Brief Articles

Synthesis and Pharmacological Evaluation of Some 8-Cyanopyrido[3′,2′:4,5]thieno[3,2-*d*]triazine Derivatives as Inhibitors of Nitric Oxide and Eicosanoid Biosynthesis

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A series of 8-cyanopyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazines, substituted at C-4 and C-7, were synthesized and evaluated as nitric oxide and prostaglandin E_2 inhibitors in murine peritoneal macrophages stimulated with bacterial endotoxin. Several compounds exhibited considerable activity, compounds **10** and **13** being the most interesting ones with IC_{50} values of 11.2 and 3.4 μM on nitrites and 0.9 and 0.6 μM on prostaglandin E_2 production, respectively. None of the examples of pyridothienotriazines that were active at 10 μM showed any effect on inducible nitric oxide synthase, cyclooxygenase-2, and cyclooxygenase-1 enzymes, suggesting that they act by modifiying the level of expression of these inducible enzymes.

Introduction

Nitric oxide (NO) is a key mediator in various physiological and pathological processes. NO is formed by the enzyme NO synthase (NOS), which exists as three distinct isoforms: namely (i) endothelial NOS, (ii) neuronal NOS, and (iii) inducible NOS (iNOS).[1] It has been reported that expression is induced in macrophages by a variety of agents, such as cytokines and bacterial endotoxins, during the inflammatory process. This enzyme is capable of producing high concentrations of NO, which contributes to the antimicrobial and antitumor action of macrophages.[2] However, NO production during inflammatory response may become "self-destructive" owing to its oxidative properties, as occurs in chronic inflammatory diseases.[3,4] Furthermore, NO contributes significantly to the circulatory failure associated with endotoxic shock.[5]

Prostanoids regulate a great number of physiological processes and are synthesized by two cyclooxygenase (COX) isoforms. COX-1 synthesizes different prostanoids involved in homeostatic functions, whereas COX-2, whose expression is restricted under basal conditions, is upregulated by inflammatory stimuli resulting in increased PGE_2 production, which has been suggested to play an important role in the pathophysiology of inflammation and arthritis.[6,7]

The co-induction of iNOS and COX-2 in cells stimulated with bacterial endotoxins and other inflammatory stimuli has been described.[8] Due to the importance of the constitutive isoenzymes in normal physiology, the selective modulation of NO and PGE_2 overproduction by iNOS and COX-2 might represent an important therapeutic goal in different inflammatory pathologies.

Many compounds described as inhibitors of NOS contain heterocyclic structures such as imidazole, triazole, or triazine.[9] During a project directed toward the identification of new inhibitors, we selected for study the pyridothieno-1,2,3-triazine nucleus and, after some screening, centered our efforts on the 8-cyanopyridothieno-1,2,3-triazine derivatives substituted at C-4 and C-7. In this paper we describe the synthesis of a series of these derivatives and their capability to inhibit the generation of NO and PGE_2 by murine macrophages. Although some 1,2,3-triazine derivatives have been described as potential antifungal drugs[10] and others possess antianaphylactic activity,[11] this is the first time pyridothieno-1,2,3-triazine derivatives have been identified as effective in vitro inhibitors of nitric oxide and eicosanoid biosynthesis.

Chemistry

The preparation of pyridothienotriazines **7**−**22** was accomplished in good yields by intramolecular condensation of a diazonium ion with an adjacent nucleophilic function. This procedure has proven useful in the synthesis of various five- and six-membered ring nitrogen heterocycles, including 1,2,3-triazines,[12] and we found in fact that the diazotization of 3-amino-2,5-dicyano-6-substituted thieno[2,3-*b*]pyridines **6** constitutes a direct and very convenient route to the title ring system. The synthetic route is represented in Scheme 1 along with the target compounds showing the substitution pattern.

