Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 1911–1925

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Antiallergic Activity of Pyridothienopyrimidines

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Received 20 November 1997; accepted 19 June 1998

Abstract—The synthesis of a series of pyridothienopyrimidines and their evaluation as inhibitors or inducers of the release of histamine from rat mast cells is reported. The activity was measured after immunological stimulation with ovoalbumin and chemical stimulation with polymer 48/80 and the drugs adryamicin and vinorelbine. The experiments were carried out with and without preincubation of the stimulus with the cells before addition of the drug. Several pyridothienopyrimidines show inhibitory IC_{50} values in the range 2–25 μM, indicating they are up to 100 times more potent than cromoglycate (DSCG) and 10 times greater than Ketotifen. Compound **9l** is a potent inhibitor in all the conditions tested and shows $IC_{50} = 9–25$ μM. Pyridothienopyrimidines **4l** and **9e** are very strong inducers of histamine release in the immunological (**4l**, 170–230%) and chemical (**9e**, 100–150%) assays, respectively. Compounds **4l** and **9i** are cytotoxic in vitro ($IC_{50} = 0.1–0.2$ μg/mL) against P-388, A-549, HT-29, and MEL-28 tumor cell lines.

Introduction

Asthma is an inflammatory disease that affects a significant portion of the population, with recent data suggesting that the numbers of asthma sufferers is increasing.[1] One of the most useful drugs for asthma treatment is disodium cromoglycate[2a,b] (DSCG, Chart 1). This compound inhibits IgE-mediated secretion of vasoactive mediators from mast cells both in vivo and in vitro, but unfortunately is not effective by oral administration and must be used as an insufflated powder. Several orally active drugs have been described and many of them are nitrogen or sulfur-containing heterocycles including tetrazoles,[3] naphthyridines,[4] pyridothienotriazines[5] such as RHC 2963, and thiophenes as Ketotifen[2c] (Chart 1).

Compounds containing a fused pyrimidine ring have attracted attention in the past few years owing to their wide range of biological activity, particularly in cancer and virus research.[6] Among these heterocycles, the thienopyrimidine class is also of interest because some derivatives, such as Tiprinast[7] (Chart 1), have been shown to be clinically effective antiallergics. In addition, antianaphilactic,[8] anti-inflammatory[9] analgesic and antipyretic,[10] and antineoplastic[11] activities have been described for these compounds.

Our previous work[12a] on the search for novel heterocyclic compounds of pharmacological interest has shown that certain guanadinocyanopyridines and pyridopyrimidines are good inhibitors, and other strong stimulants, of the release of histamine from mast rat cells. These findings, along with our interest in the development of structurally novel compounds acting as histamine release inhibitors, and the known effectiveness of pyridothienotriazine RHC2963[5] and thienopyrimidine Tiprinast[7] as immunological inhibitors of the release of histamine, prompted us to report here our results on the preparation and study of the inhibition of the release of histamine from rat mast cells and the antitumor activity of a series of new pyridothienopyrimidines shown in Table 1.

Key words: Pyridothienopyrimidine; synthesis; histamine; rat mast cells ; antiallergic; cytotoxicity.
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0968-0896/98/$—see front matter
PII: S0968-0896(98)00150-3

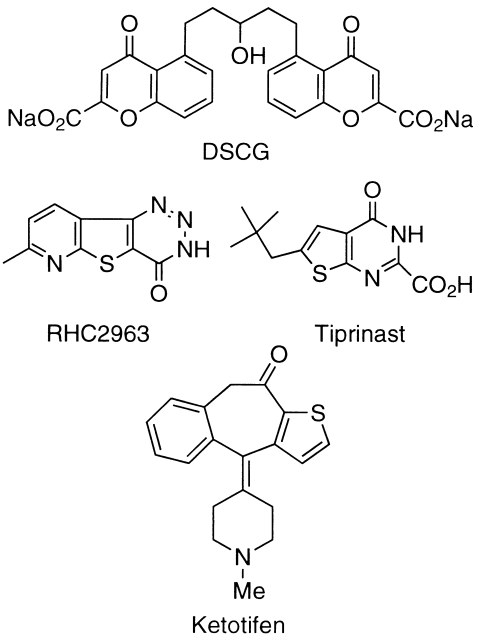


Chart 1.

