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Synthesis, antihistaminic and cytotoxic activity of pyridothieno- and pyridodithienotriazines

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Abstract – The synthesis of pyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazines **7a–n** and **8a–c** and pyrido[3′,2′:4,5]dithieno[3,2-*d*]-1,2,3-triazines **15** and **16a–c**, and their inhibitory action on the release of histamine from rat mast cells under immunological and chemical stimulus are presented. Compounds **7b** and **16a** are strong inhibitors under all the conditions tested while **16c** is a good inhibitor in all conditions except when it is preincubated with ovoalbumin. Compounds **7k**, **7m** and **7n** are good inhibitors in the immunological experiments but are practically inactive under chemical stimulus. Compounds **6a** and **15** show in vitro cytotoxic activity against several human and mouse tumoral cell lines with IC_{50} values well under 1 mg/mL. © Elsevier, Paris

pyridothienotriazines / pyridodithienoditriazines / antihistaminic / cytotoxicity

1. Introduction

The inhibition of the release of vasoactive mediators, including histamine, leukotrienes and prostaglandins, from mast cells and basophils is extensively used in the treatment of asthma [1–3] and disodium cromoglycate (DSCG, **1**) [4–6] is one of the most extensively used drugs. Although the precise biochemical mechanism by which DSCG inhibits histamine release is not known, it has been proposed that it acts by raising intracellular levels of cyclic AMP via the inhibition of cyclic nucleotide phosphordiesterase.

Very few heterocyclic systems have been explored as potential antiallergics. Very effective antiasthmatic drugs that inhibit the release of histamine have been developed based on the tetrazol [7–10] and thienopyrimidine [11] systems (i.e. tiprinast **2**), and potent inhibition of the release of leukotrienes was reported in certain naphthyridines [12, 13]. Several years ago, Youssefyeh et al. attempted to develop pyrimidothienotriazines as antiasthmatic agents [14] and found some potent inhibitors of the anaphylactically induced release of histamine [15]. More recently, and in the course of a program directed towards the exploration of antihistaminic activity of new heterocycles, we have described the result of a screening of cyanopyridines **3** and pyridopyrimidines **4** [16]. In this paper, we describe our results on the chemical synthesis and antihistaminic activity of pyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazines **7a–n** and **8a–c** (*figures 2* and *3*) and pyrido[3′,2′:4,5]dithieno[3,2-*d*]-1,2,3-triazines **16a–c** (*figure 4*), under immunological and chemical stimulus. In addition, in vitro cytotoxic activity is also presented (see *figure 1*).

The synthetic procedure was devised for the easy introduction of a variety of substituents. The release of histamine was measured in rat mast cells after immunological (with ovoalbumin as inducer) and chemical (polymer 48/80 as inducer) stimulus. In both experiments, the release of histamine was evaluated with and without preincubation of the compound with the stimulus. Both inhibition and stimulation of histamine release were

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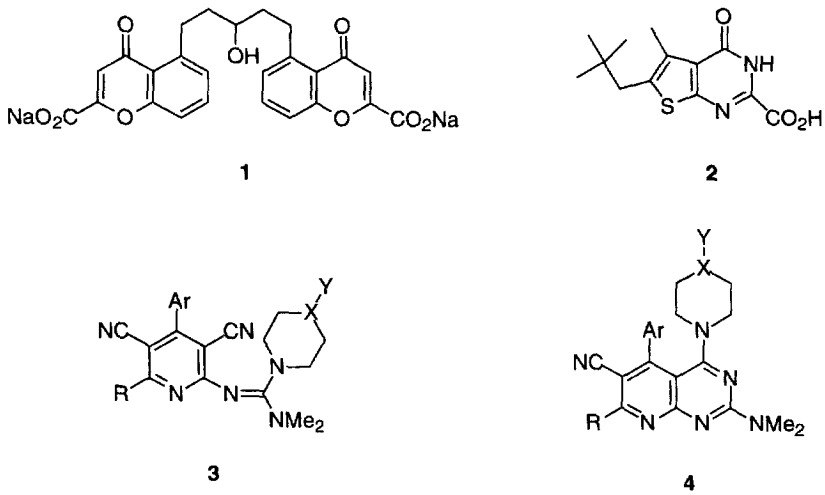


Figure 1.

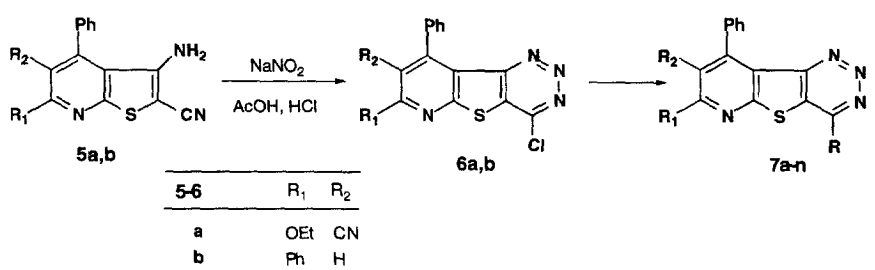
measured. For comparative purposes, some compounds were tested with the antineoplasic adriamicine and vinorelbine drugs as inducers.

The cytotoxicity was evaluated in vitro for antiproliferative activity against mouse P388 (lymphoid neoplasm) and human A-549 (colon carcinoma), HT-29 (lung carcinoma) and MEL-28 (malignant melanoma) cell lines.

2. Chemistry

The intramolecular condensation of a diazonium ion with an adjacent nucleophilic function has proven useful in the synthesis of various five- or six-membered ring nitrogen heterocycles [17]. A good example [18] of the application of this method is the preparation, using a cyanide group as the nucleophile, of 4-chloro-1,2,3-triazines. We are interested in the preparation of new pyridothienotriazines (compounds **7** and **8**) and pyridodithienotriazines (compounds **15** and **16**) and, accordingly, we used this procedure for the formation of the triazine ring. The routes shown in *figures 2–4* proceed in high yield to give the two appropriate skeletons, and were devised in order to allow the incorporation of a variety of substituents R, R_1 and R_2 at different stages.

For the synthesis of pyridothienotriazines (compounds **7** and **8**), we started with β-enaminonitriles **5a,b** [19, 20] (*figure* 2) that, by diazotization, gave a high yield of the condensed 4-chloro-8-cyano-7-ethoxy-9-phenylpyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (**6a**) and 4-chloro-7,9-diphenylpyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (**6b**) [21, 22] in analytically pure form directly from the reaction mixture. Intermediates **6a** and **6b** underwent normal halide displacement with a variety of nucleophiles, including secondary amines, the ethoxide ion, and hydrazine, to yield compounds **7a–n**.



5-6	R_1	R_2
a	OEt	CN
b	Ph	H

Figure 2.

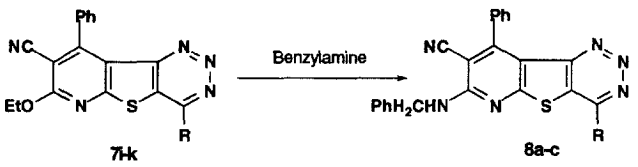


Figure 3.

Compounds **8a–c** were obtained by nucleophilic aromatic substitution of the ethoxy group of **7i–k** by a benzylamine group (*figure 3*).

The pentacyclic pyridodithienotriazines **16a–c** were prepared as shown in *figure 4*, starting from the readily available 2-amino-6-chloro-3,5-dicyano-4-phenylpyridine **9** [23]. This route is based on the preparation of the β-enaminonitrile intermediate **14** and its diazotization to form the chloro-substituted triazine **15**, which affords **16** by nucleophilic displacement.