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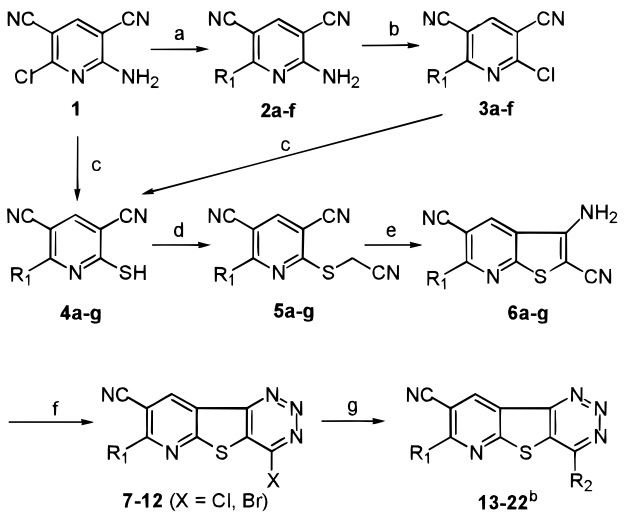
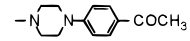
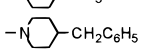
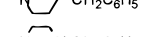
Scheme 1[a]



[a] Reagents and Conditions: (a) R_1H, EtOH/THF, reflux; (b) isoamyl nitrite, $CuCl_2$, CH_3CN, 65 °C; (c) NaHS, EtOH, rt; (d) $ClCH_2CN$, K_2CO_3, KI, acetone, rt; (e) K_2CO_3, EtOH, rt; (f) $NaNO_2$, HCl or HBr, AcOH, 5°C; (g) R_2H, THF/EtOH, reflux. [b] R_2H are shown in Table 1.

2-6	R_1	2-6	R_1
a		**e**	
b		**f**	$-NHCH_2C_6H_5$
c		**g**	$-NH_2$
d			

The introduction of substituents R_1 and R_2 is carried out using the same reaction: aromatic nucleophilic substitution of an aromatic halide, thus simplifying the process. The key β-enaminonitrile intermediates **6a**−**g** were synthesized from 2-amino-6-chloro-3,5-dicyanopyridine (**1**), as previously reported.[13] Nitrosation of **6** with sodium nitrite in HCl or HBr (**6f** to give **12**) afforded the halo-substituted triazines **7**−**12**, which produced the pyridothienotriazine derivatives **13**−**22** in good yields by halide displacement with the appropriate secondary amine as a nucleophile. All compounds prepared in this work gave satisfactory elemental analyses and spectral data (IR, ^{1}H NMR, ^{13}C NMR, and MS) that are consistent with the structures proposed.

Biological Results and Discussion

Stimulation of macrophages with bacterial lipopolysaccharide (LPS) causes expression of iNOS and COX-2 with the consequent generation of large quantities of NO and PGs.[8] To establish the activity of the title compounds, we determined the accumulation of nitrite and PGE_2 as an index of enzyme expression/activity; 24-h LPS-stimulated peritoneal macrophages produced 1018 ng of nitrite/mL and 3.5 ng of PGE_2/mL with respect to 90.5 ng of nitrite/mL and 0.4 ng of PGE_2/mL in untreated cells.

As shown in Table 1, the production of these mediators was attenuated by most of the cyanopyridothienotriazines at 10 μM. At that concentration, these compounds did not exert cytotoxic effects during a 24-h incubation period as indicated by MTT reduction (data not shown).

To evaluate the structure−activity relationship, different substituents (R_1 and R_2) were placed in two positions (C-4 and C-7) of the pyridothienotriazine skeleton. Comparative analysis of the data shown in Table 1 indicates some trends: The 8-cyanopyridothieno-1,2,3-triazines **7**−**10** with a chloro substituent (R_2 = Cl) at position C-4 of the triazine nucleus and a nitrogen heterocycle at C-7 (R_1) were found to be good inhibitors of PGE_2 production, and reductions of about 50% of the control value were obtained in most cases. Compound **10**, with an *N*′-(*p*-acetylphenyl)piperazine group as R_1, was found to be much more effective (95% inhibition), practically suppressing the generation of PGE_2. In contrast, the presence of an amino group or a linear amine at C-7 (R_1) and a bromo instead of a chloro substituent atom at C-4 (**11**, **12**) is associated with a total loss of activity on PGE_2 production.

As for the inhibition of NO formation, only three chlorine-containing compounds (**7**, **9**, and **10**) are active, suppressing around 40−50% of the nitrite accumulated. In a similar way to previous observations in the PGE_2 test, compounds **11** and **12** do not inhibit the production of NO at all. From these data, compound **10** emerges as the most interesting example within this series because it is effective in inhibiting the production of both metabolites.