Chemistry

For the preparation of the heterocycles we devised suitable procedures for the introduction of a variety of substituents with a minimum change in the nature of the chemistry used. Thus, substituents R, R_1, and R_2 are present in the pyridine or thienopyridine used as the starting material. R can be either an aryl ring or hydrogen, R_1 any alkoxy or amino group, and R_2 a cyano group or hydrogen. Substituent R_3 is introduced during the formation of the pyrimidine ring. Most of the compounds described in this paper have $R_3 = H$ or NMe_2, depending on the method employed for the cyclization. Finally, R_4 is introduced in the last step by means of an aromatic nucleophilic substitution of a chloro-substituent. Several nucleophiles of nitrogen, oxygen, and sulfur have been shown to be effective in this reaction.

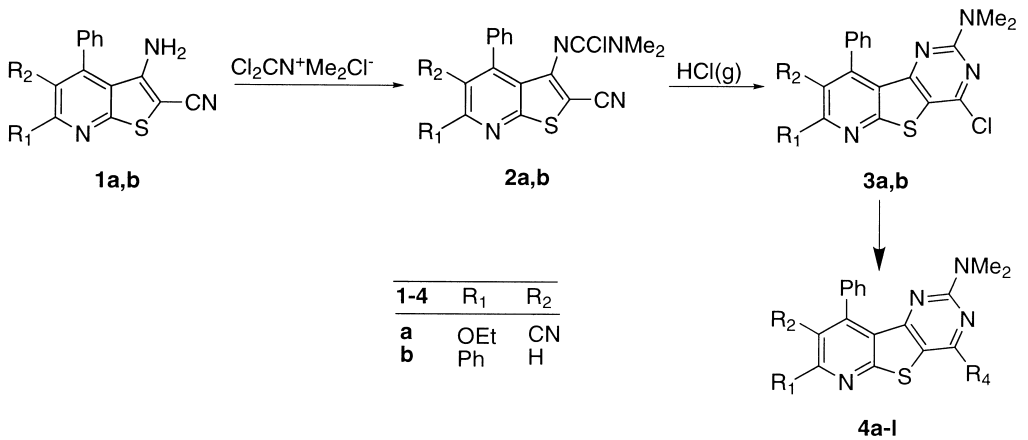
The structure of the required starting compounds is mainly determined by the nature of the substituents on the pyrimidine ring and, as a rule, systems with an amino group adjacent to another functional group are the most widely used.

In this paper we have chosen bicyclic thienopyridines with either an ortho amino nitrile (**1a–b**) or an ortho amino carboxamide (**5a–b**) functionality as the starting materials for the preparation of the tricyclic pyridothienopyrimidines shown in Table 1. In accordance with these two functionalities, two different types of cyclization reaction were needed.

In the case of pyridothienopyrimidines,[9] cyclization was carried out by the introduction of the required carbon atom with orthoformate, while in the case of pyridothienopyrimidines **4** and **21**, carrying a dimethylamino group at position 2, phosgene iminium salts were employed.

These salts are very useful synthetic reagents that can function as Vilsmeier or Mannich acceptors and lead to the insertion of a one-carbon unit bearing a dialkylamino group.[13] This makes them especially useful in one-step heterocyclization reactions such as those considered here.

Thus, treatment of 3-aminothieno[2,3-*b*]pyridine-2-carbonitriles **1a**[14] and **1b**[15] (Scheme 1) with phosgene iminium chloride in refluxing 1,2-dichloroethane gave the amide halide intermediates **2a–b**, which were reacted with dry



Scheme 1.

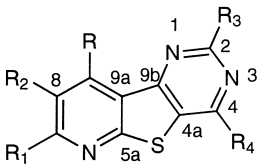
hydrogen chloride to undergo cyclization to chloropyridothienopyrimidines **3a–b**. These compounds were easily converted into a variety of pyridothienopyrimidines **4a–l** by aromatic substitution with nucleophiles (Table 1).

The structures of **2** and **3** were consistent with their elemental analyses and spectral data. The mass spectra showed the expected molecular ion peaks and the IR spectrum of **2** exhibited an absorption band at $\nu = 1640\,cm^{-1}$ due to the imino group. The ^{13}C NMR spectrum of **2a** showed two signals at $\delta = 113.8$ and 113.9 due to the cyano group, while compound **3a** showed only one signal for cyano groups at $\delta = 114.4$.