Thus, substituent R is introduced at the last stage by nucleophilic aromatic substitution of a chloro derivative. Several nucleophiles of nitrogen and oxygen have been prepared in this way (compounds **7** and **16**). Substituents R_1, and R_2 and the Ph group originate from the starting cyanopyridine (i.e. **9**). In this paper, two different combinations of R_1 and R_2, represented by 4-chloro-8-cyano-7-ethoxy-9-phenylpyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (**6a**) and 4-chloro-7,9-diphenylpyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (**6b**) are described. Nevertheless, R_1 can also be modified by nucleophilic aromatic substitution directly on the thienopyridine skeleton, and this has been carried out in the case of compounds **8a–c**.

All the compounds prepared in this work gave satisfactory elemental analyses and have spectral data (IR, MS, and ^{1}H-NMR and ^{13}C-NMR) that are consistent with the structures proposed (*tables I* and *II*).

3. Pharmacology

The inhibition of the release of histamine by the pyridothieno- and pyridodithienotriazines was measured following the same methodology as described previously [16]. We used rat mast cells and two different stimuli to

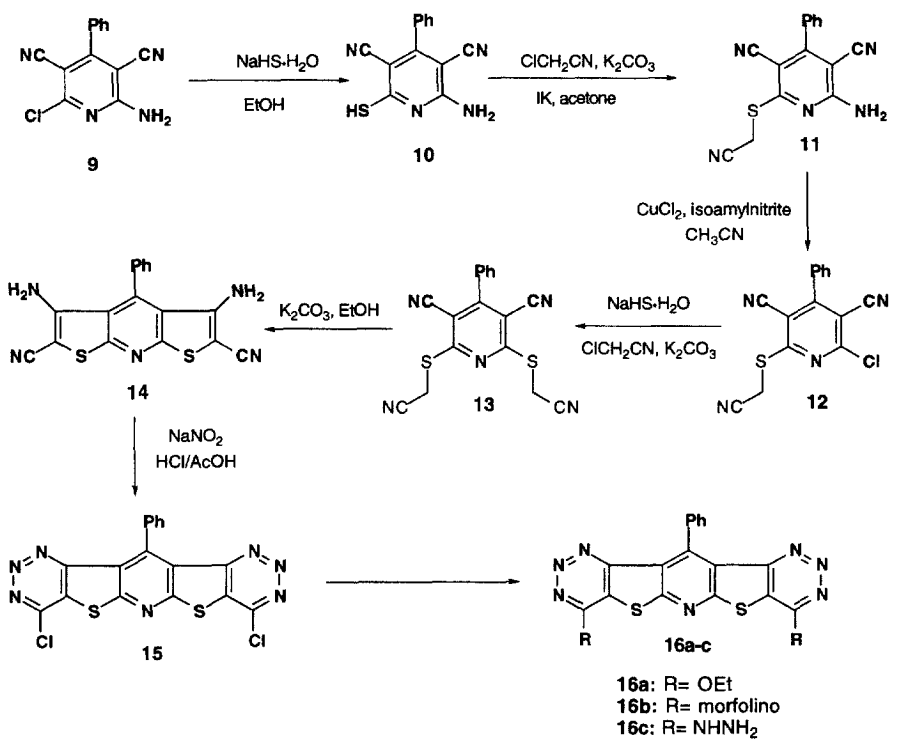
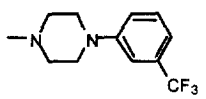
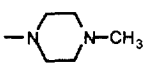
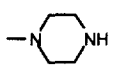
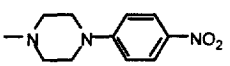
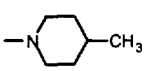
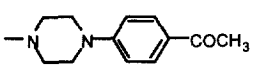
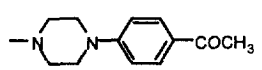
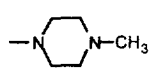
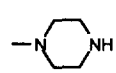
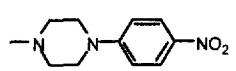
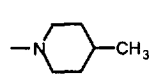
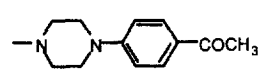


Figure 4.

Table I. Physicochemical data for pyridothienotriazines **6a**, **7a–n** and **8a–c**.

Compound [a]	R_1	R_2	R	M.p. (°C)	Yield (%)	Molecular formula [b]
6a	EtO	CN	Cl	210–212	86 [c]	$C_{17}H_{10}N_5OClS$
7a	EtO	CN	OEt	253–255	88 [d]	$C_{19}H_{15}N_5O_2S$
7b	EtO	CN	OMe	211–213	87 [e]	$C_{18}H_{13}N_5O_2S$
7c	EtO	CN	$NHNH_2$	220–222	80	$C_{17}H_{13}N_7OS$
7d	EtO	CN	NMe_2	242–244	48 [d]	$C_{19}H_{16}N_6OS$
7e	EtO	CN	H	176–179	10 [f]	$C_{17}H_{11}N_5OS$
7f	EtO	CN		255–257	56 [g]	$C_{28}H_{22}N_7OF_3S$
7g	EtO	CN		254–256	94 [h]	$C_{22}H_{21}N_7OS$
7h	EtO	CN		> 300	89 [i]	$C_{21}H_{19}N_7OS$
7i	EtO	CN		> 300	72 [f]	$C_{27}H_{22}N_8O_3S$
7j	EtO	CN		256–258	64 [d]	$C_{23}H_{22}N_6OS$
7k	EtO	CN		266–269	48 [f]	$C_{29}H_{25}N_7O_2S$
7l	Ph	H		298 dec	51 [d]	$C_{32}H_{26}N_6OS$
7m	Ph	H		209–211	70 [j]	$C_{25}H_{22}N_6S$
7n	Ph	H		> 300	72 [d]	$C_{24}H_{20}N_6S$
8a	$PhCH_2NH$–	CN		> 300	78 [f]	$C_{32}H_{25}N_9O_2S$
8b	$PhCH_2NH$–	CN		259–261	59 [f]	$C_{28}H_{25}N_7S$
8c	$PhCH_2NH$–	CN		245–247	60 [d]	$C_{34}H_{28}N_8OS$

[a] All spectra data were consistent with the assigned structures. [b] All compounds analyzed for C, H, N; analytical results were within ±0.4% of theoretical values. [c] Recrystallized from acetone. [d] Recrystallized from ethanol/dichloromethane. [e] Recrystallized from methanol/dichloromethane. [f] Purified by flash chromatography using as eluent dichloromethane. [g] Purified by flash chromatography using as eluent dichloromethane/hexane (2:1 v/v). [h] Recrystallized from ethanol/acetone. [i] Purified by flash chromatography using as eluent dichloromethane/ethanol (99:1, v/v). [j] Recrystallized from EtOH.

Table II. NMR and MS data of pyridothienotriazines **6a**, **7a–n** and **8a–c**.