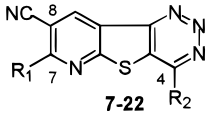
When we consider the compounds carrying a morpholine substituent at C-7 (R_1) and different heterocycles at C-4 (R_2), i.e., compounds **13**−**16**, a moderate decrease (30−40%) in accumulation of both metabolites is observed in **14**−**16** at 10 μM (Table 1), with compound **13** (R_1 = morpholine, R_2 = 4′-methylpiperidine) being much more active, producing around 90% inhibition on production of NO and PGE_2 at that concentration.

When heterocycles other than morpholine are incorporated as R_1 substituents (compounds **17**−**20**), inhibitory action on both metabolites of around 50% is obtained. Once again, the presence of acyclic amines in position R_1 (**21**, **22**) does not improve the effectiveness of these compounds.

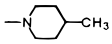
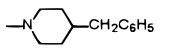
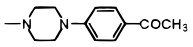
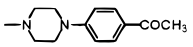
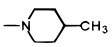
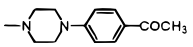
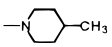
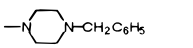
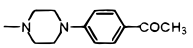
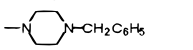
Table 2 shows the IC_{50} values for the inhibition of nitrite and PGE_2 accumulation of the two most active pyridothienotriazines examined, **10** and **13**. Comparison of these data with the reference values of aminoguanidine (AG) (an inhibitor of NOS activity), NS 398 (a selective inhibitor of COX-2), and the corticosteroid dexamethasone, shown in Table 2, illustrates the potential interest of these two pyridothienotriazines as inhibitors of the excess production of NO and prostaglandins. Compounds that inhibit excess production of NO and prostaglandins by macrophages might be of benefit for the prevention and treatment of autoimmune diseases, septic shock, and different inflammatory pathologies.[1]

Finally, to assess whether the inhibition observed in cultured cells is related, or not, to a direct action on enzymatic activities, we decided to assay the effect of the 8-cyanopyridothieno-1,2,3-triazines on iNOS, COX-2, and COX-1 enzymes and to compare them with aminoguanidine (AG), a known iNOS inhibitor that reduces the production of nitrites and citrulline formation, and with NS 398 and indomethacin, two COX inhibitors that are very effective in reducing eicosanoid synthesis in vitro. The results are shown in Table 3 and

Table 1. Structure and Chemical Data of 8-Cyanopyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazines **7**−**22** and Their Inhibitory Action (%) on the Accumulation of Nitrites and PGE_2 in Stimulated Peritoneal Macrophages



7-22

Compd[a]	R_1	R_2	% yield	mp, °C	Formula[b]	Nitrites	PGE_2
7		Cl	80	201–203	$C_{13}H_9ClN_6OS$	51.8 ± 5.7**	59.5 ± 4.2**
8		Cl	80	190–192	$C_{15}H_{13}ClN_6S$	6.1 ± 1.9	49.0 ± 5.7**
9		Cl	50	218–220	$C_{21}H_{17}ClN_6S$	48.8 ± 2.8**	57.9 ± 6.7**
10		Cl	72	236–238	$C_{21}H_{16}ClN_7OS$	44.7 ± 2.9**	94.5 ± 1.1**
11	$-NH_2$	Cl	40	254–256	$C_9H_3ClN_6S$	13.9 ± 3.0	1.0 ± 1.0
12	$-NHCH_2C_6H_5$	Br	77	208–210	$C_{16}H_9BrN_6S$	12.2 ± 4.4	0.4 ± 0.4
13			72	210–212	$C_{19}H_{21}N_7OS$	88.7 ± 3.1**	89.6+ 3.2**
14			67	235–237	$C_{17}H_{17}N_7OS_2$	31.4 ± 3.1*	36.1 ± 3.9*
15			77	295–297	$C_{17}H_{18}N_8OS$	43.3 ± 2.8**	31.3 ± 2.5*
16			69	179–181	$C_{25}H_{24}N_8O_2S$	45.6 ± 3.3**	34.6 ± 4.6*
17			79	246–248	$C_{27}H_{28}N_8OS$	42.6 ± 2.5**	45.6 ± 1.7**
18			81	186–188	$C_{21}H_{25}N_7S$	37.8 ± 4.9*	52.1 ± 6.5**
19			91	270–272	$C_{32}H_{31}N_9OS$	51.6 ± 1.7**	50.1 ± 5.1**
20			89	203–205	$C_{25}H_{27}N_9S$	50.6 ± 3.1**	55.1 ± 3.5**
21	$-NHCH_2C_6H_5$		84	265–267	$C_{21}H_{20}N_8S$	14.1 ± 4.0	56.4 ± 6.0**
22	$-NHCH_2C_6H_5$		91	279–281	$C_{20}H_{17}N_7OS$	41.1 ± 3.4**	28.5 ± 4.2*

[a] Compounds were assayed at 10 μM. [b] All elemental analyses for C, H, and N agree within ±0.4% of calculated values. Data shown are mean ± SEM (n = 6−9). **$p \leq 0.01$.

