Synthesis of a Series of Diaminobenzo[*f*]- and Diaminobenzo[*h*]pyrimido[4,5-*b*]quinolines as 5-Deaza Tetracyclic Nonclassical Antifolates

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A series of diaminobenzo[*f*]- and diaminobenzo[*h*]pyrimido[4,5-*b*]quinolines **1-11** were designed as 5-deaza tetracyclic nonclassical, lipophilic antifolates. The compounds were designed as conformationally semi-rigid and rigid analogs of 2,4-diamino-6-phenyl- **12** and 2,4-diamino-7-phenylpyrido[2,3-*d*]pyrimidines **13** and **14**. The target compounds were synthesized by cyclocondensation of chlorovinyl aldehydes obtained from appropriately substituted 1- or 2-tetralone, with 2,4,6-triaminopyrimidine. Compounds **1-11** were evaluated as inhibitors of *P. carinii* and *T. gondii* dihydrofolate reductases. These pathogens cause fatal opportunistic infections in AIDS patients. In addition, the selectivity of these agents was evaluated using rat liver dihydrofolate reductase as the mammalian source. In general the benzo[*f*]pyrimido[4,5-*b*]quinolines **1-5** were more potent than the corresponding benzo[*h*]pyrimido[4,5-*b*]quinoline analogues **6-11** against *P. carinii* and rat liver dihydrofolate reductase and were equipotent against *T. gondii* dihydrofolate reductase. Compounds **6-11** were moderately selective towards *T. gondii* dihydrofolate reductase with IC_{50}s in the 10^{-7} *M* range. In contrast analogues **1-5** lacked selectivity against *P. carinii* or *T. gondii* dihydrofolate reductase and were, in general, potent inhibitors of rat liver dihydrofolate reductase with IC_{50}s in the 10^{-8} *M* range. Analogues **1** and **4** were evaluated against a series of tumor cell lines *in vitro* and were found to have moderate antitumor activity (IC_{50} 10^{-6} *M*). The structure activity/selectivity relationships suggest that benzo[*f*]pyrimido analogues **1-5** with the phenyl ring substitution in the "upper" portion of the tetracyclic ring are better accommodated within the rat liver (mammalian) dihydrofolate reductase and *P. carinii* dihydrofolate reductase active sites compared to the benzo[*h*]pyrimido analogues **6-11** which have the phenyl ring substitution in the "lower" portion of the tetracyclic ring. In contrast *T. gondii* dihydrofolate reductase does not discriminate between the isomers and binds to both series of compounds with similar affinities.

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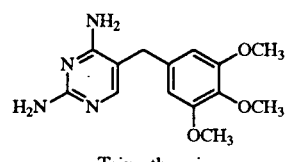
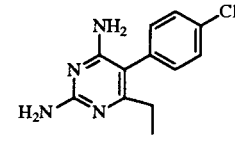
The classical antifolate methotrexate has served as a lead compound for structural modification since its introduction as an antitumor agent. Numerous analogues of methotrexate have been synthesized over a number of years with the aim of reducing the toxicity and improving the therapeutic activity against solid tumors [1]. A disadvantage of classical antifolates is that they require an active transport mechanism to enter cells, which can lead to resistance. In addition cells which lack these transport mechanisms, such as bacterial and protozoan cells, are not susceptible to the action of classical antifolates. In an attempt to overcome these problems nonclassical, lipophilic antifolates were developed which do not require the folate transport system(s) and enter cells *via* diffusion [2]. Prominent examples of lipophilic antifolates include the 2,4-diaminopyrimidines, pyrimethamine and trimethoprim, which have found clinical utility as antimalarial and antibacterial agents respectively [3]. The bicyclic 2,4-diaminoquinazoline, trimetrexate [4] and the 2,4-diaminopyrido[2,3-*d*]pyrimidine, piritrexim [5] have also been developed as anticancer drugs.

Recently, several lipophilic dihydrofolate reductase inhibitors have been used in the treatment of opportunistic infections caused by *Pneumocystis carinii* and *Toxoplasma gondii* in patients with AIDS [6,7]. Both *Pneumocystis carinii* pneumonia [8] and toxoplasmosis of the central nervous system [9] remain the principal cause of morbidity and mortality in patients with AIDS. Although lipophilic antifolates such as trimethoprim, [10] pyrimethamine, [11] trimetrexate [12,13] and piritrexim [14] are currently used for the treatment of *Pneumocystis carinii* pneumonia and toxoplasmosis or are in clinical trials, they suffer from several drawbacks. Both trimethoprim and pyrimethamine are selective but weak inhibitors of dihydrofolate reductase derived from *P. carinii* and *T. gondii* and consequently they must be used with sulfonamides to provide synergistic effects [15]. Trimetrexate and piritrexim are extremely potent inhibitors of *P. carinii* and *T. gondii* dihydrofolate reductase however they lack selectivity and have a greater affinity for the mammalian enzyme [14,16]. Thus both trimetrexate and piritrexim must be used in combination with leucovorin (5-formyl-5,6,7,8-tetrahydrofolate) to selectively protect the host tissue [14,17]. In addition to these drawbacks which include the high cost of leucovorin and lack of selectivity of the agents, the severe side effects frequently requires cessation of treatment with these antifolate regimens [6,18,19]. Thus, considerable effort has been exerted to develop potent lipophilic dihydrofolate reductase inhibitors which have greater affinity for *P. carinii* and *T. gondii* dihydrofolate reductase compared to mammalian dihydrofolate reductase. Such selective agents would obviate the necessity of leucovorin and sulfonamides and would afford safer and less expensive therapy particularly in light of the life-long requirement of antiopportunistic infection agents for AIDS patients.