Compounds **9a–l** were prepared in two ways by the reactions shown in Scheme 2. Refluxing heterocyclic carboxamides **5a**[16] and **5b**[15] with ethyl orthoformate gave the triheterocyclic compounds **7a–b**, which were converted to the corresponding chloro derivatives **8a–b** by treatment with phosphorous oxychloride. Aromatic nucleophilic substitution with a variety of nucleophiles afforded the new substituted pyridothienopyrimidine derivatives **9a–l**.

An alternative method for the preparation of **7a** consisted of the reaction of **5a** with dimethylformamide dimethylacetal to give **6**, which could readily be converted into **7a** by refluxing with sodium ethoxide.

Table 1. Physicochemical data for pyridothienopyrimidines **3a, 4a–l, 9a–l, 13**, and **21a–b**



Compd	R	R_1	R_2	R_3	R_4	mp (°C)	yield (%)	formula[a]
3a	Ph	EtO	CN	NMe_2	Cl	220–222	86[f]	$C_{20}H_{16}N_5OClS$
4a	Ph	EtO	CN	NMe_2	OEt	192–194	72[b]	$C_{22}H_{21}N_5O2S$
4b	Ph	EtO	CN	NMe_2	piperidino	204–206	71[b]	$C_{25}H_{26}N_6O_2S$
4c	Ph	EtO	CN	NMe_2	4-methylpiperidino	184 dec	64[f]	$C_{26}H_{28}N_6OS$
4d	Ph	EtO	CN	NMe_2	piperazino	225 dec	77	$C_{24}H_{25}N_7O_2S$
4e	Ph	EtO	CN	NMe_2	4-methylpiperazino	170 dec	65[d]	$C_{25}H_{27}N_7OS$
4f	Ph	EtO	CN	NMe_2	4-ethoxycarbonylpiperazino	217–219	77[b]	$C_{27}H_{29}N_7O_3S$
4g	Ph	EtO	CN	NMe_2	4-(4-acetylphenyl) piperazino	212–214	65[b]	$C_{32}H_{31}N_7O_2S$
4h	Ph	EtO	CN	NMe_2	4-(trifluoro-*m*-tolyl)-piperazino	249–251	72[b]	$C_{31}H_{28}N_7OF_3S$
4i	Ph	Ph	H	NMe_2	4-methylpiperidino	138–140	59[e]	$C_{29}H_{29}N_5S$
4j	Ph	Ph	H	NMe_2	4-methylpiperazino	200–202	64[b]	$C_{28}H_{28}N_6S$
4k	Ph	Ph	H	NMe_2	4-(4-acetylphenyl) piperazino	214–216	58[b]	$C_{35}H_{32}N_6OS$
4l	Ph	Ph	H	NMe_2	piperazino	> 300	72[d]	$C_{27}H_{26}N_6S$
9a	Ph	EtO	CN	H	piperidino	189–191	75[b]	$C_{23}H_{21}N_5OS$
9b	Ph	EtO	CN	H	4-methylpiperidino	210–212	74[c]	$C_{24}H_{23}N_5OS$
9c	Ph	EtO	CN	H	morpholino	220–222	80[b]	$C_{22}H_{19}N_5O_2S$
9d	Ph	EtO	CN	H	piperazino	212–214	74[d]	$C_{22}H_{20}N_6OS$
9e	Ph	EtO	CN	H	4-methylpiperazino	212–214	65[d]	$C_{23}H_{22}N_6OS$
9f	Ph	EtO	CN	H	4-benzylpiperazino	229–231	81[d]	$C_{29}H_{26}N_6OS$
9g	Ph	EtO	CN	H	4-(4-acetylphenyl) piperazino	226–228	85[d]	$C_{30}H_{26}N_6O_2S$
9h	Ph	EtO	CN	H	4-(trifluoro-*m*-tolyl)-piperazino	213–215	83[d]	$C_{29}H_{23}N_6OF_3S$
9i	Ph	Ph	H	H	piperazino	191–193	68[c]	$C_{25}H_{21}N_5S$
9j	Ph	Ph	H	H	4-methylpiperidino	145–148	65[e]	$C_{27}H_{24}N_4S$
9k	Ph	Ph	H	H	4-methylpiperazino	190–192	84[b]	$C_{26}H_{23}N_5S$
9l	Ph	Ph	H	H	4-(4-acetylphenyl) piperazino	230–232	62[d]	$C_{33}H_{27}N_5OS$
13	Ph	EtO	CN	$2NO_2C_6H_4$	4-benzylpiperazino	209–211	71[d]	$C_{35}H_{29}N_7O_3S$
21a	H	morpholino	CN	NMe_2	morpholino	235–237	77[b]	$C_{20}H_{23}N_7O_2S$
21b	H	morpholino	CN	NMe_2	thiomorpholino	218–220	75[b]	$C_{20}H_{23}N_7OS_2$