Table 2. IC_{50} Values of Selected 8-Cyanopyrido[3′,2′:4,5]-thieno[3,2-*d*]-1,2,3-triazines for the Inhibition of Nitrite and PGE_2 Accumulation in Stimulated Macrophages

	IC_{50}[a] (μM)	
compd	nitrites	PGE_2
10	11.2 (10.5−12.0)	0.9 (0.4−1.7)
13	3.4 (1.0−5.2)	0.6 (0.3−1.0)
aminoguanidine	25.1 (22.9−28.8)	ND
NS 398	ND	3.1 (1.3−5.4) nM
dexamethasone	35.8 (12.2−89.1) nM	1.0 (0.7−2.4) nM

[a] Values represent the concentration required to produce 50% inhibition of the response, along with the 95% confidence limits. ND, not determined.

indicate that none of the compounds active at 10 μM significantly modified the enzymatic activities in vitro. This fact suggests that the effect of these compounds could be due to their action on the degree of induction/expression of NOS and COX proteins caused by LPS in macrophages.

In summary, we report here that 8-cyanopyridothieno-1,2,3-triazines **10** and **13** are potent inhibitors of NO and PGE_2 generation on murine macrophages and that this action is not related to a direct effect on NOS or COX activities. Further studies are underway to elucidate the mechanisms of action and to optimize the inhibitory effect.

Experimental Section

Pharmacology. [5,6,8,11,12,14,15(*n*)-^{3}H]PGE_2, [5,6,8,9,11,-12,14,15(*n*)-^{3}H]TXB_2, and l-[^{3}H]arginine were obtained from Amersham Iberica, (Madrid, Spain). NS 398 was purchased from Universal Biologicals Ltd. (London, U.K.). The rest of the reagents were obtained from Sigma Chemical Co. (MO).

Table 3. Effect of a Selection of 8-Cyanopyridothieno-1,2,3-triazines on iNOS, COX-1, and COX-2 Activities in Vitro[a]

compd	iNOS (*p*mol citrulline·mg protein/min)	COX-1 (ng TXB_2/mg protein	COX-2 (ng PGE_2/mg protein)
control	14.1 ± 1.0	128.1 ± 5.1	16.6 ± 0.9
7	14.0 ± 0.4	112.5 ± 11.3	16.5 ± 1.8
8	ND	130.5 ± 10.8	16.2 ± 1.5
9	13.1 ± 0.2	127.6 ± 23.1	13.8 ± 1.2
10	14.1 ± 0.4	104.1 ± 2.1	15.9 ± 1.3
13	13.1 ± 0.6	130.3 ± 5.9	13.0 ± 1.1
15	15.1 ± 0.9	149.3 ± 31.0	16.5 ± 1.9
16	14.2 ± 0.6	141.5 ± 20.8	16.6 ± 1.4
17	12.5 ± 0.6	127.2 ± 33.7	14.3 ± 0.9
18	12.4 ± 0.5	124.1 ± 13.5	13.9 ± 2.0
19	13.5 ± 0.9	134.1 ± 15.3	15.8 ± 1.4
20	13.1 ± 1.0	123.1 ± 11.2	14.6 ± 1.2
NS 398	ND	ND	8.5 ± 0.8**
indomethacin	ND	63.8 ± 4.8**	8.6 ± 1.0**
aminoguanidine	5.4 ± 0.3**	ND	ND

[a] Data shown are mean ± SEM (n = 6−9). **$p \leq 0.01$. ND., not determined. All compounds were assayed at a concentration of 10 μM, except aminoguanidine which was assayed at 100 μM.