We [20,21] and others [22-24] have reported several bicyclic lipophilic antifolates, incorporating the 2,4-diaminopyrimidine ring system, as potent and/or selective inhibitors of dihydrofolate reductases isolated from *P. carinii* and *T. gondii*. Rosowsky and coworkers have reported the dihydrofolate reductase inhibitory activity of several tricyclic lipophilic antifolates such as the diaminopyrimido[4,5-*c*]isoquinolines [25]. As part of our continuing interest in nonclassical 5-deazafolates and in order to further develop the structure activity relationship of these 5-deaza derivatives, we have synthesized a series of 9,11-diamino-5,6-dihydrobenzo[*f*]pyrimido[4,5-*b*]-quinolines **1-3** and 8,10-diamino-5,6-dihydrobenzo[*h*]-pyrimido[4,5-*b*]quinolines **6-9** as 5-deaza tetracyclic lipophilic antifolates [26,27]. These compounds were designed as conformationally restricted semi-rigid 2,4-diaminopyrido[2,3-*d*]pyrimidine derivatives. The corresponding conformationally flexible analogues 2,4-diamino-6-phenylpyrido[2,3-*d*]pyrimidine (**12**), 2,4-diamino-7-phenylpyrido[2,3-*d*]pyrimidine (**13**) and 2,4-diamino-6-ethyl-7-phenylpyrido[2,3-*d*]pyrimidine (**14**) were reported several years ago by Hurlbert *et al.* as antibacterial agents [28]. In addition the aromatic 9,11-diaminobenzo[*f*]pyrimido[4,5-*b*]quinolines **4** and **5** and, the 8,10-diaminobenzo[*h*]pyrimido[4,5-*b*]quinolines **10** and **11** were also synthesized as rigid, 5-deaza lipophilic antifolates. The methoxy substituent present in several of these analogues was chosen on the basis of previous reports from our laboratory [20,21] which indicated that in several bicyclic ring fused lipophilic antifolates this substituent provided for potency and/or selectivity against *T. gondii* and/or *P. carinii* dihydrofolate reductase. It was anticipated that these rigid analogues would define further the relationship of the side chain phenyl ring orientation and the potency and/or selectivity towards *P. carinii* and *T. gondii* dihydrofolate reductase.


Trimethoprim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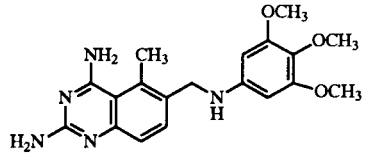
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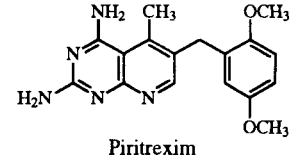
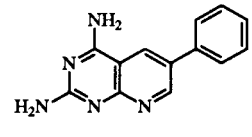

Piritrexim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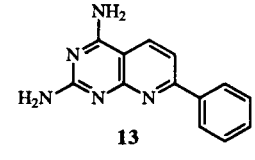
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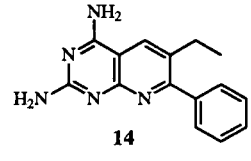
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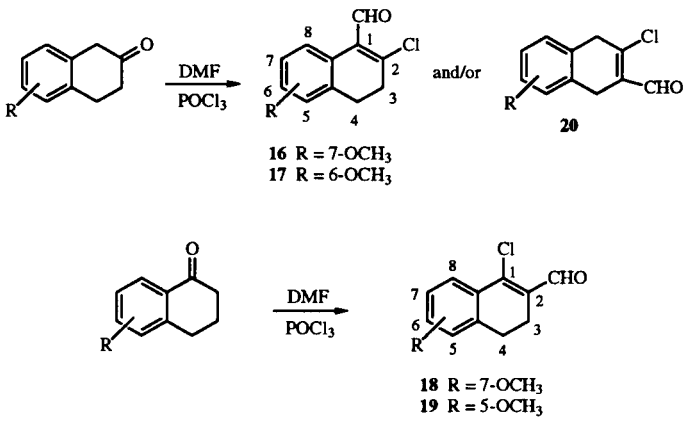
Compounds **2**, **3**, **7** and **9** were synthesized following procedures similar to that reported [26,27] previously for analogues **1**, **6** and **8** utilizing a cyclocondensation reaction of a chlorovinylaldehyde derived from a suitably substituted tetralone and 2,4,6-triaminopyrimidine (**15**). The requisite chlorovinylaldehydes **16-19** were obtained in good yields (Scheme 1) from the appropriately substituted 2-tetralone or 1-tetralone by Vismeier chloroformylation with *N,N*-dimethylformamide and phosphorus oxychloride. Chloroformylation of 2-tetralone could afford either or both possible regioisomeric products, the desired

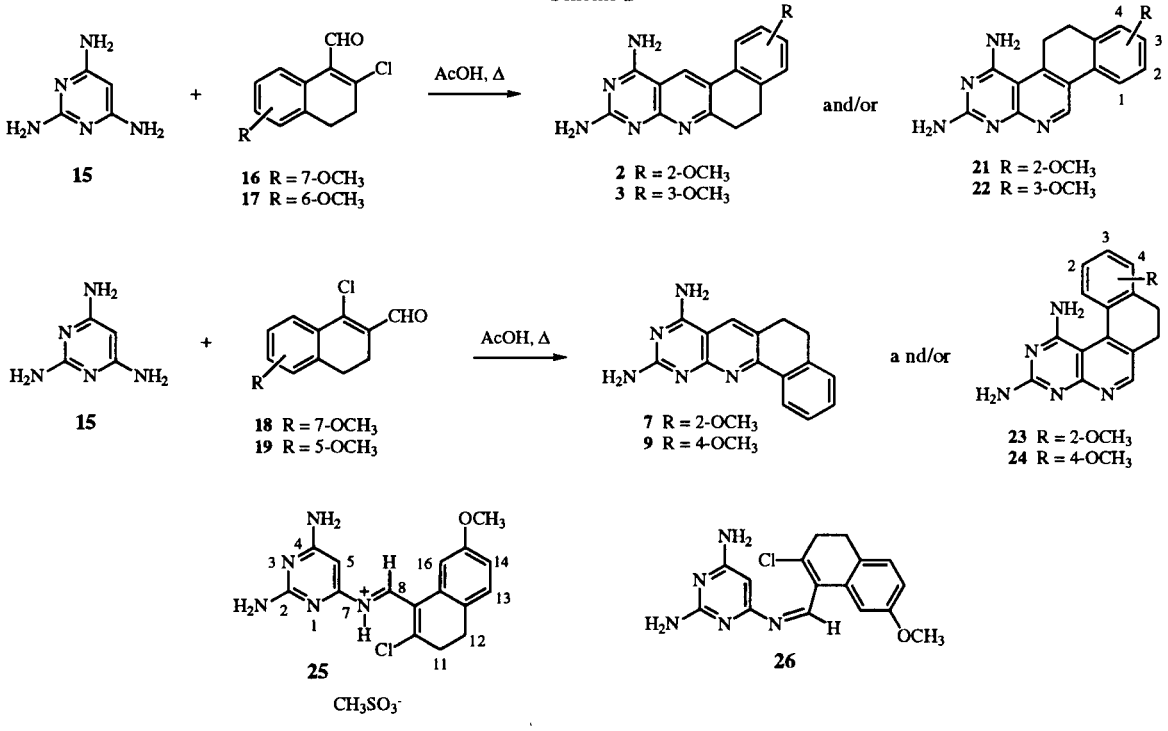
Scheme 1



1-formyl-2-chloro products **16** and **17** or the undesired 3-formyl-2-chloro isomer **20**. The ^{1}H nmr data, however, established that for 2-tetralone the desired isomers **16** and **17** were obtained exclusively. Due to their instability these chlorovinyl aldehydes were not characterized further but used directly in the cyclocondensations.