Compound	^{1}H-NMR (δ, ppm) [a]	MS (FAB, *m/z*): (%) [b]
6a	1.58 (t, 3H, *J* = 7.1 Hz, CH_3); 4.75 (q, 2H, *J* = 7.1 Hz, CH_2O); 7.53–7.64 (m, 5H, C_6H_5)	367 (M^+, 20); 339 ($M^+ - N_2$, 57); 311 (100); 305 (43); 277 (89)
7a	1.53 (t, 3H, *J* = 7.1 Hz, CH_3); 1.58 (t, 3H, *J* = 7.1 Hz, CH_3); 4.71 (q, 2H, *J* = 7.1 Hz, CH_2O); 4.85 (q, 2H, *J* = 7.1 Hz, CH_2O); 7.58 (m, 5H, C_6H_5)	377 (M^+, 30); 349 ($M^+ - N_2$, 7); 320 (73); 292 (100); 264 (55)
7b	1.57 (t, 3H, *J* = 7.1 Hz, CH_3); 4.38 (s, 3H, CH_3O); 4.74 (q, 2H, *J* = 7.1 Hz, CH_2O); 7.58–7.64 (m, 5H, C_6H_5)	364 [$(MH)^+$, 100]; 336 [$(MH)^+ - N_2$, 25]; 320 (26); 292 (13)
7c [c]	1.44 (t, 3H, *J* = 7.1 Hz, CH_3); 4.64 (q, 2H, *J* = 7.1 Hz, CH_2O); 5.14 (br s, 2H, NH_2); 7.52–7.57 (m, 5H, C_6H_5); 9.77 (br s, 1H, NH)	
7d	1.43 (t, 3H, *J* = 7.1 Hz, CH_3); 3.41 (s, 6H, NMe_2); 4.60 (q, 2H, *J* = 7.1 Hz, CH_2O); 7.55–7.58 (m, 5H, C_6H_5)	377 [$(MH)^+$, 100]; 349 [$(MH)^+ - N_2$, 42]; 306 (7); 300 (20); 278 (13)
7e	1.52 (t, 3H, *J* = 7.1 Hz, CH_3); 4.64 (q, 2H, *J* = 7.1 Hz, CH_2O); 7.53–7.63 (m, 6H, C_6H_5 + CH)	305 ($M^+ - N_2$, 54); 277 (100); 249 (22)
7f	1.55 (t, 3H, *J* = 7.1 Hz, CH_3); 3.44 (t, 4H, *J* = 5.1 Hz, $N(CH_2)_2$); 4.23 (t, 4H, *J* = 5.1 Hz, $N(CH_2)_2$); 4.69 (q, 2H, *J* = 7.1 Hz, CH_2O); 7.13–7.60 (m, 9H, $C_6H_5 + C_6H_4$)	533 ($M^+ - N_2$, 22); 347 (61); 306 (77); 278 (100)
7g	1.64 (t, 3H, *J* = 7.1 Hz, CH_3); 2.17 (s, 3H, CH_3); 2.78 (t, 4H, *J* = 5.0 Hz, $N(CH_2)_2$); 3.25 (t, 4H, *J* = 5.0 Hz, $N(CH_2)_2$); 4.72 (q, 2H, *J* = 7.1 Hz, CH_2O); 7.60–7.65 (m, 5H, C_6H_5)	403 ($M^+ - N_2$, 86); 360 (90); 347 (87); 331 (93)
7h	1.52 (t, 3H, *J* = 7.1 Hz, CH_3); 3.46 (br s, 4H, $N(CH_2)_2$); 4.00 (br s, 4H, $N(CH_2)_2$); 4.70 (q, 2H, *J* = 7.1 Hz, CH_2O); 7.63 (m, 5H, C_6H_5)	417 (M^+, 5); 389 (2); 349 (100); 306 (18)
7i [c]	1.64 (t, 3H, *J* = 7.1 Hz, CH_3); 3.77 (br s, 4H, $N(CH_2)_2$); 4.20 (br s, 4H, NCH_2); 4.64 (q, 2H, *J* = 7.1 Hz, CH_2O); 7.00 (d, 2H, *J* = 9.3 Hz, C_6H_4); 8.10 (d, 2H, *J* = 9.3 Hz, C_6H_4); 7.57 (s, 5H, C_6H_5)	
7j	1.00 (d, 3H, *J* = 6.2 Hz, CH_3); 1.34 (m, 2H, CH_2); 1.53 (t, 3H, *J* = 7.1 Hz, CH_3); 1.60–1.91 (m, 3H,CH_2CH); 3.21 (dt, 2H, *J* = 2.3 Hz, *J* = 12.5 Hz, NCH_2); 4.66 (q, 2H, *J* = 7.1 Hz, CH_2O); 4.72 (dt, 2H, *J* = 12.5 Hz, *J* = 2.0 Hz, NCH_2); 7.51–7.59 (m, 5H, C_6H_5)	430 (M^+, 16); 402 (14); 373 (15); 331 (13)
7k	1.55 (t, 3H, *J* = 7.1 Hz, CH_3); 2.55 (s, 3H, CH_3); 3.59 (t, 4H, *J* = 5.0 Hz, $N(CH_2)_2$); 4.26 (t, 4H, *J* = 5.0 Hz, $N(CH_2)_2$); 4.67 (q, 2H, *J* = 7.1 Hz, CH_2O); 6.98 (d, 2H, *J* = 8.7 Hz, C_6H_4); 7.93 (d, 2H, *J* = 8.7 Hz, C_6H_4); 7.56 (s, 5H, C_6H_5)	536 [$(MH)^+$, 15]; 508 ($M^+ - N_2$, 8); 307 (17)
7l	2.56 (s, 3H, $COCH_3$); 3.66 (t, 3H, *J* = 5.0 Hz, NCH_2); 4.30 (t, 3H, *J* = 4.8 Hz, NCH_2); 6.94 (d, 2H, *J* = 8.8 Hz, C_6H_4); 7.93 (d, 2H, *J* = 8.8 Hz, C_6H_4); 7.52–7.73 (m, 8H, C_6H_5); 7.92 (s, 1H, H-8); 8.17–8.22 (m, 2H, C_6H_5)	543 [$(MH)^+$, 26]; 515 [$(MH)^+ - N_2$, 61]; 430 (23); 313 (25)
7m	2.35 (s, 3H, NCH_3); 2.58 (br s, 4H, NCH_2); 4.06 (br s, 4H, NCH_2); 7.45–7.64 (m, 8H, C_6H_5); 7.81 (s, 1H, H-8); 8.10 (s, 2H, C_6H_5)	439 [$(MH)^+$, 100]; 411 [$(MH)^+ - N_2$, 26]; 313 (13)
7n	4.21 (br s, 4H, NCH_2); 7.56–7.73 (m, 8H,C_6H_5); 8.16 (s, 1H, H-8); 8.32–8.34 (m, 2H, C_6H_5); 8.98 (br s, 1H, NH)	425 [$(MH)^+$, 100]; 397 [$(MH)^+ - N_2$, 49]; 363 (13); 354 (11); 313 (36)
8a	3.40 (s, 4H, NCH_2); 4.11 (s, 4H, NCH_2); 4.71 (s, 2H, $NHCH_2$); 7.01 (d, 2H, *J* = 8.3 Hz, C_6H_4); 8.09 (d, 2H, *J* = 8.3 Hz, C_6H_4); 7.33–7.54 (m, 11H, $2C_6H_5$ + NH)	600 [$(MH)^+$, 2]; 571 [$(MH)^+ - N_2$, 2]; 560 (3); 514 (4); 499 (3); 391 (23)
8b	0.99 (d, 3H, *J* = 6.1 Hz, CH_3); 1.27–1.33 (m, 2H, CH_2); 1.81–1.87 (m, 3H, CH_2 + CH); 3.11–3.24 (m, 2H, NCH_2); 4.73–4.82 (m, 2H, NCH_2); 4.83 (d, 2H, *J* = 5.5 Hz, CH_2NH); 6.07 (t, 1H, *J* = 5.5 Hz, NH); 7.31–7.58 (m, 10H, $2C_6H_5$)	492 [$(MH)^+$, 19]; 464 (9), 389 (100)
8c	2.53 (s, 3H, CH_3); 3.57 (t, 4H, *J* = 4.8 Hz, $N(CH_2)_2$); 4.20 (t, 4H, *J* = 4.8 Hz, $N(CH_2)_2$); 4.84 (d, 2H, *J* = 5.5 Hz,CH_2NH); 6.16 (t, 1H, *J* = 5.5 Hz, NH); 6.91 (d, 2H, *J* = 8.7 Hz, C_6H_4); 7.90 (d, 2H, *J* = 8.7 Hz, C_6H_4); 7.34–7.58 (m, 10H, $2C_6H_5$)	597 [$(MH)^+$,10]; 583 (24); 569 [$(MH)^+ - N_2$, 10]; 541 (30); 527 (21); 396 (14); 391 (34)

[a] All the spectra were run in $CDCl_3$ except for compounds **7c**, **7d**, **7h**, **7i**, **7n** and **8a** (CD_3SOCD_3). [b] MS (EI) *m/z* (%) for **6a**, **7a**, and **7f–j**. [c] No useful FAB or EI mass spectra data could be obtained from compounds **7c** and **7i**.