[a]All compounds analyzed for C, H, N; analytical results are within ±0.4% of theoretical values.
[b]Recrystallized from ethanol/acetone.
[c]Recrystallized from ethanol.
[d]Recrystallized from ethanol/dichloromethane.
[e]Purified by flash chromatography with dichloromethane/hexane (3/1, v/v) as eluent.
[f]Purified by flash chromatography with dichloromethane/hexane (1/2, v/v) as eluent.

Table 2. Selected spectroscopic data for pyridothienopyrimidines

Compd	^{1}H NMR ($CDCl_3$) δ[a,b]	^{13}C NMR ($CDCl_3$) δ[a,b]	MS (FAB)[c] m/z (%)
3a	2.82 (br s, 6H, NMe_2)	36.9 (NMe_2)	411 ($M^+ + 2$, 9), 410 (40), 409 (M^+, 26), 408 (100), 380 (54), 352 (33)
4a	1.52 (t, 3H, $J = 7.1$ Hz, CH_3), 2.80 (s, 6H, NMe_2), 4.52 (q, 2H, $J = 7.1$ Hz, OCH_2)	14.4 (CH_3), 36.7 (NMe_2), 62.3 (CH_2O)	419 (M^+, 6), 391 (100), 376 (23), 362 (30)
4b	1.69 (br s, 6H, $CH_2CH_2CH_2$), 2.74 (s, 6H, NMe_2), 3.78 (br s, 4H, $N(CH_2)_2$)	24.8, 25.8 (CH_2), 36.4 (NMe_2), 47.5 (NCH_2)	458 (M^+, 100), 443 (33), 429 (26), 415 (29), 403 (12)
4c	0.96 (d, 2H, $J = 6.0$ Hz, $CHCH_3$), 1.23–1.34 (m, 2H, $CHCH_2$), 1.59–1.76 (m, 3H, $CHCH_2$, $CHCH_2$), 2.74 (s, 6H, NMe_2), 3.00 (t, 2H, $J = 12.7$ Hz, NCH_2), 4.56–4.66 (m, 2H, NCH_2)	21.8 ($CHCH_3$), 31.4 (CH_3CH), 36.5 (NMe_2), 46.8 (NCH_2)	473 [$(MH)^+$, 100], 445 (23), 429 (8)
4d	2.66 (s, 6H, NMe_2), 2.90 (br s, 4H, NCH_2), 3.75 (br s, 4H, NCH_2)		460 [$(MH)^+$, 100], 432 (19), 403 (16)
4e	2.33 (s, 3H, NCH_3), 2.51 (t, 4H, $J = 4.9$ Hz, NCH_2), 2.74 (s, 6H, NMe_2), 3.84 (t, 4H, $J = 4.9$ Hz, NCH_2)	36.5 (NMe_2), 46.1, 54.7 (NCH_2)	474 [$(MH)^+$, 100], 472 (12), 446 (10), 403 (12)
4f	1.28 (t, 3H, $J = 7.1$ Hz, CH_3), 2.74 (s, 6H, NMe_2), 3.58–3.83 (m, 8H, NCH_2), 4.17 (c, 2H, $J = 7.1$ Hz, OCH_2) 14.6 (CH_3), 36.5 (NMe_2), 43.4, 46.0 (NCH_2), 61.6 (CH_2O), 155.4 (CO) 531 (M^+, 58), 403 (100), 375 (16)		
4g	2.52 (s, 3H, CH_3CO), 2.75 (s, 6H, NMe_2), 3.49 (t, 4H, $J = 5.1$ Hz, NCH_2), 4.00 (t, 4H, $J = 5.1$ Hz, NCH_2), 6.87, 7.89 (AB system, 4H, $J = 9.0$ Hz, C_6H_4)	26.1 (CH_3CO), 36.5 (NMe_2), 45.5, 46.8 (NCH_2), 113.2, 128.1, 130.3, 153.6 (C_6H_4), 194.6 (CO)	578 [$(MH)^+$, 39], 403 (12), 217 (100)