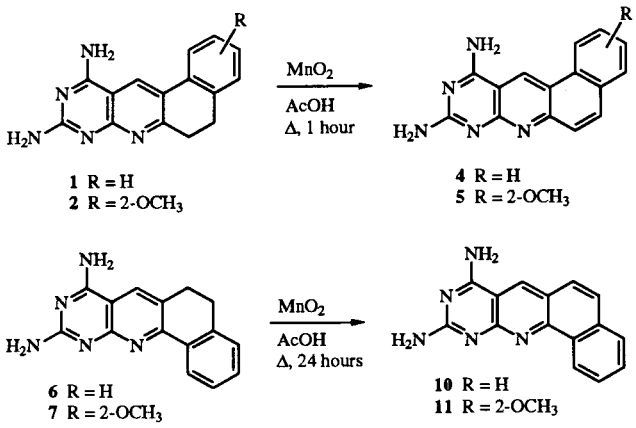
Gangjee *et al.* [29] and Taylor and Warner [30] have demonstrated that the direction of ring closure of cyclic chlorovinylaldehydes with substituted 6-aminopyrimidines is dependent on the nature of the biselectrophile, the pyrimidine and the solvent. Thus, condensation of the chlorovinyl aldehydes **16** and **17** with pyrimidine **15** (Scheme 2) could afford the linear isomers **2** and **3** and/or the angular isomers **21** and **22**. Similarly the reaction of **15** with chlorovinyl aldehydes **18** and **19** could afford the linear isomers **7** and **9** and/or the angular isomers **23** and **24**. We found that the condensation of **16** and **17** with **15** in acetic acid at reflux was regioselective. Reaction of chlorovinyl aldehyde **16** with **15** afforded the linear isomer **2** and a compound that was assigned the structure **25** based on its ^{1}H nmr. The formation of **25** could result from reaction of the 6-amino group of **15** with the aldehyde of **16**. The resulting imine may exist in the *E* -**25** or the *Z* -**26** isomeric forms across the C8-N7 double bond. The *Z* isomer could undergo ring closure *via* reaction of the 5-position of the pyrimidine with the electrophilic C-10 carbon to afford the angular isomer **21**. However, no angular isomer was isolated. Thus the *E* isomer **25** must be the predominant isomer under the reaction conditions, which forms the linear isomer **2** and precludes the formation of the angular isomer **21**. Similarly, reaction of **15** with **17** afforded the linear isomer **3** and another compound which was not isolated in a pure form. The ^{1}H nmr of the crude solid however indicated that it was not angular isomer **22**. Cyclocondensation of chlorovinyl aldehydes **18** and **19** with **15** in acetic acid at reflux were regiospecific and afforded the linear isomers **7** and **9** respectively. No angular isomers **23** and **24** were obtained nor was any other intermediate isolated from the reaction mixture. The assignment of linear structures for compounds **2**, **3**, **7** and **9** was based on the characteristic ^{1}H nmr and ^{13}C nmr data which has been previously established by us [26,27,31] and others [32-35] for similar systems. Additionally, X-ray crystal studies and two-dimensional H,C-correlated nmr spectroscopic studies of the

Scheme 2

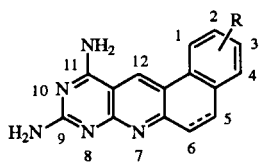


products of similar cyclocondensation reactions indicate that linear rather than angular products are formed [36]. Oxidation (Scheme 3) of selected componds **1**, **2**, **6** and **7**

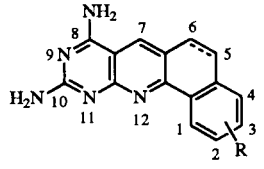
Scheme 3



with activated manganese dioxide in hot acetic acid afforded the completely aromatic analogues **4**, **5**, **10** and **11** in moderate to good yields. It was interesting to note that oxidation of the 9,11-diamino-5,6-dihydrobenzo-[*f*]pyrimido[4,5-*b*]quinolines **1** and **2** was extremely rapid compared to the oxidation of the corresponding benzo[*h*] analogues **6** and **7** under identical reaction conditions.



Compound	R	5,6-position
1	H	CH_2-CH_2
2	2-OCH_3	CH_2-CH_2
3	3-OCH_3	CH_2-CH_2
4	H	CH=CH
5	2-OCH_3	CH=CH



Compound	R	5,6-position
6	H	CH_2-CH_2
7	2-OCH_3	CH_2-CH_2
8	3-OCH_3	CH_2-CH_2
9	4-OCH_3	CH_2-CH_2
10	H	CH=CH
11	2-OCH_3	CH=CH

Compounds **1-11** were evaluated as inhibitors (IC_{50}) of dihydrofolate reductases from *P. carinii*, *T. gondii* and rat liver, and the results are listed in Table 1 [16] along with rat liver dihydrofolate reductase which was used as the mammalian source to determine selectivity ratios (rat liver/*P. carinii*, rat liver/*T. gondii*) and are also listed in Table 1. The results revealed that the benzo[*f*]pyrimido[4,5-*b*]quinolines **1-5** with the phenyl ring substitution in the "upper" portion of the tetracyclic molecule are in general more potent than the benzo[*h*]pyrimido[4,5-*b*]-quinolines **6-11** with the phenyl ring in the "lower" region

Table 1

Inhibitory Concentrations (IC_{50}, μM) against Dihydrofolate Reductase and Selectivity Ratios [a]