Table III. Cytotoxic activity of selected pyridothienotriazines.

Compound	IC_{50} (μg/mL)			
	P-388	A-549	HT-29	MEL
6a	0.25	0.25	0.25	0.25
7a	10	> 10	> 10	> 10
7d	10	> 10	> 10	> 10
7g	2.5	2.5	2.5	2.5
7h	1	2.5	2.5	1
7j	> 2	> 2	> 2	> 2
7k	5	10	> 10	10
7l	5	5	10	5
7m	2	2	2	2
7n	1	2	2	2
8b	5	10	1	20
15	0.05	0.05	0.05	0.5

provoke the relase of histamine; the polymer 48/80 as the chemical stimulus [24] and ovoalbumin as the immunological stimulus. In addition, the inhibitory action was measured under two different sets of experimental conditions; simultaneous addition of the stimulus and the drug to the cells, and with preincubation (i.e. by addition of the chemical 10 min before the stimulus).

The results of all these experiments are shown in *figures 5–8* and are discussed in the following section. The cytotoxicity was also measured and is presented in *table III*.

4. Results and discussion

4.1. Histamine release by immunological stimulus

Most of the thienotriazines and dithienotriazines studied show an inhibitory effect on the release of histamine when they are added, simultaneously with the immunological stimulus, to the rat mast cells (*figure 5*). Particularly active are **7b**, **7d**, **7f**, **7k**, **7m**, **7n** and the dithienotriazines **16a**, **16b** and **16c**, which all produce inhibition values around 40–50%.

Only one of the compounds studied, **7a**, acts as an inducer of the liberation of histamine, and its action is very weak.

In the experiments performed with the compound added 10 min before the stimulus (preincubation; *figure 6*), the general pattern of activity is similar and practically all the same compounds as before show inhibitory action. It is interesting to note that **16a** is, in this assay, a much stronger inhibitor (85%) than in the

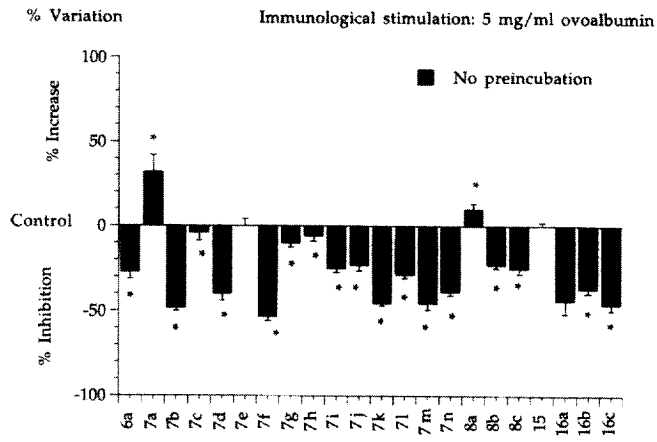


Figure 5. Histamine release in mast rat cells stimulated with 5 mg/mL of antigen (ovoalbumin) in the presence of thienotriazines. The compounds were added to the cells simultaneously with the stimulus (no preincubation). The activity data are normalized to the response of the control experiments (45.2 ± 1.1% in the presence of 5 mg/mL of antigen). The asterisks indicate the statistical significant results.

previous study (without preincubation), and an inversion in behaviour is observed in **7a**, which is now an inhibitor (30%), and **7l**, which is now an inducer of the liberation of histamine.

4.2. Histamine release by chemical stimulus

In addition to the experiments carried out with immunological stimulus, pyridothieno- and dithienotriazines

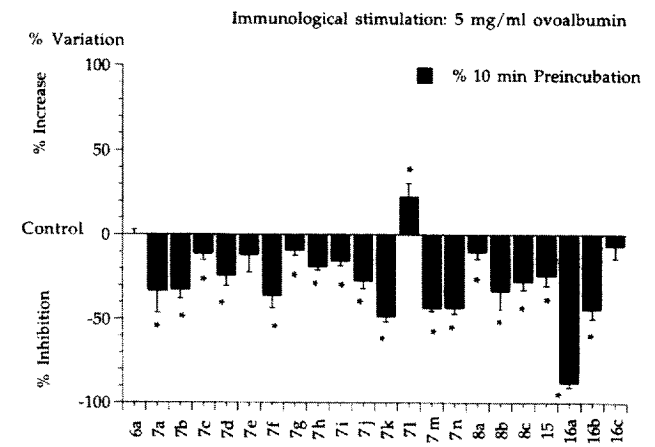


Figure 6. Histamine release in mast rat cells stimulated with 5 mg/mL of antigen (ovoalbumin) in the presence of thienotriazines. The compounds were added to the cells 10 min before the addition of the stimulus (10 min preincubation). The activity data are normalized to the response of the control experiments (47.4 ± 4.3% in the presence of 5 mg/mL of antigen). The asterisks indicate the statistical significant results.

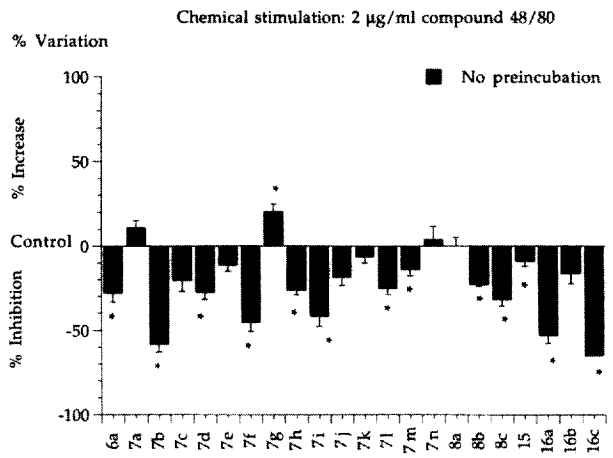


Figure 7. Histamine release in mast rat cells stimulated with 2 µg/mL of compound 48/80 in the presence of thienotriazines. The compounds were added to the cells simultaneously with the stimulus (no preincubation). The activity data are normalized to the response of the control experiments (21.05 ± 2.4% in the presence of 2 µg/mL of compound 48/80). The asterisks indicate the statistical significant results.

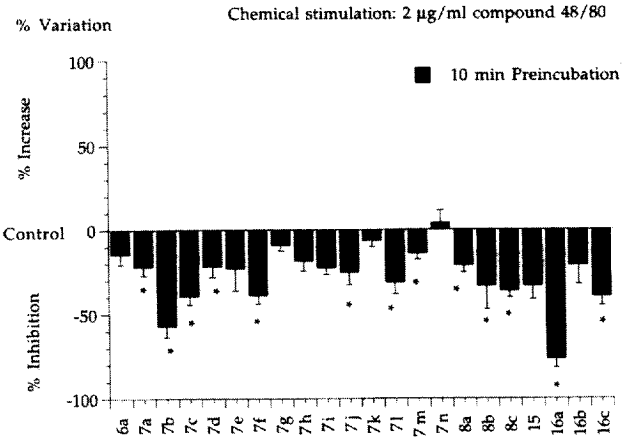


Figure 8. Histamine release in mast rat cells stimulated with 2 µg/mL of compound 48/80 in the presence of thienotriazines. The compounds were added to the cells 10 min before the addition of the stimulus (10 min preincubation). The activity data are normalized to the response of the control experiments (32.7 ± 6.2% in the presence of 2 µg/mL of compound 48/80). The asterisks indicate the statistical significant results.

were tested for their action when the release of histamine was induced by a non-immunological stimulus such as the polymer 48/80.

When the experiments were carried out without preincubation (*figure 7*), five compounds (**7b**, **7f**, **7i**, **16a**, and **16c**) showed an inhibitory action of 40% or higher, with **7b** and the pyridodithienotriazines **16a** and **16c** being the most active in this experiment by producing up to 65% inhibition.