Mouse Macrophages Culture. Highly purified peritoneal macrophages were harvested by peritoneal lavage 4 days after ip injection of 1 mL of 10% thioglycolate broth. Cells were resuspended in culture medium (120 nM NaCl, 4.7 mM KCl, 1.2 mM $CaCl_2 \cdot 7H_2O$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 mM HEPES, 1 mM arginine, and 10 mM glucose) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100

IU mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and incubated at 37 °C for 2 h. The adherent cells were incubated with *E. coli* lipopolysaccharide (10 μg/mL) at 37 °C for 24 h in the presence of test compounds or vehicle. Nitrite and PGE_2 levels were assayed in culture supernatants by a fluorimetric method[14] and by radioimmunoassay,[15] respectively. The mitochondrial-dependent reduction of 3-(4,5-dimethylthioxol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan was used to assess the possible cytotoxic effects of compounds.

Assay of Cyclooxygenase-1 Activity. Human platelets were sonicated at 4 °C in an ultrasonicator at maximum potency. Microsomes were prepared by centrifugation at 2000*g* for 5 min at 4 °C followed by centrifugation of the supernatant at 100000*g* for 100 min at 4 °C. Microsomes (20 μg of protein/tube) were incubated with arachidonic acid and test compounds or vehicle.[16] TXB_2 levels were determined by radioimmunoassay.[15]

Assay of Cyclooxygenase-2 Activity. Murine peritoneal macrophages incubated with *E. coli* lipopolysaccharide (10 μg/mL) at 37 °C for 24 h were collected and sonicated at 4 °C in an ultrasonicator at maximum potency, and microsomes were prepared as above.[16] Microsomes (40 μg of protein/tube) were used as a source of cyclooxygenase-2, and reactions were carried out in the same conditions as above. PGE_2 synthesis was determined by radioimmunoassay.[15]

Assay of iNOS Activity. High-speed (100000*g*) supernatants from sonicated peritoneal macrophages were obtained as described above. NOS activity was determined in supernatants by monitoring the conversion of L-[^{3}H]arginine to L-[^{3}H]-citrulline.[17]

Statistical Analysis. The results are presented as mean ± SEM; *n* represents the number of experiments. Inhibitory concentration 50% (IC_{50}) values were calculated from at least four significant concentrations ($n = 6$). The level of statistical significance was determined by analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons.

Chemical Methods. All reagents used were commercial grade chemicals from freshly opened containers. Melting points were determined on a Büchi 510 apparatus and are uncorrected. IR spectra were recorded as potassium bromide disks on a Perkin-Elmer 783 spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AC 200F instrument at room temperature, and values are reported as δ units. Mass spectra were obtained on a VG QUATTRO spectrometer in electron impact (EI) or fast atom bombardment (FAB) mode using thioglycerol as the matrix. The silica gel 60 $HF_{254+366}$ used for analytical thin-layer chromatography and the silica gel 60 (230−400 mesh) employed for flash chromatography were purchased from Merck. Microanalyses for C, H, and N were performed by the Elemental Analysis General Service of the University of La Coruña. Compounds **2a**−**f**, **3a**−**f**, **4a**−**g**, **5a**−**g**, and **6a**−**g** were prepared according to modified literature procedures.[13]

Synthesis of Pyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazines 7−12. General Procedure. To an ice-cooled solution of the corresponding aminonitrile **6** (6.73 mmol) in 1:1 (v/v) HCl/AcOH or HBr/AcOH (50 mL) was added dropwise a solution of sodium nitrite (10.1 mmol) in water (10 mL). The solution was stirred at room temperature for 3 h, and then the mixture was poured into water (200 mL). The resulting solid was filtered off and purified by flash chromatography on silica gel.

8-Cyano-4-chloro-7-morpholinopyrido[3′,2′:4,5]thieno-[3,2-*d*]-1,2,3-triazine (7): purified by flash chromatography using CH_2Cl_2/AcOEt (25:1) as eluent; mp 201−203 °C; ^{1}H NMR ($CDCl_3$) 3.90 (t, 4H, $J = 4.6$ Hz), 4.07 (t, 4H, $J = 4.6$ Hz), 8.94 (s, 1H); ^{13}C NMR ($CDCl_3$) 48.2 (CH_2N), 66.4 (CH_2O), 93.7 (C-8), 115.3 (C-4a), 116.9 (CN), 129.5 (C-9a), 141.5 (C-9), 150.9, 152.4, 159.7, 166.5; MS (EI) *m/e* 332 (M^+), 334 ($M^+ + 2$). Anal. ($C_{13}H_9ClN_6OS$) C, H, N.