Compound	*P. carinii*	rat liver	rl/pc [a]	*T. gondii*	rl/tg [a]
1	0.65	0.032	0.05	0.16	0.2
2	0.22	0.023	0.10	0.091	0.25
3	7.7	1.6	0.21	4.7	0.34
4	0.19	0.055	0.29	0.30	0.18
5	0.70	0.092	0.13	0.34	0.27
6	15.8	0.64	0.04	0.18	3.56
7	38.4	3.2	0.08	0.57	5.6
8	6.7	0.24	0.04	0.11	2.18
9	8.6	0.60	0.07	0.15	4.0
10	>0.4	2.4	<6.0	0.46	5.22
11	>1.85	>18.5	-	1.85	>10.0

[a] Selectivity ratios, rl/pc = IC_{50} rat liver dihydrofolate reductase/IC_{50} *P. carinii* dihydrofolate reductase; rl/tg = IC_{50} rat liver dihydrofolate reductase/IC_{50} *T. gondii* dihydrofolate reductase.

against both, *P. carinii* and rat liver dihydrofolate reductase. Generally, both regioisomers (*i.e.* **1-5** and **6-11**) were equipotent as inhibitors of *T. gondii* dihydrofolate reductase. However the benzo[*h*]pyrimido[4,5-*b*]quinolines **6-11** were more selective for *T.gondii* dihydrofolate reductase than their benzo[*f*] regioisomers **1-5**.

Within the series with the phenyl ring "up", (*i.e.* benzo[*f*] series) the addition of a methoxy group at the 2-position (**2**) caused a slight increase in inhibitory potency against all three enzymes, however, the analogue with the methoxy group in the 3-position (**3**) was greater than an order of magnitude less potent against the three enzymes. Compound **2** was also the most potent rat liver and *T. gondii* dihydrofolate reductase inhibitor in this series with an IC_{50} of 0.023 μM and 0.091 μM respectively. Aromatization of **1** to the conformationally rigid analogue **4**, caused a slight drop in potency against both rat liver dihydrofolate reductase and *T. gondii* dihydrofolate reductase, however there was an increase in the activity against *P. carinii* dihydrofolate reductase and consequently **4** was about six times more selective than **1** against *P. carinii* dihydrofolate reductase. Analogue **4** was the most potent *P. carinii* dihydrofolate reductase inhibitor in this series with an IC_{50} of 0.19 μM. This trend was however not observed in the 2-methoxy substituted aromatic analogue **5** which was three to four fold less active than its dihydro derivative **2** against all three enzymes.

The unsubstituted dihydrobenzo[*h*]pyrimido[4,5-*b*]-quinoline **6** had an IC_{50} of 0.18 μM against *T. gondii* dihydrofolate reductase and had a selectivity ratio of 3.56 for *T. gondii* dihydrofolate reductase. Introduction of the methoxy group in the phenyl ring of **6** afforded varied results. Thus substitution with a methoxy group at the 2-position (**7**) produced a decrease in potency against all three dihydrofolate reductases, substitution at the 3-position (**8**) and the 4-position (**9**) produced analogues with

greater potency against all three dihydrofolate reductases. This trend in potency was reversed for selectivity *i.e.*, compound **7** with the lowest potency in this series (**6-9**) showed the highest selectivity towards *P. carinii* and *T. gondii* dihydrofolate reductase. Aromatization of compounds **6** and **7** to the conformationally rigid analogues **10** and **11** caused a decrease in inhibitory activity against both rat liver and *T. gondii* dihydrofolate reductase. However the decrease in potency against rat liver dihydrofolate reductase was greater than that for *T. gondii* dihydrofolate reductase thus both **10** and **11** were more selective than their corresponding dihydro analogues **6** and **7** against *T. gondii* dihydrofolate reductase. The dihydrofolate reductase inhibitory results indicated that analogues **6**, **7**, **10** and **11** with the phenyl ring "down" (*i.e.* benzo[*h*] series) were at least 17-33 times more selective towards *T. gondii* dihydrofolate reductase than the corresponding analogues **1**, **2**, **4** and **5** with the phenyl ring "up" (*i.e.* benzo[*f*] series).

Table 2

Growth Inhibitory Concentrations, IC_{50} in μM, of **1** and **4** against Tumor Cells in Culture [a]

Cell Line	1	4
CCRF-CEM Leukemia	1.7	1.8
SK-5 Melanoma	16.2	6.3
ACHN Renal Cancer	32.4	6.3
HCT-116 Colon Cancer	25.0	3.88
MCF-7 Breast Cancer	32.4	8.6
PC-3 Prostrate Cancer	8.4	4.6
KB Epidermoid Cancer	9.5	6.7

[a] IC_{50} = concentration of drug required to decrease cell viability as measured by MTA (MTT assay) by 50% after 3 days of treatment.

The activity of the benzo[*f*]pyrimido[4,5-*b*]quinolines **1** and its aromatic analogue **4** against rat liver dihydrofolate reductase prompted us to evaluate them against a series of tumor cells *in vitro* [37-39] and their IC_{50} values are listed in Table 2. The analogues displayed moderate antitumor activity with IC_{50}s in the range of 10^{-5}-10^{-6} *M* against various tumor cell lines. The aromatic analogue **4** was more active than the dihydro analogue **1** against various tumor cell lines including breast cancer, colon cancer, renal cancer, melanoma, leukemia, and prostrate cancer. This is probably the result of better cellular penetration of **4** compared to **1**. Both **1** and **4** were also active against the human epidermoid solid tumor cell line (KB) with IC_{50} values of 6.7 and 9.5 μM respectively.

