The inhibitory values for **1** (Y = NCH_3, R_2 = 3,4,5-triOMe) and trimetrexate are also included for comparison. The most potent of the three target compounds against *P. carinii* and *T. gondii* dihydrofolate reductase was the dehydrogenated lactam **5**. More significantly this compound was 93-fold more selective against *P. carinii* dihydrofolate reductase and about 110-fold more selective against *T. gondii* dihydrofolate reductase than trimetrexate. All three compounds, **4, 5** and **7**, were more selective than trimetrexate against both *P. carinii* and *T. gondii* dihydrofolate reductase. The significant increase in both potency and selectivity of **5** compared to **4** strongly suggests that partial planarity of the C-ring with the pyrido[2,3-*d*]pyrimidine system was important for potency and selectivity in this series. Thus, conformationally restricting τ_1 and τ_2 of **1** with partial planarity of the C-ring as in compound **5** significantly increases selectivity against both *P. carinii* and *T. gondii* dihydrofolate reductase compared to **1** and trimetrexate. A comparison of **4** and **7** suggests that in the absence of planarity in the C-ring the lactam is somewhat detrimental to potency as well as selectivity (except for *T. gondii* dihydrofolate reductase). Since compound **5** was the most potent and selective analogue against both *P. carinii* and *T. gondii* dihydrofolate reductase in this series and compound **5** also contains the nitrogen least likely to be protonated in the C-ring, due to conjugation, it is also possible that the increased potency and selectivity of **5** could arise due to its inability to be protonated at the nitrogen atom. Thus the selectivity and potency of **5** could be attributed to the planarity of the tricyclic systems and/or the inability of **5** to be protonated compared to **4** and **7**.

Table I

Inhibitory Concentrations (IC_{50}) in μM against Dihydrofolate Reductase and Selectivity Ratios of **4, 5** and **7**.

Compound	*P. carinii* [a]	Rat liver [b]	Selectivity Ratio rl/pc	*T. gondii* [c]	Selectivity Ratio rl/tg
1	0.013	0.007	0.58	0.0008	8.90
Trimetrexate	0.042	0.003	0.07	0.001	0.30
4	94.5	42.9	0.45	9.00	4.80
5	3.10	20.3	6.50	0.62	32.7
7	12.6	11.7	0.90	5.30	2.20

[a] Pneumocystis carinii dihydrofolate reductase. [b] Rat liver dihydrofolate reductase. [c] Toxoplasma gondii dihydrofolate reductase.

EXPERIMENTAL

All evaporations were carried out *in vacuo* with a rotary evaporator. Analytical samples were dried *in vacuo* (0.2 mm mercury) in an Abderhalden drying apparatus over phosphorus pentoxide and refluxing ethanol or toluene. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Nuclear Magnetic Resonance spectra for proton (^{1}H nmr) were recorded on a Bruker WH-300 (300 MHz) spectrometer. Data was accumulated by 16 K size with a 0.5 s delay time and 70° tip angle. The chemical shift values are expressed in ppm (parts per million) relative to tetramethylsilane as internal standard; s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet. The relative integrals of peak areas agreed with those expected for the assigned structures. Thin layer chromatography was performed on POLYGRAM Sil G/UV_{254} silica gel plates with fluorescent indicator and the spots were visualized under 254 nm and 366 nm illumination. Proportions of solvents used for thin layer chromatography are by volume. Elemental analysis were performed by Atlantic Microlabs Inc., Norcoss, GA. Analytical results indicated by element symbols are within ± 0.4% of the

calculated values. Fractional moles of water or organic solvents frequently found in some analytical samples of antifolates could not be removed in spite of 24-48 hours of drying *in vacuo* and were confirmed, where possible, by their presence in the ^{1}H nmr spectrum. All solvents and chemicals were purchased from Aldrich Chemical Co. and Fisher Scientific and were used as received.

1,3-Diamino-8-(3',4',5'-trimethoxybenzyl)-7,9-dihydropyrrolo-[3,4-*c*]pyrido[2,3-*d*]pyrimidin-6(5*H*,8*H*)-one (**4**).