8-Cyano-4-chloro-7-(4-methylpiperidino)pyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (8): purified by flash chromatography using CH_2Cl_2/AcOEt (40:1) as eluent; mp 190−192 °C; ^{1}H NMR ($CDCl_3$) 1.02 (d, 3H, $J = 6.2$ Hz), 1.40 (m, 2H), 1.83 (m, 3H), 3.21 (m, 2H), 4.75 (m, 2H), 8.84 (s, 1H); ^{13}C NMR ($CDCl_3$) 21.5 (CH_3), 30.8 (CH), 34.1 (CH_2), 48.7 (CH_2N), 93.2 (C-8), 114.2 (C-4a), 117.2 (CN), 129.2 (C-9a), 141.5 (C-9), 151.1, 152.1, 159.4, 165.8; MS (EI) *m/e* 344 (M^+), 346 ($M^+ + 2$). Anal. ($C_{15}H_{13}ClN_6S$) C, H, N.

7-(4-Benzylpiperidino)-4-chloro-8-cyanopyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (9): purified by flash chromatography using CH_2Cl_2/AcOEt (20:1) as eluent; mp 218−220 °C; ^{1}H NMR ($CDCl_3$) 1.45 (m, 2H), 1.93 (m, 3H), 2.63 (d, 2H, $J = 6.7$ Hz), 3.17 (m, 2H), 4.81 (m, 2H), 7.14−7.37 (m, 5H), 8.87 (s, 1H); ^{13}C NMR ($CDCl_3$) 32.0 (CH_2), 37.9 (CH), 42.7 (CH_2Ph), 48.7 (NCH_2), 93.3 (C-8), 114.4 (C-4a), 117.2 (CN), 129.2 (C-9a), 126.1, 128.3, 129.0, 139.5 (C_6H_5), 141.5 (C-9), 151.1, 152.2, 159.4, 165.8; MS (FAB) *m/e* 421 $(MH)^+$, 423 $[(MH)^+ + 2]$. Anal. ($C_{21}H_{17}ClN_6S$) C, H, N.

7-(*N*-4′-Acetylphenylpiperazino)-8-cyano-4-chloropyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (10): purified by flash chromatography using AcOEt/hexanes (2:1) as eluent; mp 236−238 °C; ^{1}H NMR ($CDCl_3$) 2.55 (s, 3H), 3.64 (t, 4H, $J = 5.3$ Hz), 4.25 (t, 4H, $J = 5.3$ Hz), 6.91 (d, 2H, $J = 9.0$ Hz), 7.94 (d, 2H, $J = 9.0$ Hz), 8.97 (s, 1H); MS (FAB) *m/e* 450 $(MH)^+$, 452 $[(MH)^+ + 2]$. Anal. ($C_{21}H_{16}ClN_7OS$) C, H, N.

7-Amino-4-chloro-8-cyanopyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (11): purified by flash chromatography using CH_2Cl_2/AcOEt (10:1) as eluent; mp 254−256 °C; ^{1}H NMR (DMSO-d_6) 8.25 (s, 2H), 9.13 (s, 1H); ^{13}C NMR (DMSO-d_6) 91.7 (C-8), 113.4 (C-4a), 115.6 (CN), 128.4 (C-9a), 139.9 (C-9), 151.5, 151.7, 161.1, 166.5; MS (FAB) *m/e* 263 $(MH)^+$, 265 $[(MH)^+ + 2]$. Anal. ($C_9H_3ClN_6S$) C, H, N.

7-Benzylamino-4-bromo-8-cyanopyrido[3′,2′:4,5]thieno-[3,2-*d*]-1,2,3-triazine (12): purified by flash chromatography using CH_2Cl_2/AcOEt (20:1) as eluent; mp 208−210 °C; ^{1}H NMR (CD_3COCD_3) 4.92 (d, 2H, $J = 6.1$ Hz), 7.22−7.38 (m, 3H), 7.46−7.50 (m, 2H), 8.81 (s, 1H), 9.02 (s, 1H); MS (EI) *m/e* 396 (M^+), 398 $[(M)^+ + 2]$. Anal. ($C_{16}H_9BrN_6S$) C, H, N.

Synthesis of Pyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazines 13−22. General Procedure. A solution of the corresponding halogenated derivative (**7**−**12**) (0.60 mmol) and the appropriate secondary amine (0.72 mmol) in 3:1 (v/v) THF/EtOH (15 mL) was refluxed until the starting material disappeared (TLC). After cooling, the solid was filtered off and recrystallized from EtOH/acetone.