In summary, a series of tetracyclic 5-deaza nonclassical antifolates were synthesized as potential inhibitors of *P. carinii* dihydrofolate reductase, *T. gondii* dihydrofolate reductase and rat liver dihydrofolate reductase. In contrast to the benzo[*f*] analogues **1-5** which did not display any selectivity for *T. gondii* dihydrofolate reductase, the benzo[*h*] analogues **6-11** showed reasonable selectivity for *T. gondii* dihydrofolate reductase with IC_{50} values in the 10^{-7} *M* range. This indicated that in contrast to *P. carinii* dihydrofolate reductase and rat liver dihydrofolate reductase, *T. gondii* dihydrofolate reductase tolerates significant steric bulk appended at the 7-position of the 2,4-pyrido[2,3-*d*]pyrimidine ring. In general compounds **1-5** with the phenyl ring substituted at the 6-position of the 2,4-diaminopyrido[2,3-*d*]pyrimidine ring afforded analogues with potent inhibitory activity against mammalian rat liver dihydrofolate reductase. Within this series substitution with a 3-methoxy group (**3**) was not tolerated by any of the three dihydrofolate reductases evaluated. Complete aromatization yielded analogues that were less potent than their dihydro counterparts, but were more selective against both *P. carinii* dihydrofolate reductase (**4** *versus* **1**) and *T. gondii* dihydrofolate reductase (**6**, **7** *versus* **10**, **11**). These results suggest that the combination of potency and selectivity was difficult to acheive against *P. carinii* dihydrofolate reductase within this series of tetracyclic 5-deaza nonclassical antifolates. However the potency and moderate selectivity of the semi-rigid and rigid benzo[*h*] analogues **6-11** towards *T. gondii* dihydrofolate reductase indicate the

APPENDIX

Compound	Molecular Formula	Found					Calculated				
		C	H	N	S	Cl	C	H	N	S	Cl
1	$C_{15}H_{13}N_5 \cdot 1.0HCl \cdot 0.5H_2O$	58.36	4.81	22.47		11.47	58.35	4.90	22.68		11.48
2	$C_{16}H_{15}N_5O \cdot 1.0CH_3SO_3H$	52.47	4.89	17.97	8.26		52.44	4.88	18.00	8.23	
3	$C_{16}H_{15}N_2O \cdot 0.95CH_3SO_3H$	52.96	4.87	18.24	8.08		52.93	4.93	18.21	7.92	
4	$C_{15}H_{11}N_5 \cdot 1.0HCl \cdot 0.5CH_3COOH$	58.36	4.41	21.71		11.02	58.63	4.31	21.37		10.82
5	$C_{16}H_{13}N_5O \cdot 0.8CH_3SO_3H$	54.64	4.39	19.05	7.02		54.80	4.43	19.02	6.97	
6	$C_{15}H_{13}N_2 \cdot 1.0HCl.0 \cdot 82H_2O$	57.66	4.88	21.87		10.94	57.28	4.98	22.27		11.30
7	$C_{16}H_{15}N_2O \cdot 0.9CH_3SO_3H$	53.32	5.00	17.30*	7.80		52.93	4.93	18.21	7.92	
8	$C_{16}H_{15}N_5O \cdot 1.0HCl \cdot 1.2H_2O$	54.29	5.36	19.66		10.01	54.69	5.28	19.93		10.09
9	$C_{16}H_{15}N_5O \cdot 0.5CH_3SO_3H \cdot 1.0H_2O$	55.55	5.09	18.48*	4.34		55.14	5.33	19.49	4.46	
10	$C_{15}H_{11}N_5 \cdot 0.9HCl \cdot 0.2CH_3COOH$	60.53	4.11	22.81		10.55	60.43	4.18	22.88		10.42
11	$C_{16}H_{13}N_5O \cdot 0.75CH_3SO_3H \cdot 0.5CH_3COOH$	54.58	4.51	17.79	6.30		54.19	4.61	17.80	6.11	

*The nitrogen values are not within ±0.4% of the calculated values.

subtle differences that exist between *T. gondii* dihydrofolate reductase and mammalian dihydrofolate reductase, which can be further exploited to afford tetracyclic analogues with high potency and selectivity.

EXPERIMENTAL

Melting points were determined on a Mel-Temp or Fisher-Johns melting point apparatus and are uncorrected. Infrared spectra (ir) were recorded with a Perkin Elmer 1430 or 1320 Infrared Spectrophotometer in nujol mulls and are reported in reciprocal centimeters (cm^{-1}). The ^1H nmr spectra were recorded on a Varian EM 390 (300 MHz) or a Bruker WH-300 (300 MHz) spectrometers. The ^{13}C nmr spectra were recorded on a Varian EM 390 instrument. The chemical shift (δ) values are expressed in parts per million (ppm) relative to tetramethylsilane as an internal standard: s = singlet, d = doublet, t = triplet, m = multiplet; Ar-CH = aromatic proton. Thin layer chromatography (tlc) was performed on cellulose or silica gel plates with fluorescent indicator and were visualized with uv light at 254 and 350 nm. Flash chromatography was performed on 230-400 mesh silica gel purchased from Aldrich, Milwaukee, Wisconsin. Samples for microanalysis were dried *in vacuo* over phosphorus pentoxide with heating over refluxing ethanol or toluene. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA. Fractional moles of solvents in the analytical samples frequently found in such antifolates could not be prevented in spite of vigorous drying *in vacuo* and were confirmed, where possible, by their presence in the nmr spectrum.

General Procedure for the Synthesis of Chlorovinyl Aldehydes **16-19**.

The detailed procedure for the synthesis of 2-chloro-7-methoxy-3,4-dihydronaphthalene-1-carboxaldehyde (**16**) is described below. Synthesis of chlorovinylaldehydes **17-19** followed similar procedures using the appropriate starting ketones.

2-Chloro-7-methoxy-3,4-dihydronaphthalene-1-carboxaldehyde (**16**).

In a three-necked flask fitted with a drying tube, a thermometer and an argon inlet tube, was placed 4.18 g (57 mmoles) of dimethylformamide and 20 ml of methylene chloride. After cooling in an ice bath to 0-5°, 7 g (46 mmoles) of phosphorus oxychloride was added dropwise. Addition was regulated to maintain the temperature of the mixture below 20°. After the addition of phosphorus oxychloride was complete (about 40 minutes), the reaction was continued at 27° for 2 hours. Following this period, the mixture was cooled again in an ice-bath and a solution of 5 g (28 mmoles) of 7-methoxy-2-tetralone in 30 ml of methylene chloride was added dropwise to the mixture while maintaining the temperature of the reaction below 20° with external cooling (ice-bath). After addition was completed (45 minutes), the reaction was continued at 27° for 8 hours and then poured over crushed ice and solid sodium bicarbonate was added until evolution of carbon dioxide ceased. The mixture was then stirred vigorously for 15 minutes and the methylene chloride layer was separated. The aqueous portion was extracted twice with 80 ml of methylene chloride. The combined methylene chloride extracts were washed twice with 50 ml of water, dried with magnesium sulfate and evaporated under reduced pressure (water aspirator) to afford 12.6 g (98%) of **16**; ir (neat): 1675 cm^{-1} (CHO); ^1H nmr (deuteriochloroform): δ 2.56 (m, 4 H, CH_2CH_2), 3.76 (s, 3 H, OCH_3), 6.72 (d, 1 H, Ar-CH), 7.80 (d, 1 H, Ar-CH), 7.88 (dd, 1 H, Ar-CH), 10.38 (s, 1 H, CHO).