4h	2.75 (s, 6H, NMe_2), 3.34 (t, 4H, $J = 5.0$ Hz, NCH_2), 3.98 (t, 4H, $J = 4.9$ Hz, NCH_2), 7.05–7.12 (m, 4H, $C_6H_4CF_3$)	36.5 (NMe_2), 45.8, 48.4 (NCH_2), 112.1, 112.2, 129.6, 131.7 (C_6H_4)	604 [$(MH)^+$, 10], 403 (4), 217 (100)
4i	1.00 (d, 2H, $J = 6.1$ Hz, $CHCH_3$), 1.33–1.40 (m, 2H, $CHCH_2$), 1.61–1.81 (m, 3H, $CHCH_2$, $CHCH_2$), 2.88 (s, 6H, NMe_2), 3.06 (dt, 2H, $J = 1.3$ Hz, $J = 12.7$ Hz, NCH_2), 4.71 (br s, 2H, NCH_2)	21.9 ($CHCH_3$), 31.4 (CH_3CH), 34.1 (CH_2CH), 36.6 (NMe_2), 46.9 (NCH_2)	480 [$(MH)^+$, 100], 437 (13), 410 (4)
4j	2.37 (s, 3H, NCH_3), 2.57 (t, 4H, $J = 4.8$ Hz, NCH_2), 2.86 (s, 6H, NMe_2), 3.94 (t, 4H, $J = 4.8$ Hz, NCH_2)	36.7 (NMe_2), 46.1 (NCH3), 46.2 (NCH_2), 54.9 (NCH2)	481 [$(MH)^+$, 100], 467 (3), 424 (16), 410 (21)
4k	2.53 (s, 3H, $COCH_3$), 2.86 (s, 6H, NMe_2), 3.54 (t, 3H, $J = 5.1$ Hz, NCH_2), 4.07 (t, 3H, $J = 5.2$ Hz, NCH_2), 6.90, 7.91 (AB system, 4H, $J = 9.0$ Hz, C_6H_4)	26.1 (CH_3CO), 36.7 (NMe_2), 45.7, 47.0 (NCH_2), 113.3, 127.5, 130.4, 153.8 (C_6H_4), 196.5 (CO)	585 [$(MH)^+$, 40], 451 (69), 410 (46)
4l	2.85 (s, 6H, NMe_2), 3.00 (t, 3H, $J = 5.0$ Hz, NCH_2), 3.87 (t, 3H, $J = 5.0$ Hz, NCH_2)	36.6 (NMe_2), 45.9, 47.5 (NCH_2)	467 (M^+, 25), 410 (100), 397 (60), 382 (28), 368 (29)
9a	1.74 (br s, 6H, CH_2-CH_2-CH_2), 3.89 (br s, 4H, NCH_2), 8.35 (s, 1H, H-2)	24.6 (CH_2), 26.0 (CH_2), 47.6 (NCH_2)	415 (M^+, 100), 386 (43), 359 (17), 332 (22)
9b	0.99 (d, 3H, $J = 6.2$ Hz, $CHCH_3$), 1.20–1.38 (m, 2H, $CHCH_2$), 1.69–1.84 (m, 3H, $CHCH_2 + CHCH_2$), 3.10 (t, 2H, $J = 12.3$ Hz, NCH_2), 4.63–4.70 (m, 2H, NCH_2), 8.35 (s, 1H, H-2)	21.7 ($CHCH_3$), 31.2 (CH_3CH), 34.2 (CH_2CH), 46.9 (NCH_2)	430 (M^+, 95), 417 (87), 232 (16)
9c	3.83–3.94 (m, 8H, CH_2-CH_2), 8.39 (s, 1H, H-2)	46.6 (NCH_2), 66.7 (OCH_2)	417 (M^+, 100), 388 (18), 386 (23), 360 (49)
9d	3.03 (t, 4H, $J = 4.9$ Hz, NCH_2), 3.91 (t, 4H, $J = 4.9$ Hz, NCH_2), 8.38 (s, 1H, H-2)	47.4 (NCH_2), 52.9 (NCH_2)	417 [$(MH)^+$, 100], 389 (9)

(continued)