Figure 8 shows that if the cells are preincubated with the compounds before addition of the chemical inducer, practically all the compounds tested produce inhibition values that are statistically significative. However, only one pyridothienotriazine **7b** (60% inhibition) and one pyridodithienotriazine **16a** (75% inhibition) are really active in this series.

The antineoplastic drugs adryamicin and vinorelbine have been reported to produce an effect similar to that of 48/80 on mast cells [25, 26]. For this reason we decided to measure the release of histamine induced by these two drugs in the presence of thienotriazines **7c** and **7f–7j**.chosen at random.

The results, which are not shown, indicate that in the experiments carried out with preincubation of the cells with the thienotriazines before addition of the antineoplastic drug adryamicin, all six compounds behave as similar inhibitors (30–50% inhibition). Similar experiments with vinorelbine as the histamine releaser, added without preincubation, showed a very selective behaviour of the compounds examined: **7f**, **7h**, **7i**, and **7j** are inhibitors (**7f** and **7h**: 40–50%; **7j**: 70% and **7i**: 90% inhibition), while **7g** and **7c** are very weak inducers (10–20%).

4.3. Structure–activity relationship

For the SAR analysis, the pyridothienotriazines examined have been divided into groups according to their substituents R, R_1, R_2 and the type of skeleton.

We first analysed the influence of the diverse R substituents (nitrogen, oxygen, chlorine and hydrogen) in the series of compounds where R_1 and R_2 are EtO and CN respectively. Thus, among the nitrogen-substituted compounds, only **7d**, **7f**, and **7k** show significant inhibitory action. The oxygen-substituted compound **7b** is a good inhibitor in practically all the experiments, while its analogue **7a** surprisingly acts as an inducer of the liberation of histamine in some assays. For their part, the hydrogen and chloro derivatives **6a** and **7e** are inactive.

Subsequent comparison of compounds **7g**, **7i** and **7k** with **8a**, **8b** and **8c** was used to analyse the influence of substituents R_1/R_3. The data show that the change from R_1 = OEt to R_1 = aminobenzyl does not produce any modification in the activity for the first two compounds

mentioned, while the effect on the last one is unclear: **7k** is more active than **8c** under immunological conditions, but the opposite occurs when chemical stimulus is used.

A different combination of substituents R_1 and R_2 was also examined. This is illustrated by comparison of **7g**, **7h**, **7k** (R_1 = EtO, R_2 = CN) with **7l**, **7m**, **7n** where R_1 and R_2 are Ph and H respectively. The data in *figures 5–8* show that this change is associated with a significant increase in inhibitory activity and, while **7g** and **7h** are inactive, **7m** and **7n** are good inhibitors.

The comparative activity of **7k** and its counterpart **7l** is far from clear. Both are moderate inhibitors when stimulated by 48/80 (simultaneous addition and preincubated) and ovoalbumin (without incubation), but when the immunologically driven experiment was carried out with preincubation **7l** is an inducer of histamine liberation.

The third structural change studied is related to the skeletal change represented by dithienopyridotriazines **15** and **16a–c**. *Figures 5–8* show that **16a** and **16c**, both carrying simple oxygen and nitrogen substituents, are two of the most active compounds described in this paper. Comparison with thienotriazines **6a**, **7a**, and **7c**, which have the same R substituent, illustrates the importance of this structural change for inhibitory activity: **6a** and **15** are both very moderate inhibitors but **16a** and **16c** are much more active than their counterparts **7a** and **7c**.

4.4. Cytotoxic activity

Pyridothieno- and pyridodithienotriazines have been tested in vitro for their potential antitumoral activity against four standard tumoral cell lines P388 (lymphoid neoplasm) and human A-549 (colon carcinoma), HT-29 (lung carcinoma) and MEL-28 (malignant melanoma) cell lines. The selection of IC_{50} values presented in *table III* shows that some thienotriazines and one dithienotriazine are active in these assays. The most active compounds are **6a** (R = Cl; IC_{50} = 0.25 μg/mL) and **15** (IC_{50} = 0.05 μg/mL). These two compounds are characterized by R = Cl, suggesting a common mechanism of action that is perhaps related to the alkylating properties of the masked imminiun ion.

5. Conclusions

In conclusion, many compounds containing the pyridothienotriazine heterocyclic system show some inhibitory action on the liberation of histamine. Among those studied, the most interesting are the thieno- (**7b**) and the dithieno- (**16a**) compounds which are strong inhibitors under all the conditions tested and, to a lesser extent, compound **16c** that is a very good inhibitor in tall conditions except when the immunological stimulus is preincubated. Other thieno derivatives, such as **7k**, **7m** and **7n**, were good inhibitors in the immunological experiments but practically inactive under chemical stimulus.

In contrast to related heterocyclic series examined [16], there are no histamine-release inducers of importance among the thieno- and dithienotriazines.

The in vitro cytotoxic activity of several of the heterocycles presented in this work is interesting. In particular, the pyridothieno- (**6a**) and pyridodithieno- (**15**) compounds, which show IC_{50} values well under 1 μg/mL against several human and mouse tumoral cell lines, are remarkable.

6. Experimental protocols

6.1. Biological methods

The histamine releaser, Compound 48/80, a condensation product of N-methyl-*p*-methoxy-phenethylamine, was obtained from Sigma Chemical Co. (St Louis, MO, USA); orthophtalaldehyde from Merck (Darmstadt, Germany), and *Bordetella pertussis* from Wako (Germany).

6.1.1. Mast cell preparation

Mast cells were obtained by lavage of pleural and peritoneal cavities of Sprague–Dawley rats (200–400 g) as previously described [27]. Physiological saline composition was (mM): Na^+, 142.3; K^+, 5.94; Ca^{+2}, 1; Mg^{+2}, 1.2; Cl^-, 126.1; CO_3^{-2}, 22.85; $PO_4H_2^-$, 1.2; SO_4^{-2}, 1.2, giving a final osmotic pressure of 300 ± 5 mOms/Kg H_2O. One mg/mL of sucrose and bovine serum albumin (BSA) was also added to the solution, final pH being 7.0. The impurified cellular suspension contained 4–8% mast cells, with an average of 1.5–2 × 10^6 mast cells per rat.

6.1.2. Sensitization of rat mast cells

Sprague–Dawley rats weighting 200–300 g were sensitized by intramuscular injection in the back extremities of egg albumin (15 mg each rat) and adjuvant (9 × 10^9 killed *Bordetella pertussis* each rat) in saline solution. Two weeks later, the rats were sacrificed and the mast cells isolated.

6.1.3. Cell incubation

Twenty-five microliters of a freshly prepared concentrated solution of each drug in dimethylsulfoxide were added to 0.9 mL of incubation medium. When the medium reached 37 °C, 25 μL of cell suspension, containing 1–1.5 × 10^5 mast cells, were added and the cells were then incubated for 10 min (in the experiments performed without preincubation this step was omitted), and for 10 more minutes after addition of the stimulus. Since we used compound 48/80 as a reference drug, each experiment was carried out with parallel controls of compound 48/80. Incubations were stopped by immersing the tubes in a cold bath. After centrifugation at 1000 g_{max} for 5 min, the supernatants were collected and decanted into other tubes for histamine determination. We used trichloroacetic acid (7%, final concentration) to precipitate the

protein and avoid its interference with histamine determination (the presence of 1 mg/mL BSA interferes slightly with histamine determination). Appropriate controls to determine spontaneous histamine release in the absence of stimuli were executed in every experiment. Spontaneous histamine release was never higher than 8%.

6.1.4. Histamine release assay

Histamine was assayed spectrofluorometrically both in the pellet (residual histamine) and supernatants (released histamine) by Shore's method [28] in a spectrofluorometer Perkin-Elmer LS-50. Briefly, histamine reacts with 0.1% orthophtalaldehyde at alkaline pH, and the reaction is then stabilized by acidification, the fluorophore so obtained is stable for at least 2 h at room temperature. To ensure total histamine release, pellets were sonnicated for 60 s in 0.8 mL of 0.1 N HCl.