2-Chloro-6-methoxy-3,4-dihydronaphthalene-1-carboxaldehyde (**17**).

Chloroformylation of 6-methoxy-2-tetralone as described for the synthesis of **16** afforded the desired compound **17** in 87% yield; ir (neat): 1672 cm^{-1} (CHO); ^1H nmr (deuteriochloroform): δ 2.60 (m, 4 H, CH_2CH_2), 3.74 (s, 3 H, OCH_3), 7.20 (d, 1 H, Ar-CH), 7.67 (d, 1 H, Ar-CH), 7.86 (dd, 1 H, Ar-CH), 10.40 (s, 1 H, CHO).

1-Chloro-7-methoxy-3,4-dihydronaphthalene-2-carboxaldehyde (**18**).

Chloroformylation of 7-methoxy-1-tetralone as described for the synthesis of **16** afforded crude **18**. The crude product was purified by flash chromatography on silica gel using hexanes/acetone (2:1) as the eluant. The eluant was evaporated under reduced pressure to afford **18** in 92% yield; ir (neat): 1672 cm^{-1} (CHO); ^1H nmr (deuteriochloroform): δ 2.57 (m, 4 H, CH_2CH_2), 3.74 (s, 3 H, OCH_3), 6.73 (d, 1 H, Ar-CH), 7.66 (d, 1 H, Ar-CH), 7.85 (dd, 1 H, Ar-CH), 10.28 (s, 1 H, CHO).

1-Chloro-5-methoxy-3,4-dihydronaphthalene-2-carboxaldehyde (**19**).

Chloroformylation of 5-methoxy-1-tetralone as described for the synthesis of **16** afforded compound **19** as a pale yellow solid in 73% yield; mp 73°; ir (nujol): 1670 cm^{-1} (CHO); ^1H nmr (deuteriochloroform): δ 2.60 (m, 4 H, CH_2CH_2), 3.76 (s, 3 H, OCH_3), 6.82 (d, 1 H, Ar-CH), 7.62 (dd, 1 H, Ar-CH), 7.85 (d, 1 H, Ar-CH), 10.26 (s, 1 H, CHO).

9,11-Diamino-5,6-dihydrobenzo[*f*]pyrimido[4,5-*b*]quinoline (**1**).

Compound **1** was synthesized as previously reported [26], mp >300°; ir (nujol): 3140 cm^{-1} (NH_2); ^1H nmr (TFA-*d*): δ 3.18 (t, 2 H, 5-CH_2), 3.72 (t, 2 H, 6-CH_2), 7.54 (m, 3 H, Ar-CH), 7.82 (br s, 1 H, Ar-CH), 9.22 (s, 1 H, 12-CH); ^{13}C nmr (TFA-*d*): 141.2 ppm (12-CH, $^1J_{CH}$ = 190 Hz).

Anal. Calcd. for $C_{15}H_{13}N_5 \cdot HCl \cdot 0.5H_2O$: C, 58.35; H, 4.90; N, 22.68; Cl, 11.48. Found: C, 58.36; H, 4.81; N, 22.47; Cl, 11.47.

9,11-Diamino-2-methoxy-5,6-dihydrobenzo[*f*]pyrimido-[4,5-*b*]quinoline (**2**).

To a solution of 3.49 g (28 mmoles) of 2,4,6-triaminopyrimidine (**15**) in acetic acid at reflux was added, dropwise, a solution of 6.2 g (28 mmoles) of **16** in 20 ml of acetic acid and the reflux continued for 19 hours after addition was completed. The mixture was allowed to cool to room temperature and the precipitated yellow solid was filtered, suspended in 10% ammonium hydroxide solution and stirred for 30 minutes. The solid was isolated by filtration and washed with water until the washings were neutral to litmus to afford **2** as the free base. Compound **2** was air dried, suspended in 50 ml absolute ethanol and the *p*H adjusted to **4** by dropwise addition of methanesulfonic acid. The acidic suspension was stirred for 10 minutes and the solid was filtered to afford **2** as the mesylate salt in 42% yield, mp 292°; ir (nujol): 3336 cm^{-1} (NH_2); ^1H nmr (TFA-*d*): δ 3.04 (t, 2 H, 5-CH_2), 3.65 (t, 2 H, 6-CH_2), 4.04 (s, 3 H, OCH_3), 7.18 (dd, 1 H, 3-CH), 7.37 (d, 1 H, 4-CH), 7.46 (d, 1 H, 1-CH), 9.21 (s, 1 H, 12-CH); ^{13}C nmr (TFA-*d*): 141 ppm (12-CH, $^1J_{CH}$ = 190 Hz).

Anal. Calcd. for $C_{16}H_{15}N_5O{\cdot}CH_3SO_3H$: C, 52.44; H, 4.88; N, 18.00; S, 8.23. Found: C, 52.47; H, 4.89; N, 17.97; S, 8.26.

The acidic filtrate from the reaction mixture was neutralized by the dropwise addition of concentrated ammonium hydroxide solution (temperature <15°) and the solid that precipitated was filtered, air dried and converted to the mesylate salt as described above to afford a brown solid (1.37 g). This solid was identified as compound **25** based on its proton nmr data, mp 252-254°; ^{1}H nmr (DMSO-d_6): δ 2.45 (t, 2 H, 12-CH_2), 2.73 (t, 2 H, 11-CH_2), 3.73 (s, 3 H, OCH_3), 5.84 (s, 1 H, 5-CH), 6.68 (dd, 1 H, 14-CH), 6.91 (d, 1 H, 13-CH), 7.08 (d, 1 H, 16-CH), 7.49 (br s, 4 H, NH_2), 8.02 (d, 1 H, 8-CH), 11.57 (d, 1 H, 7-NH^+). The protons at positions 2, 4 and 7 were exchangeable with deuterium oxide. The imine CH at position 8 collapsed from a doublet to a singlet upon deuterium oxide addition.

9,11-Diamino-3-methoxy-5,6-dihydrobenzo[*f*]pyrimido-[4,5-*b*]quinoline (**3**).