A mixture of triaminopyrimidine (**19**) 0.113 g, (0.90 mmole), **18** 0.30 g, (0.90 mmole) and glacial acetic acid (10 ml) in a three necked flask fitted with a Dean-Stark trap was immersed in an oil bath heated to 120 °C. The suspension was stirred at this temperature under nitrogen for 12 hours. The reaction mixture was then cooled to room temperature and ether (50 ml) was added to the yellow suspension which was stirred for 30 minutes, filtered and washed with ether to yield crude lactam **4**. The crude lactam was then dissolved in methanol and silica gel (1.00 g) was added to this solution and the solvent evaporated under reduced pressure to form a uniformly coated silica gel plug which was dried under vacuum and loaded onto a dry silica gel column (2.4 x 20 cm) and eluted with a 10%-50% methanol in chloroform (1% ammonium hydroxide) gradient. Fractions containing the product (tlc) were pooled, concentrated under reduced pressure and neutralized with glacial acetic acid to yield 0.19 g (55%) of **4** as a tan powder: mp > 300 °C, tlc R_f 0.60 (chloroform/methanol.ammonium hydroxide 9:4:0.1, silica gel), ^{1}H nmr (DMSO-d_6): δ 3.17 (s, 2H, CH_2), 3.41 (s, 2H, CH_2), 3.67 (s, 3H, 4'-OCH_3), 3.78 (s, 6H, 3',5'-OCH_3), 4.43 (s, 2H, N-CH_2), 5.60 (s, 2H, NH_2), 5.98 (s, 2H, NH_2), 6.56 (s, 2H, 2',5'-CH), 8.98 (s, 1H, 5-NH).

Anal. Calcd. for $C_{19}H_{22}N_6O_4\bullet0.3H_2O\bullet0.5CH_3COOH$: C, 55.37; H, 5.72; N, 19.37. Found: C, 55.02; H, 5.42; N, 19.70.

1,3-Diamino-8-(3',4',5'-trimethoxybenzyl)-9-hydropyrrolo-[3,4-*c*]pyrido[2,3-*d*]pyrimidin-6-(8*H*)-one (**5**).

A mixture of triaminopyrimidine (**19**) 0.75 g, (6.0 mmoles), **18** 2.00 g, (6.0 mmoles) and Dowtherm-A (20 ml) in a three necked flask fitted with a Dean-Stark trap was immersed in an oil bath preheated to 240 °C. The suspension was stirred at this temperature under nitrogen for 2 hours until no increase was observed in the ethanol/water level in the Dean-Stark trap. The reaction mixture was cooled to room temperature. Ether (10 ml) was added to the mixture, which was further cooled to 0 °C and filtered to yield the crude lactam. This was evaporated under reduced pressure to form a uniformly coated silica gel plug which was dried under vacuum and loaded onto a dry silica gel column (2.4 x 20 cm) which was eluted with a 10-50% chloroform:methanol gradient. Fractions containing the product were pooled and evaporated to yield 0.80 g (40%) of **5** as a yellow solid. mp > 300 °C, tlc R_f 0.62 (chloroform/-methanol/ammonium hydroxide 9:4:0.1, silica gel), ^{1}H nmr (DMSO-d_6): δ 3.64 (s, 3H, 4'-OCH_3), 3.75 (s, 6H, 3',5'-OCH_3), 4.38 (s, 2H, CH_2), 4.65 (s, 2H, CH_2), 6.62 (s, 2H, 2',5'-CH), 6.76 (bs, 2H, NH_2), 7.73 (bs, 2H, NH_2), 8.82 (s, 1H, 7-CH).

Anal. Calcd. for $C_{19}H_{20}N_6O_4\bullet0.5H_2O$: C, 56.29; H, 5.22; N, 20.73. Found: C, 56.33; H, 5.30; N, 20.40.

1,3-Diamino-(8*H*)-(3',4',5'-trimethoxybenzyl)-7,9-dihydro-pyrrolo[3,4-*c*]pyrido[2,3-*d*]pyrimidine (**7**).