8-Cyano-4-(4-methylpiperidino)-7-morpholinopyrido-[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (13): mp 210−212 °C; ^{1}H NMR ($CDCl_3$) 1.01 (d, 3H, $J = 6.1$ Hz), 1.34 (m, 2H), 1.85 (m, 3H), 3.24 (m, 2H), 3.89 (m, 8H), 4.81 (m, 2H), 8.81 (s, 1H); ^{13}C NMR ($CDCl_3$) 21.6 (CH_3), 31.0 (CH), 34.1 (CH_2), 46.6 (NCH_2), 48.5 (NCH_2), 66.8 (OCH_2), 93.7 (C-8), 111.2 (C-4a), 117.1 (C-9a), 117.4 (CN), 140.4 (C-9), 148.6, 152.4, 160.1, 164.1; MS (EI) *m/e* 395 (M^+). Anal. ($C_{19}H_{21}N_7OS$) C, H, N.

8-Cyano-7-morpholino-4-thiomorpholinopyrido-[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (14): mp 235−237 °C; ^{1}H NMR ($CDCl_3$) 2.83 (t, 4H, $J = 5.1$ Hz), 3.91 (m, 8H), 4.37 (t, 4H, $J = 5.1$ Hz), 8.82 (s, 1H); ^{13}C NMR ($CDCl_3$) 27.1 (SCH_2), 48.4 (NCH_2), 49.1 (NCH_2), 66.5 (OCH_2), 93.8 (C-8), 111.6 (C-4a), 116.8 (C-9a), 117.3 (CN), 140.3 (C-9), 148.9, 152.3, 160.1, 164.1; MS (FAB) *m/e* 400 $(MH)^+$. Anal. ($C_{17}H_{17}N_7OS_2$) C, H, N.

8-Cyano-7-morpholino-4-piperazinopyrido[3′,2′:4,5]-thieno[3,2*d*]-1,2,3-triazine (15): mp 295−297 °C; ^{1}H NMR (DMSO-d_6) 3.27 (t, 4H, $J = 4.6$ Hz), 3.79 (m, 8H), 4.15 (t, 4H, $J = 4.6$ Hz), 9.04 (s, 1H); ^{13}C NMR (DMSO-d_6) 42.6 (NCH_2), 42.9 (NCH_2), 48.2 (NCH_2), 65.8 (OCH_2), 93.7 (C-8), 111.5 (C-4a), 115.9 (C-9a), 117.4 (CN), 141.5 (C-9), 148.9, 152.5, 159.9, 163.7; MS (FAB) *m/e* 383 $(MH)^+$. Anal. ($C_{17}H_{18}N_8OS$) C, H, N.

4-(*N*-4′-Acetylphenylpiperazino)-8-cyano-7-morpholinopyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3 triazine (16): mp 179−181 °C; ^{1}H NMR ($CDCl_3$) 2.55 (s, 3H), 3.62 (t, 4H, $J = 5.2$ Hz), 3.92 (m, 8H), 4.28 (t, 4H, $J = 5.2$ Hz), 6.92 (d, 2H, $J = 9.0$ Hz), 7.93 (d, 2H, $J = 9.0$ Hz), 8.88 (s, 1H); MS (FAB) *m/e* 501 $(MH)^+$. Anal. ($C_{25}H_{24}N_8O_2S$) C, H, N.

4-(*N*-4′-Acetylphenylpiperazino)-8-cyano-7-(4-methylpiperidino)pyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (17): mp 246−248 °C; ^{1}H NMR ($CDCl_3$) 1.02 (d, 3H, $J = 6.2$ Hz), 1.41 (m, 2H), 1.85 (m, 3H), 2.55 (s, 3H), 3.16 (m, 2H), 3.61 (t,

4H, J = 5.2 Hz), 4.27 (t, 4H, J = 5.2 Hz), 4.67 (m, 2H), 6.93 (d, 2H, J = 8.9 Hz), 7.93 (d, 2H, J = 8.9 Hz), 8.83 (s, 1H); MS (FAB) *m/e* 513 $(MH)^+$. Anal. ($C_{27}H_{28}N_8OS$) C, H, N.