Results are expressed as a percentage of histamine released with respect to the total histamine content. Calculations were done substracting spontaneous histamine values from numerator and denominator. The trypan blue exclusion test was used in order to ensure that histamine release was not due to cytotoxicity.

Statistical analysis. Results were analyzed using the Student's *t*-test for unpaired data. A probability level of 0.05 or smaller was used for statistical significance. Results are expressed as the mean ± SEM, and all experiments were at least repeated three times in duplicate. Controls with cromoglycate were also carried out. A maximum 40% inhibitory effect was obtained with cromoglycate at 500 μg/mL, while at 100 μg/mL (the concentration used for all the compounds in this study), there was no inhibitory effect.

6.1.5. Antineoplasic assays

Eagle's minimum essential medium, Earle's balanced salts, nonessential aminoacids (EMEM/neaa), and L-glutamine were purchased from Biosciences and Fetal Calf Serum (FCS) and Trypsin from Seromed.

In vitro antitumor assays were performed by an adaptation of the method described by Bergeron et al. [29] and the activity screened against the following cell lines: P-388 (ATCC CCL 46; suspension culture of a lymphoid neoplasm from a DBA/2 mouse), A-549 (ATCC CCL 185; monolayer culture of a human lung carcinoma), HT-29 (ATCC HTB-38; monolayer culture of a human colon carcinoma), and MEL-28 (ATCC HTB-72; monolayer culture of a human melanoma). The cells were maintained in logarithmic growth in EMEM/neaa, supplemented with 5% Fetal Calf Serum (FCS), 10^{-2} M sodium bicarbonate and 0.1 g/L penicillin G + 0.1 g/L streptomycin sulfate and placed in 16 mm diameter wells at 1×10^4 (P-388), 2×10^4 (A-549, MEL-28, and HT-29) cells per well, respectively. The cytotoxicity was evaluated by addition of 1 mL aliquots of a solution of the compound in EMEM 5% FCS. A separate set of cultures without test compounds to be added, was used as control and to ensure that the cells remained in an exponential phase of growth all along the test. After three days of incubation at 37 °C in a 10% CO_2 and 98% H_2O atmosphere, the cells were trypsinized and counted in a Coulter Counter ZM. All counts (net cells per well), represent the average of duplicate wells. Comparison of the counts with those of control cultures, allows the calculation of the percentage of growth produced by each compound. The results obtained from cultures treated with different concentrations of each compound, are used to generate dose–response curves from which IC_{50} values were derived.

6.2. Chemistry

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. ^1H and ^{13}C-NMR spectra were obtained on a Bruker AC 200F instrument at room temperature. MS spectra were obtained on a VG QUATTRO spectrometer. 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. Analyses indicated by the symbols of the elements or functions were within ±0.4% of theoretical values and were performed by the Elemental Analyses General Service of the University of La Coruña.

6.2.1. 4-Chloro-8-cyano-7-ethoxy-9-phenylpyrido[3',2':4,5]thieno[3,2-d]-1,2,3-triazine ***6a***

To an ice-cooled solution of **5a** (2.0 g, 6.2 mmol) in 1:1 (v/v) HCl/AcOH (50 mL) a solution of sodium nitrite (0.83 g, 12.0 mmol) in H_2O (12.5 mL) was added. The mixture was stirred at room temperature for 24 h. The solution was poured into water and the solid material was filtered and recrystallized from acetone to yield **6a** (2.0 g, 86%) (*tables I* and *II*).

6.2.2. 8-Cyano-7-ethoxy-9-phenylpyrido[3',2':4,5]thieno[3,2-d]-1,2,3-triazines ***7a,b***

General procedure: A solution of **6a** (0.20 g, 0.54 mmol) in ethanol **7a** or methanol **7b** (10 mL), 10% NaOH (0.1 mL) was added. The mixture was stirred at room temperature for 15 min. The solid was filtered off and recrystallized to yield **7a** and **7b** (*tables I* and *II*).

6.2.3. 8-Cyano-7-ethoxy-4-hydrazino-9-phenylpyrido[3',2':4,5]thieno[3,2-d]-1,2,3-triazine ***7c***

A solution of **6a** (0.10 g, 0.27 mmol) in 3:1 (v/v) THF/EtOH (20 mL), 80% $NH_2NH_2 \bullet H_2O$ (0.01 mL, 0.27 mmol) was added at 5 °C. The mixture was stirred at room temperature for 8 h. The solid was filtered off and the crude solid was used in the next step without further purification (*tables I* and *II*).

6.2.4. 8-Cyano-7-ethoxy-4-dimethylamino-9-phenylpyrido[3',2':4,5]thieno[3,2-d]-1,2,3-triazine ***7d***

A solution of **6a** (0.40 g, 1.08 mmol) and 10% NaOH (1 mL) in DMF (2 mL) was stirred at 50–60 °C for 30 min. After cooling, the reaction mixture was poured into H_2O (50 mL). The mixture was extracted with CH_2Cl_2 (3 × 50 mL) and washed with 20% HCl (2 × 30 mL). The solvent was removed under reduced pressure and the resulting solid was purified by recrystallized from ethanol/dichloromethane to yield **7d** (0.18 g, 48%) (*tables I* and *II*).

6.2.5. 8-Cyano-7-ethoxy-9-phenylpyrido[3',2':4,5]thieno[3,2-d]-1,2,3-triazine ***7e***

A solution of **7c** (0.20 g, 0.55 mmol) and HgO (0.15 g, 0.68 mmol) in H_2O (14 mL) was refluxed for 48 h. After cooling, the reaction mixture was filtered off. The solid in dichloromethane (15 mL) was treatment with activated charcoal. The mixture was filtered off and the solvent was removed under reduced pressure. The resulting solid was purified by flash chromatography using CH_2Cl_2 as eluent to yield **7e** (0.02 g, 10%) (*tables I* and *II*).

6.2.6. 9-Phenylpyrido[3',2':4,5]thieno[3,2-d]-1,2,3-triazines ***7f–n***

General procedure: A solution of **6a** or **6b** (0.54 mmol) and the appropriate amine (0.32 mmol) in 1:1 (v/v) THF/EtOH (30 mL) was refluxed until starting material had disappeared as checked by tlc. The solvent was removed under reduced pressure and the residue was purified by flash chromatography or recrystallized to yield **7f–n** (*tables I* and *II*).

6.2.7. 7-Benzylamino-8-cyano-9-phenylpyrido[3',2':4,5]thieno [3,2-d]-1,2,3-triazines ***8a–c***

General procedure: A solution of the appropriate triazine **7i–k** (0.22 mmol) in benzylamine (5 mL) was refluxed until starting material had disappeared as checked by tlc. The reaction mixture was poured into H_2O (30 mL) and the solution was neutralized with 2 N HCl. The mixture was extracted with CH_2Cl_2 (3 × 15). The solvent was removed under reduced pressure and the resulting solid was purified by flash chromatography or the solid was filtered off and recrystallized to yield **8a–c** (*tables I* and *II*).

6.2.8. 2-Amino-3,5-dicyano-4-phenylpyridine-6-(1H)-thione ***10***

A mixture of **9** (0.18 g, 0.70 mmol) and $NaHS{\bullet}H_2O$ (0.18 g) in ethanol (100 mL) was stirred at room temperature for 24 h. The solvent was removed under reduced pressure and water (50 mL) was added. The solution was acidified with 2 N HCl and the deposited solid was filtered off to yield **10** (0.13 g, 73%). The crude solid was used in the next step without further purification. M.p.: 229–231 °C; Anal. $C_{13}H_8N_4S$; (C, H, N); ^{1}H-NMR (acetone-d_6): δ =7.55 (s, 5H, C_6H_5), 12.10 (br s, 1H) ppm; ^{13}C-NMR (acetone-d_6): δ =68.8, 76.7 (C-3, C-5), 114.8 (CN), 116.6 (CN), 128.9, 129.5, 131.3, 135.5 (C_6H_5), 159.6, 161.0, 164.3 ppm. MS (EI): *m/z* (%) = 252 (M^+, 9), 238 (21), 222 (11), 216 (13); IR (KBr): ν =3300, 3200 (NH), 2210 (CN), 1650 cm^{-1}.