A 1.0 *M* borane-tetrahydrofuran complex 8.00 ml, (8.00 mmoles) was added to a cold suspension of the dihydrol-actam **3** 0.40 g, (1.00 mmole) in tetrahydrofuran (25 ml). On completion of the addition, a clear brown solution formed that was stirred at room temperature for 16-18 hours. The mixture was then cooled to 0 °C, acidified carefully with 6 *N* hydrochloric acid to pH 2 and stirred at 0 °C for 1 hour and evaporated under reduced pressure. Cold distilled water (25 ml) was added to the residue to afford a thick white suspension, which was neutralized at 0 ° C with 1 *N* sodium hydroxide and stirred at 0 °C for 3 hours. The white suspension was filtered, washed with water and dried under vacuum. Tlc of this showed the presence of the starting lactam **3**, hence, the mixture was retreated with another portion of 1.0 *M* borane-tetrahydrofuran complex 6.40 ml, (6.40 mmole) for 16 hours. The product obtained on acidification, evaporation, addition of water, neutralization and filtration did not show the presence of the lactam (tlc) and was triturated in refluxing methanol (20 ml) for 5 hours. Filtration and evaporation of the filtrate under reduced pressure afforded a residue which was dissolved in glacial acetic acid (5 ml), stirred at room temperature for 18 hours and concentrated under reduced pressure. The concentrate was trituated with anhydrous diethyl ether for 3-4 hours and the precipitate obtained was filtered to yield 0.12 g (31%) of **7** as a light tan solid. mp > 300 °C, tlc R_f 0.4 (chloroform/methanol/ammonium hydroxide 9:4:0.1, silica gel) MS m/z 383 (M^+), ^{1}H nmr (DMSO-d_6): δ 3.55 (s, 2H, CH_2), 3.60 (s, 2H, CH_2), 3.68 (s, 3H, OCH_3), 3.72 (s, 6H, $(OCH_3)_2$), 5.51 (s, 2H, NH_2), 5.68 (s, 2H, NH_2), 6.30 (s, 1H, NH^+), 6.57 (m, 3H, 2',6'-CH, 5-CH).

Anal. Calcd. for $C_{19}H_{21}N_6O_3\bullet0.5CH_3OH\bullet1.0H_2O$: C, 49.60; H, 6.08; N, 17.80. Found: C, 49.92; H, 6.11; N, 17.63.

N-(3',4',5'-Trimethoxybenzyl)-2,5-dimethylpyrrole (**10**).

Acetonyl acetone (**8**) (1.97 ml, 10.0 mmole) and 3,4,5-trimethoxybenzylamine (**9**) 1.97 g, (10.0 mmole) were dissolved in 5 ml of toluene and heated under reflux in a flask fitted with a Dean-Stark trap until separation of water ceased (4 hours). The reaction was cooled to room temperature and allowed to stand overnight. The sandy precipitate obtained was filtered and washed with methanol to yield 1.92 g (70%) of **10** as a tan solid: mp 104-106 °C; tlc R_f 0.72 (ethyl acetate, silica gel); ^{1}H nmr (60 MHz, DMSO-d_6): δ 2.10 (s, 6H, 2,5-CH_3), 3.65 (s, 9H, OCH_3), 5.00 (s, 2H, N-CH_2), 5.7 (s, 2H, 3,4-CH), 6.2 (s, 2H, 2',6'-CH).

N-(3',4',5'-Trimethoxybenzyl)-2,5-dimethyl-3-formylpyrrole (**11**).

To dimethylformamide (1.85 ml) in a three neck flask under nitrogen at 0 °C was added phosphorus oxychloride ($POCl_3$) (0.56 ml) dropwise with stirring over a period of 15 minutes. To this mixture was added a solution of **10** 1.0 g, (3.60 mmoles) in dimethylformamide (7 ml) over a period of 30 minutes at 0 °C. The reaction mixture was then heated at 90 °C for 2 hours, cooled to room temperature and poured into ice. The mixture was then made basic to pH 10 with 30% sodium hydroxide. The precipitate formed was filtered and air dried to yield 0.96 g (91%) of **11** as a tan solid. mp 110 °C; tlc R_f 0.59 (ethyl acetate , silica gel); ^{1}H nmr (60 MHz, DMSO-d_6): δ 2.10 (s, 3H, 5-CH_3), 2.45 (s, 3H, 2-CH_3), 3.60 (d, 9H, OCH_3), 5.10 (s, 2H, N-CH_2), 6.25 (s, 3H, 4-CH, 2',6'-CH), 9.75 (s, 1H, CHO).

3-[2-(2,4-Diaminopyrimidin-6-yl)hydrazo]methenyl-1-(3',4',5'-trimethoxybenzyl)-2,5-dimethylpyrrole (**13**).