8-Cyano-4,7-bis(4-methylpiperidino)pyrido[3′,2′:4,5]-thieno[3,2-*d*]-1,2,3-triazine (18): mp 186−188 °C; ^{1}H NMR ($CDCl_3$) 0.99 (d, 3H, J = 6.1 Hz), 1.00 (d, 3H, J = 6.1 Hz), 1.36 (m, 4H), 1.78 (m, 6H), 3.14 (m, 4H), 4.60 (m, 2H), 4.79 (m, 2H), 8.75 (s, 1H); ^{13}C NMR ($CDCl_3$) 21.6 (CH_3), 30.8 (CH), 30.9 (CH), 33.9 (CH_2), 34.0 (CH_2), 46.5 (NCH_2), 48.7 (NCH_2), 93.0 (C-8), 110.8 (C-4a), 115.9 (C-9a), 117.7 (CN), 140.0 (C-9), 148.8, 152.4, 159.8, 164.3; MS (EI) *m/e* 407 (M^+). Anal. ($C_{21}H_{25}N_7S$) C, H, N.

4-(*N*-4′-Acetylphenylpiperazino)-7-(4-benzylpiperazino)-8-cyanopyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (19): mp 270−272 °C; ^{1}H NMR (DMSO-d_6) 2.45 (s, 3H), 3.50−3.62 (m, 10H), 4.10−4.62 (m, 8H), 6.98 (d, 2H, J = 8.8 Hz), 7.34−7.63 (m, 5H), 7.84 (d, 2H, J = 8.8 Hz), 9.11 (s, 1H); MS (FAB) *m/e* 590 $(MH)^+$. Anal. ($C_{32}H_{31}N_9OS$) C, H, N.

7-(4-Benzylpiperazino)-8-cyano-4-(4-methylpiperazino)-pyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (20): mp 203−205 °C; ^{1}H NMR ($CDCl_3$) 2.38 (s, 3H), 2.63 (m, 8H), 3.60 (s, 2H), 3.95 (t, 4H, J = 4.9 Hz), 4.09 (t, 4H, J = 4.9 Hz), 7.30−7.37 (m, 5H), 8.81 (s, 1H); ^{13}C NMR ($CDCl_3$) 45.8 (NCH_2), 45.9 (CH_3), 48.2 (NCH_2), 52.7 (NCH_2), 54.7 (NCH_2), 62.8 ($CH_2C_6H_5$), 93.5 (C-8), 111.4 (C-4a), 116.5 (C-9a), 117.0 (CN), 127.3, 128.3, 129.2, 137.5 (C_6H_5), 140.4 (C-9), 149.2, 152.8, 159.9, 164.3; MS (FAB) *m/e* 486 $(MH)^+$. Anal. ($C_{25}H_{27}N_9S$) C, H, N.

7-Benzylamino-8-cyano-4-(4-methylpiperazino)pyrido-[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (21): mp 265−267 °C; ^{1}H NMR (DMSO-d_6) 2.58 (s, 3H), 3.35 (m, 4H), 4.08 (m, 4H), 4.64 (d, 2H), 7.27−7.35 (m, 5H), 8.72 (s, 1H), 8.90 (s, 1H); ^{13}C NMR (DMSO-d_6) 42.8 (CH_3), 43.1 (NCH_2), 44.6 ($CH_2C_6H_5$), 52.1 (NCH_2), 91.9 (C-8), 110.2 (C-4a), 113.9 (C-9a), 115.9 (CN), 126.8, 127.4, 128.3, 138.9 (C_6H_5), 138.4 (C-9), 149.3, 152.2, 158.5, 165.0; MS (FAB) *m/e* 417 $(MH)^+$. Anal. ($C_{21}H_{20}N_8S$) C, H, N.

7-Benzylamino-8-cyano-4-morpholinopyrido[3′,2′:4,5]-thieno[3,2-*d*]-1,2,3-triazine (22): mp 279−281 °C; ^{1}H NMR (DMSO-d_6) 3.77 (m, 4H), 3.87 (m, 4H), 4.64 (d, 2H, J = 5.8 Hz), 7.18−7.39 (m, 5H), 8.67 (t, 1H, J = 5.8 Hz), 8.85 (s, 1H); ^{13}C NMR (DMSO-d_6) 44.5 ($NHCH_2$), 45.6 (NCH_2), 65.8 (OCH_2), 91.9 (C-8), 110.3 (C-4a), 114.2 (C-9a), 115.9 (CN), 126.9, 127.5, 128.3, 138.9 (C_6H_5), 138.6 (C-9), 149.3, 152.7, 158.5; MS (FAB) *m/e* 404 $(MH)^+$. Anal. ($C_{20}H_{17}N_7OS$) C, H, N.

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