6.2.9. 2-Amino-3,5-dicyano-6-cyanomethylthio-4-phenylpyridine ***11***

A mixture of **10** (0.13 g, 0.51 mmol), chloroacetonitrile (45 mg, 0.51 mmol), potassium carbonate (84 mg, 0.61 mmol) and a catalytic amount of KI in acetone (15 mL) was stirred at room temperature for 30 min. The insoluble solid was removed by filtration, and washed with acetone. The filtrate and the washings were combined and evaporated. The residual solid was purified by flash chromatography using CH_2Cl_2 as eluent to yield **11** (0.15 g, 98%). M.p.: 269–271 °C; Anal. $C_{15}H_9N_5S$ (C, H, N); ^{1}H-NMR (DMSO-d_6): δ =4.33 (s, 2H, CH_2), 7.56 (s, 5H, C_6H_5), 8.24 (br s, 2H, NH_2) ppm; ^{13}C-NMR (DMSO-d_6): δ = 15.6 (SCH_2), 87.1, 93.3 (C-3, C-5), 114.9, 115.0 (CN), 117.4 (CN), 128.4, 128.8, 130.5, 133.7 (C_6H_5), 158.8, 159.7, 163.3 ppm; MS (EI): *m/z* (%) = 291 (M^+, 8),288 (5), 278 (5), 252 (12), 236 (7), 231 (14), 215 (22); IR (KBr): ν = 3400, 3300, 3250 (NH), 2220, 2210 (CN), 1650, 1540, 1510 cm^{-1}.

6.2.10. 2-Chloro-3,5-dicyano-6-cyanomethylthio-4-phenylpyridine ***12***

To a stirred solution of anhydrous $CuCl_2$ (0.5 g, 4.0 mmol), isoamyl nitrite (0.5 g, 4.0 mmol) in dry CH_3CN (100 mL), **11** (1.0 g, 3.5 mmol) was added. The reaction mixture was stirred at room temperature for 48 h under Ar. The reaction mixture was poured into 20% HCl (200 mL). The mixture was extracted with CH_2Cl_2 (3 × 50 mL). The organics layers were combined and evaporated under reduced pressure and the resulting solid was purified by flash chromatography using CH_2Cl_2 as eluent to yield **12** (0.28 g, 53%). M.p.: 206–209 °C; Anal. $C_{15}H_7N_4ClS$ (C, H, N); ^{1}H-NMR ($CDCl_3$): δ =4.10 (s, 2H, CH_2), 7.53–7.72 (m, 5H, C_6H_5) ppm; ^{13}C-NMR ($CDCl_3$): δ = 16.7 (CH_2), 106.3, 107.6 (C-3, C-5), 112.3 (CN), 113.0 (CN), 114.6 (CN), 128.5, 129.5, 131.3, 132.1 (C_6H_5), 156.4, 159.3, 164.2 ppm; MS (FAB): *m/z* (%) = 313 [$(MH)^+$ + 2, 16], 311 [$(MH)^+$, 22], 299 (39), 292 (88); IR (KBr): ν = 2230 (CN), 2220 (CN), 1570, 1540, 1520, 1440, 1400 cm^{-1}.

6.2.11. 3,5-Dicyano-2,6-dicyanomethylthio-4-phenylpyridine ***13***

A mixture of **12** (0.20 g, 0.64 mmol) and $NaSH{\bullet}H_2O$ (2.40 g) in acetone (20 mL) was stirred at room temperature for 15 min. A solution of chloroacetonitrile (80 mg, 1.0 mmol), potassium carbonate (91 mg, 0.65 mmol) and a catalytic amount of KI in ethanol (15 mL) was added. The mixture was stirred at room temperature for 15 h. The insoluble solid was removed by filtration, and washed with acetone. The filtrate and the washings were combined and evaporated. The residual solid purified by flash chromatography using CH_2Cl_2 as eluent to yield **13** (0.21 g, 98%). M.p.: 236–238 °C; Anal. $C_{17}H_9N_5S_2$ (C, H, N); ^{1}H-NMR ($CDCl_3$): δ =4.18 (s, 2H, CH_2), 7.56–7.58 (m, 5H, C_6H_5) ppm; ^{13}C-NMR ($CDCl_3$): δ = 17.0 (CH_2), 103.7 (C–CN), 112.8 (CN), 116.4 (CN), 128.5, 129.4, 131.3, 131.9 (C_6H_5), 157.2, 164.6 ppm; MS (EI): *m/z* (%) = 347 (M^+, 3), 321 (3), 308 (4), 305 (6); 231 (25); IR (KBr): ν = 2220 (CN), 2210 (CN), 1550, 1390, 1330 cm^{-1}.

6.2.12. 3,5-Diamino-2,6-dicyano-4-phenyldithieno[3',2'-e:2,3-b]pyridine ***14***

A mixture of **13** (0.15 g, 0.43 mmol) and potassium carbonate (0.12 g, 0.89 mmol) in ethanol (20 mL) was stirred at room temperature for 30 min. The solid was filtered off to yield **14** (0.10 g, 67%). The solid was used in the next step without further purification. M.p.: > 270 °C; Anal. $C_{17}H_9N_5S_2$ (C, H, N); ^{1}H-NMR (DMSO-d_6): δ =5.39 (br s, 4H, exchangeable with D_2O, NH_2), 7.67–7.78 (m, 5H, C_6H_5) ppm; ^{13}C-NMR (DMSO-d_6): δ =73.1 (C-2), 114.9 (CN), 118.6 (C-3a), 128.5, 129.6, 131.0 (C_6H_5), 143.6 (C-2), 150.4 (C-7a), 160.8 (C-4) ppm; MS (EI): *m/z* (%) = 347 (M^+, 77), 319 (7), 221 (12); IR (KBr): ν = 3500, 3450 (NH), 2200 (CN), 1610, 1560, 1540, 1240 cm^{-1}.

6.2.13. 4,8-Dichloro-12-phenylpyrido[5',6':4'',5'';3',2':4,5] dithieno[3'',2''-d':3,2-d]-1,2,3-ditriazine ***15***

To an ice-cooled solution of **14** (0.2 g, 0.57 mmol) in HCl/AcOH (1:1, 30 mL) a solution of sodium nitrite (0.15 g, 2.17 mmol) in H_2O (2.5 mL) was added. The mixture was stirred for 15 h. The solution was poured into water and the solid material was filtered and purified by flash chromatography using CH_2Cl_2 as eluent to yield **15** (0.18 g, 71%). M.p.: 245–247 °C; Anal. $C_{17}H_5N_7Cl_2S_2$ (C, H, N); ^{1}H-NMR ($CDCl_3$): δ =7.53–7.94 (m, 5H, C_6H_5) ppm; ^{13}C-NMR ($CDCl_3$): δ = 122.8 (C-11a, C-12a), 128.3, 128.9, 130.7, 131.4 (C_6H_5), 131.5, 151.8, 151.9, 153.3 (C-5a, C-6a), 165.1 (C-12) ppm; MS (FAB): *m/z* (%) = 444 [$(MH)^+$ + 2, 5], 442 [$(MH)^+$, 8], 441 (14), 415 (4), 401 (8), 384 (8), 355 (12); IR (KBr): ν = 1550, 1500, 1450, 1440, 1280, 1220 cm^{-1}.