A mixture of **11** 0.29 g, (1.25 mmoles) and **12** 0.195 g, (1.25 mmoles) in ethanol (10 ml) containing glacial acetic acid (0.35 ml) was heated at 90 °C for 48 hours. The reaction mixture was cooled to room temperature and poured into crushed ice. The pH of the mixture was adjusted to 10 with 60% sodium hydroxide. The solid obtained was filtered to yield 0.22 g (54%) of **13** as a tan solid; mp 170-180 °C; tlc R_f 0.45 (chloroform/-methanol/glacial acetic acid 9:4:0.1, silica gel); MS *m/z* 426 (M^+); ^{1}H nmr (DMSO-d_6): δ 2.11 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 3.61 (s, 3H, 4"-OCH_3), 3.66 (s, 6H, 3", 5"-OCH_3), 5.01 (s, 2H, N-CH_2), 5.56 (s, 2H, NH_2), 5.74 (s, 2H, NH_2), 6.10 (s, 2H, 2'-CH, 5-CH), 6.21 (s, 2H, 2",6"-CH), 7.96 (s, 1H, N=CH), 9.87 (s, 1H, NH).

Ethyl *N*-(3,4,5-trimethoxybenzyl)-β-amino Propionate (**16**).

A mixture of 3,4,5-trimethoxybenzylamine (**9**) 39.4 g, (200 mmoles) and ethyl acrylate (**15**) 21.6 ml, (199.6 mmoles) in tetrahydrofuran (THF) (50 ml) was stirred at room temperature for 2 days. The mixture was concentrated *in vacuo* and the residue was flash distilled to obtain 35.0 g (59%) of **16** as a viscous colorless liquid; bp 145-150 °C at 0.05 mm mercury, tlc R_f 0.32 (ethyl acetate/1 drop acetic acid, silica gel). The compound was used without further purification in the next step.

N-[β-(Ethoxycarbonyl)ethyl]-*N*-(3,4,5-trimethoxybenzyl)-glycinate (**17**).

Ethylbromoacetate 6.75, (40.44 mmole) was added dropwise over a period of 2 hours at room temperature to a mixture of **16** 10.0 g, (33.7 mmoles) and anhydrous potassium carbonate (K_2CO_3) 6.28 g, (45.5 mmoles) in 25 ml of dimethylformamide. The mixture was stirred at 60 °C for 5 hours after which it was cooled to room temperature and poured in a thin stream into 20 ml of ice-cold 0.1 *N* sodium hydroxide solution. This was then stirred at room temperature for 4-5 hours (until the characteristic odor of ethyl-bromoacetate dissipated). The aqueous solution was extracted with ether (4 x 25 ml) and the combined extracts were washed with water (4 x 20 ml), dried (magnesium sulfate), filtered and the ether evaporated under reduced pressure and the resulting oily product was purified by flash distillation which afforded 11.6 g (90%) of **17** as a pale yellow oil; bp 185-195 °C/0.05 mm mercury; tlc R_f 0.61 (ethyl acetate/1 drop acetic acid, silica gel); ^{1}H nmr ($CDCl_3$): δ 1.26 (t, 6H, CH_3), 2.52 (t, 2H, N-C$H_2$$CH_2$), 3.07 (t, 2H, CH_2CH_2COO), 3.77 (s, 2H, NCH_2CO), 3,85 (m, 9H, OCH_3), 4.15 (m, 6H, $(OCH_2)_2$, N-CH_2), 6.58 (s, 2H, 2',6'-CH).

4-(Ethoxycarbonyl)-*N*-(3',4',5'-trimethoxybenzyl)pyrrolidin-3-one (**18**).

Compound **17** 40.0 g, (107.82 mmoles) was added to an ice-cold slurry of sodium ethoxide 11.32 g, (155.07 mmoles) in freshly distilled toluene (80 ml) containing 4 Å molecular sieves. The mixture was stirred at room temperature for 5 hours after which it was extracted with ice-cold water (4 x 50 ml). The aqueous layer was washed with ether (4 x 20 ml) and then neutralized to pH 7 with concentrated hydrochloric acid. The neutral solution was extracted with ether (4 x 25 ml). The organic layer was dried (magnesium sulfate) and the ether evaporated to yield a viscous orange-yellow oil. A solid separated from the oil on refrigeration which upon filtration afforded 21.7 g (61.5%) of the ketoester **18** as an off-white solid: mp 115-120 °C; tlc R_f 0.38 (ethyl acetate/1 drop acetic acid, silica gel).

Anal. Calcd. for $C_{17}H_{22}O_6N\bullet0.2H_2O$: C, 60.06; H, 6.69; N, 4.12. Found: C, 59.97; H, 6.92; N, 4.17.

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