To a solution of 1.8 g (14.38 mmoles) of 2,4,6-triaminopyrimidine (**15**) in acetic acid at reflux was added, dropwise, a solution of 3.2 g (14.38 mmoles) of **17** in 10 ml of acetic acid and reflux was continued for 20 hours after addition was completed. The mixture was allowed to cool to room temperature and the precipitated orange solid was filtered, air dried and suspended in 10% ammonium hydroxide solution, stirred for 30 minutes and filtered. The residue was washed with water until the washings were neutral to litmus paper, air dried and further dried *in vacuo*. This solid was converted to the mesylate salt as described for **2** to afford **3** (0.97 g) in 23% yield, mp 294°; ir (nujol): 3150 cm^{-1} (NH_2); ^{1}H nmr (TFA-*d*): δ 3.07 (t, 2 H, 5-CH_2), 3.68 (t, 2 H, 6-CH_2), 4.02 (s, 3 H, OCH_3), 7.03 (br s, 1 H, Ar-CH), 7.12 (m, 1 H, Ar-CH), 7.72 (dd, 1 H, Ar-CH), 9.02 (s, 1 H, 12-CH); ^{13}C nmr (TFA-*d*): 141 ppm (12-CH, $^1J_{CH}$ = 189 Hz).

Anal. Calcd. for $C_{16}H_{15}N_5O{\cdot}0.95CH_3SO_3H$: C, 52.93; H, 4.93; N, 18.21; S, 7.92. Found: C, 52.96; H, 4.87; N, 18.24; S, 8.08.

The filtrate from the reaction mixture was poured into 150 ml of ice-water mixture and basified with concentrated ammonium hydroxide solution to *p*H 8 to afford 1.41 g (33%) of an orange brown solid. Attempted purification of this solid by flash chromatography or crystallization was unsuccessful. The ^{1}H nmr data of the crude solid however indicated that it was not the angular regioisomer.

9,11-Diaminobenzo[*f*]pyrimido[4,5-*b*]quinoline (**4**).

A mixture 0.04 g (0.15 mmole) of **1** in 8 ml of 25% aqueous acetic acid was heated to 100° and 0.13 g (1.5 mmoles) of activated manganese dioxide was added to the hot solution. The solution was refluxed for 1 hour and filtered through a pad of Celite. The Celite was washed with 15 ml of hot 25% aqueous acetic acid and the filtrate was evaporated to dryness under reduced pressure. The residue was coevaporated twice with 20 ml of absolute ethanol, stirred with 20 ml of absolute ethanol and filtered. The residue was washed with ethanol, acetone, followed by ether and air dried. This solid was suspended in glacial acetic acid at room temperature and dissolved by dropwise addition of methanolic hydrochloric acid. The solution was stored at 0° and filtered to afford 0.035 g (88%) of **4** as a brown solid, mp >300°; ir (nujol): 3160 cm^{-1} (NH_2); ^{1}H nmr (TFA-*d*): δ 8.08-8.30 (m, 3 H, Ar-CH), 8.61 (br peak, 1 H, Ar-CH), 8.86-8.90 (m, 2 H), 10.50 (s, 1 H, 12-CH).

Anal. Calcd. for $C_{15}H_{11}N_5{\cdot}HCl{\cdot}0.5\text{-}CH_3COOH$: C, 58.63; H, 4.31; N, 21.37; Cl, 10.82. Found: C, 58.36; H, 4.41; N, 21.71; Cl, 11.02.

9,11-Diamino-2-methoxybenzo[*f*]pyrimido[4,5-*b*]quinoline (**5**).

In a similar manner as described above for the synthesis of **4**, 0.04 g (0.13 mmole) of compound **2** was oxidized with activated manganese dioxide. The crude solid was converted to the mesylate salt as described for **2** to afford 0.032 g (80%) of **5** as a tan solid, mp >300°; ir (nujol): 3300 cm^{-1} (NH_2); ^{1}H nmr (TFA-*d*): δ 4.26 (s, 3 H, OCH_3), 7.83 (d, 1 H, Ar-CH), 7.97 (d, 1 H, Ar-CH), 8.18 (2 overlapping d, 2 H, Ar-CH), 8.57 (s, 1 H, 1-CH), 10.03 (s, 1 H, 12-CH).

Anal. Calcd. for $C_{16}H_{13}N_5O{\cdot}0.8CH_3SO_3H$: C, 54.80; H, 4.43; N, 19.02; S, 6.97. Found: C, 54.64; H, 4.39; N, 19.05; S, 7.02.

8,10-Diamino-5,6-dihydrobenzo[*h*]pyrimido[4,5-*b*]quinoline (**6**).

Compound **6** was synthesized as previously reported [27] from 2,4,6-triaminopyrimidine (**15**) and 1-chloro-3,4-dihydronaphthalene-2-carboxaldehyde in 65% yield, mp >300°; ir (nujol): 3140 cm^{-1} (NH_2); ^{1}H nmr (TFA-*d*): δ 3.27 (s, 4 H, 5-CH_2 and 6-CH_2), 7.80 (m, 3 H, Ar-CH), 8.40 (d, 1 H, Ar-CH), 9.17 (s, 1 H, 7-CH); ^{13}C nmr (TFA-*d*): 137.9 ppm (7-CH, $^1J_{CH}$ = 161 Hz).

Anal. Calcd. for $C_{15}H_{13}N_5{\cdot}HCl{\cdot}0.82H_2O$: C, 57.28; H, 4.98; N, 22.27; Cl, 11.30. Found: C, 57.66; H, 4.88; N, 21.87; Cl, 10.94.

8,10-Diamino-2-methoxy-5,6-dihydrobenzo[*h*]pyrimido-[4,5-*b*]quinoline (**7**).

To a refluxing solution of 3 g (24.0 mmoles) of 2,4,6-triaminopyrimidine (**15**) in acetic acid was added dropwise a solution of 5.25 g (24 mmoles) of **18** in 20 ml of acetic acid and reflux continued for 20 hours after the addition was completed. The mixture was allowed to cool to room temperature and the precipitated yellow solid was filtered, washed with cold acetic acid and air-dried. The residue was suspended in 10% ammonium hydroxide solution, stirred for 30 minutes and filtered. The residue was washed with water until the washings were neutral to litmus paper. The solid was air-dried, suspended in 50 ml of absolute ethanol and made acidic (*p*H 4) by dropwise addition of methanesulfonic acid. The acidic suspension was stirred for 10 minutes and the solid obtained was filtered to afford **7** as the mesylate salt in 49% yield, mp >300°; ir (nujol): 3326 cm^{-1} (NH_2); ^{1}H nmr (TFA-*d*): δ 3.25 (t, 2 H, 5-CH_2), 3.34 (t, 2 H, 6-CH_2), 4.20 (s, 3 H, OCH_3), 7.55 (dd, 1 H, 3-CH), 7.62 (d, 1 H, 4-CH), 7.99 (d, 1 H, 1-CH), 9.22 (s, 1 H, 7-CH); ^{13}C nmr (TFA-*d*): 141.1 ppm (7-CH, $^1J_{CH}$ = 166 Hz).