6.2.14. 4,8-Diethoxy-12-phenylpyrido[(5',6':4''),(5'';3',2':4,5)] dithieno[3'',2''-d':3,2-d]-1,2,3-ditriazine ***16a***

A solution of **15** (0.16 g, 0.36 mmol) in ethanol (20 mL), 10% NaOH (0.1 mL) was added. The mixture was stirred at room

temperature for 15 min. The solid was filtered off and recrystallized from CH_2Cl_2/EtOH to yield **16a** (0.14 g, 83%). M.p.: > 270 °C; Anal. $C_{21}H_{15}N_7O_2S_2$ (C, H, N); ^{1}H-NMR ($CDCl_3$): δ =1.58 (t, 6H, *J* = 7.1 Hz, CH_3), 4.88 (q, 4H, *J* = 7.1 Hz, CH_2), 7.58–7.69 (m, 5H, C_6H_5) ppm; ^{13}C-NMR ($CDCl_3$): δ = 14.4 (CH_3) 65.3 (CH_2), 122.8 (C-11a), 128.3, 128.5, 128.7, 130.0 (C_6H_5), 132.6, 149.6, 152.4 (C-5a), 159.8 (C-4), 164.7 (C-12) ppm; MS (EI): *m/z* (%) = 461 (M^+, 14), 348 (70), 346 (75), 320 (56), 304 (34); IR (KBr): ν = 1550, 1470, 1450, 1380 cm^{-1}.

6.2.15. 4,8-Dimorpholino-12-phenylpyrido[(5',6':4'',5''),(3',2': 4,5)]dithieno[3'',2''-d':3,2-d]-1,2,3-ditriazine ***16b***

A solution of **15** (0.15 g, 0.34 mmol) and morpholine (0.07 g, 0.81 mmol) in DMF (25 mL) was stirred at 50–60 °C for 30 min. The solid was filtrated off and recrystallized from ethanol/acetone to yield **16b** (0.13 g, 70%). M.p.: > 270 °C; Anal. $C_{25}H_{21}N_9O_2S_2$ (C, H, N); ^{1}H-NMR ($CDCl_3$): δ =3.87 (br s, 8H, CH_2N), 4.03 (br s, 8H, CH_2O), 7.23–7.59 (m, 5H, C_6H_5) ppm; ^{13}C-NMR ($CDCl_3$): δ = 46.3 (CH_2N), 66.5 (CH_2O), 114.1, 122.7 (C-11a), 128.2, 128.6, 129.5, 133.4 (C_6H_5), 149.5, 150.8, 153.1, 163.1 (C-12) ppm; MS (FAB): *m/z* (%) = 544 [$(MH)^+$, 25], 527 (12), 516 (9), 471 (60), 412 (11), 363 (21); IR (KBr): ν = 1550, 1440, 1360 cm^{-1}.

6.2.16. 4,8-Dihydrazino-12-phenylpyrido[(5',6':4'',5''),(3',2': 4,5)]dithieno[3'',2''-d':3,2-d]1,2,3-ditriazine ***16c***

To an ice-cooled solution of **15** (0.15 g, 0.34 mmol) in DMF (15 mL), 80% $NH_2NH_2 \bullet H_2O$ (0.03 mL, 0.81 mmol) was added at 5 °C. The mixture was stirred at room temperature for 24 h. The solid was filtered off to yield **16c** (0.13 g, 88%). The crude solid was used in the next step without further purification. M.p.: > 270 °C; Anal. $C_{17}H_{11}N_{11}S_2$ (C, H, N); ^{1}H-NMR ($CDCl_3$): δ =5.15 (br s, 2H), 7.47–7.57 (m, 5H, C_6H_5), 9.77 (br s, 1H) ppm; MS (EI): *m/z* (%) = 347 (100); IR (KBr): ν = 3310, 3200 (NH), 1650, 1550, 1450, 1380 cm^{-1}.

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References

[1] Thomas L.L., Chem. Immunol. 61 (1995) 186–207.

[2] Fleming D.M., Crombie D.L., Br. Med. J. 294 (1987) 279–28.

[3] Sano A., Masami I., Yoshihara J., Sumino M., Nawa H., Chem. Pharm. Bull. 43 (1995) 683–685.

[4] Cox J., Beach J., Blair M., Clarke A., King J., Lee T., Loveday D., Moss G., Orr T., Ritchie J., Sheard P., Adv. Drugs Res. 5 (1970) 115–196.

[5] Assem E., Ritcher A., Immunol. Lond. 21 (1971) 729–730.

[6] Foreman J., Garland L., Br. Med. J. 1 (1976) 820–821.

[7] Unangst P.C., Brown R.E., Herzig D.J., J. Med. Chem. 23 (1980) 1251–1255.

[8] Klaubert D.H., Sellstedt J.H., Ginosso C.J., Bell S.C., Capetola S.J., J. Med. Chem. 24 (1981) 748–752.

[9] Phillip A., Jirkousky I., Martel R.R., J. Med. Chem. 23 (1980) 1372–1376.

[10] Schwender C.F., Sunday B.R., Kerbleski J.J, Herzig D.J., J. Med. Chem. 23 (1980) 964–972.

[11] Madding G.D., Thompson M.D., J. Heterocycl. Chem 24 (1987) 581–586.

[12] Sherlock M.H., Kaminski J.J., Tom W.C., Lee J.F., Wong S.C., Kretner W., Bryant R.W., McPhail A.T., J. Med. Chem. 31 (1988) 2108–2121.

[13] Kreutner W., Sherwood J., Sehring S., Rizzo C., Chapman R.W., Siegel M.I., Egan R.W., J. Pharmacol. Exp. Ther. 247 (1988) 997–1003.

[14] Youssefyeh R.D., Brown R.E., Uresh Shah J.W., Jones H., Loev B., Khandwala A., Liebowitz M.J., Jonnino-Goldman P., J. Med. Chem. 27 (1984) 1639–1643.

[15] Khandwala A., Van Inwegen R., Coutts S., Dally-Meade V., Youssefyeh R.D., Int. J. Immunopharmacol. 5 (1983) 491–502.

[16] Quintela J.M., Peinador C., Botana L., Estevez M., Riguera R., Biorganic Med. Chem 5 (1997) 1543–1553.

[17] Beck J.R., Yahner J.A., J. Org. Chem. 41 (1976) 1733–1734.

[18] Clark J., Hitiris G., J. Chem. Soc. Perkin Trans. I (1984) 2005–2008.

[19] Peinador C., Moreira M.J., Quintela J.M., Tetrahedron 50 (1994) 6705–6714.

[20] Soto J.L., Seoane C., Rubio M.J., Botija J.M., Org. Prep. Proc. Int. 16 (1984) 11–24.

[21] Wagner G., Leistner S., Vieweg H., Krasselt U., Prantz J., Pharmazie 48 (1993) 514–518.

[22] Guerrera F., Salerno L., Sarva M.C., Siracusa M.A., Farmaco 48 (1993) 1725–1733.

[23] Peinador C., Veiga M.C., Quintela J.M., Heterocycles 38 (1994) 1299–1305.

[24] Scharemberg A.M., Kinet J.P., Chem. Immunol. 61 (1995) 72–87.

[25] Estevez M.D., Vieytes M.R., Louzao M.C., Alfonso A., Vilariño N., Botana L.M., Inflamm. Res. 46 (1997) 119–124.

[26] Estevez M.D., Vieytes M.R., Botana L.M., Agents Actions 42 (1994) 86–91.

[27] Estevez M.D., Vieytes M.R., Louzao M.C., Botana L.M., Biochem. Pharmacol. 47 (1994) 591–593.

[28] Shore P.A., Meth. Biochem. Anal. Suppl. (1971) 89–97.

[29] Bergeron R.J., Cavanaugh P.F., Kline S.J., Hughes R.G., Elliot G.T., Porter C.W., Biochem. Biophys. Res. Comm. 121 (1984) 848–854.