Anal. Calcd. $C_{16}H_{15}N_5O{\cdot}0.95CH_3SO_3H$: C, 52.93; H, 4.93; N, 18.21; S, 7.92. Found: C, 53.32; H, 5.00; N, 17.30; S, 7.80.

The percentage of nitrogen in this compound is outside the permitted limits of ± 0.4% so we further established the identity of the compound by determining its molecular mass with ms (FAB) using glycerol/methanol/water as the matrix. $(M+H)^+$ = 294.3.

8,10-Diamino-3-methoxy-5,6-dihydrobenzo[*h*]pyrimido-[4,5-*b*]quinoline (**8**).

Compound **8** was synthesized as previously reported [27] from 2,4,6-triaminopyrimidine (**15**) and 1-chloro-6-methoxy-3,4-dihydronaphthalene-2-carboxaldehyde in 63% yield, mp >300°; ir (nujol): 3140 cm^{-1} (NH_2); ^{1}H nmr (TFA-*d*): δ 3.28 (s, 4 H, 5- and 6-CH_2), 4.10 (s, 3 H, OCH_3), 7.22 (m, 2 H, 1-CH and 2-CH), 8.35 (d, 1 H, 4-CH), 8.98 (7-CH); ^{13}C nmr (TFA-*d*): 141 ppm (7-CH, $^1J_{CH}$ = 167 Hz).

Anal. Calcd. for $C_{16}H_{15}N_5O \cdot HCl \cdot 1.2H_2O$: C, 54.69; H, 5.28; N, 19.93; Cl, 10.09. Found: C, 54.29; H, 5.36; N, 19.66; Cl, 10.01.

8,10-Diamino-4-methoxy-5,6-dihydrobenzo[*h*]pyrimido-[4,5-*b*]quinoline (**9**).

Reaction of 1.24 g (10 mmoles) of 2,4,6-triaminopyrimidine (**15**) with 2.2 g (10 mmoles) of **19** following the procedure described above for the synthesis of compound **7**, afforded the desired compound **9** as the mesylate salt in 48% yield, mp >300°; ir (nujol): 3366 cm^{-1} (NH_2); ^1H nmr (TFA-*d*): δ 2.26 (d, 1 H, 5-CH_2), 3.32 (m, 3 H, 5- and 6-CH_2), 4.18 (s, 3 H, OCH_3), 7.42 (d, 1 H, 3-CH), 7.60 (t, 1 H, 2-CH), 7.89 (d, 1 H, 1-CH), 9.20 (s, 1 H, 7-CH); ^{13}C nmr (TFA-*d*): 141.1 ppm (7-CH, $^1J_{CH}$ = 166 Hz).

Anal. Calcd. for $C_{16}H_{15}N_5O \cdot 0.5CH_3SO_3H \cdot H_2O$: C, 55.14; H, 5.33; N, 19.49; S, 4.46. Found: C, 55.55; H, 5.09; N, 18.48; S, 4.34.

The percentage of nitrogen was outside the permitted limits of ± 0.4% so we further established the identity of the compound by determining its molecular mass with ms (FAB) using glycerol/methanol as the matrix. $(M+H)^+ = 294.3$.

8,10-Diaminobenzo[*h*]pyrimido[4,5-*b*]quinoline (**10**).

A mixture of 0.04 g (0.15 mmole) of **6** in 8 ml of 30% aqueous acetic acid was brought to reflux and 0.26 g (3.0 mmoles) of activated manganese dioxide was added to the hot solution. The solution was refluxed for 24 hours and filtered through a pad of Celite. The Celite was washed with 25 ml of hot 30% aqueous acetic acid and the filtrate was evaporated to dryness under reduced pressure. The residue was coevaporated twice with 20 ml of absolute ethanol, stirred with 30 ml of absolute ethanol and filtered. The residue was washed with ethanol, acetone, ether and air dried. The solid was purified as described for **4** to afford 0.025 g (63%) of **10** as an orange solid, mp >300°; ir (nujol): 3140 cm^{-1} (NH_2); ^1H nmr (TFA-*d*): δ 8.08-8.24 (m, 5 H, Ar-CH), 9.15 (br s, 1 H, Ar-CH), 10.02 (s, 1 H, 7-CH).

Anal. Calcd. for $C_{15}H_{11}N_5 \cdot 0.9HCl \cdot 0.2CH_3COOH$: C, 60.43; H, 4.18; N, 22.88; Cl, 10.42. Found: C, 60.53; H, 4.11; N, 22.81; Cl, 10.55.

8,10-Diamino-2-methoxybenzo[*h*]pyrimido[4,5-*b*]quinoline (**11**).

In a similar manner as described above for the synthesis of **10**, 0.04 g (0.13 mmole) of compound **7** was oxidized with activated manganese dioxide. The crude solid was suspended in acetic acid at room temperature and dissolved by the dropwise addition of methanesulfonic acid. This solution was stored at 0° and filtered to afford 0.024 g (60%) of **11** as an orange solid, mp >300°; ir (nujol): 3300 cm^{-1} (NH_2); ^1H nmr (TFA-*d*): δ 4.24 (s, 3 H, OCH_3), 7.71 (d, 1 H, Ar-CH), 8.20 (d, 1 H, Ar-CH), 8.37 (br s, 2 H, Ar-CH), 8.75 (s, 1 H, Ar-CH), 10.56 (s, 1 H, 7-CH).

Anal. Calcd. for $C_{16}H_{13}N_5O \cdot 0.75CH_3SO_3H \cdot 0.5CH_3COOH$: C, 54.19; H, 4.61; N, 17.80; S, 6.11. Found: C, 54.58; H, 4.51; N, 17.79; S, 6.30.

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