Table 2—*contd*

9e	2.36 (s, 3H, NCH_3), 2.56 (t, 4H, $J=4.9$ Hz, NCH_2), 3.95 (t, 4H, $J=4.9$ Hz, NCH_2), 8.37 (s, 1H, H-2)	46.0 (NCH_3), 46.2 (NCH_2), 54.8 (NCH_2)	431 [$(MH)^+$, 100], 429 (10), 374 (13), 346 (20)
9f	2.59 (t, 4H, $J=4.8$ Hz, NCH_2), 3.56 (s, 2H, CH_2Ph), 3.93 (t, 4H, $J=4.9$ Hz, NCH_2), 7.24–7.35 (m, 5H, C_6H_5), 8.35 (s, 1H, H-2)	46.3 (CH_2), 52.9 (NCH_2), 62.9 (NCH_2)	506 [$(MH)^+$, 20], 217 (100)
9g	2.54 (s, 3H, CH_3CO), 3.55 (t, 4H, $J=5.2$ Hz, NCH_2), 4.12 (t, 4H, $J=5.2$ Hz, NCH_2), 6.90, 7.91 (AB system, 4H, $J=9.0$ Hz, C_6H_4), 8.40 (s, 1H, H-2)	26.0 (CH_3CO), 45.5, 47.0 (NCH_2), 112.3, 113.4, 128.3, 130.4 (C_6H_4), 196.5 (CO)	535 [$(MH)^+$, 12], 360 (2)
9h	3.40 (t, 4H, $J=5.1$ Hz, NCH_2), 4.11 (t, 4H, $J=5.1$ Hz, NCH_2), 7.09–7.43 (m, 4H, $C_6H_4CF_3$), 8.42 (s, 1H, H-2)	45.6, 48.6 (NCH_2), 112.4 (C-6′), 116.5, 116.6 (C-2′, C-4′), 122.1 (CF_3), 131.4 (C-1′), 131.9 (C-3′), 151.0 (C-5′)	561 [$(MH)^+$, 20], 217 (100)
9i	3.11 (t, 4H, $J=4.2$ Hz, NCH_2), 4.01 (t, 4H, $J=4.5$ Hz, NCH_2), 8.50 (s, 1H, H-2)		424 [$(MH)^+$, 100], 381 (25), 367 (14), 355 (11)
9j	0.89 (d, 2H, $J=6.2$ Hz, $CHCH_3$), 1.16–1.31 (m, 2H, $CHCH_2$), 1.57–1.75 (m, 3H, $CHCH_2+CHCH_2$), 3.01 (dt, 2H, $J=2.3$ Hz, $J=12.4$ Hz, NCH_2), 4.63 (br s, 2H, NCH_2), 8.37 (s, 1H, H-2)	21.7 ($CHCH_3$), 31.3 (CH_3CH), 34.2 (CH_2CH), 46.9 (NCH_2), 153.9 (C-2)	437 [$(MH)^+$, 100), 435 (10), 367 (9)
9k	2.37 (s, 3H, NCH_3), 2.60 (t, 4H, $J=4.8$ Hz, NCH_2), 4.01 (t, 4H, $J=4.8$ Hz, NCH_2), 8.51 (s, 1H, H-2)	46.0 (NCH_3), 46.2 (NCH_2), 54.9 (NCH_2), 153.8 (C-2)	438 [$(MH)^+$, 100], 481 (59), 367 (23)
9l	2.53 (s, 3H, $COCH_3$), 3.55 (t, 3H, $J=4.8$ Hz, NCH_2), 4.13 (t, 3H, $J=5.3$ Hz, NCH_2), 6.89, 7.90 (AB system, 4H, $J=8.9$ Hz, C_6H_4), 8.50 (s, 1H, H-2)	26.1 ($COCH_3$), 45.6, 47.0 (NCH_2), 153.9 (C-2), 113.3, 114.2, 129.9, 130.4 (C_6H_4), 196.4 (CO)	542 [$(MH)^+$, 40], 486 (19), 367 (19)
13	2.61 (s, 4H, NCH_2), 3.58 (s, 2H, CH_2Ph), 3.90 (s, 4H, NCH_2)	46.2 (NCH_2), 52.9 (NCH_2), 62.8 ($C_6H_5CH_2$)	609 (18), 518 (4), 481 (9), 146 (11), 91 (100)
21a	3.81–3.88 (m, 16H, CH_2)	46.4 (NCH_2), 66.7 (CH_2O)	425 (M^+, 41), 381 (4), 368 (24), 281 (15)
21b	2.74 (t, 4H, $J=5.0$ Hz, SCH_2), 3.78–3.90 (m, 8H, CH), 4.77 (t, 4H, $J=5.0$ Hz, NCH)	26.8 (SCH_2), 49.1 (NCH_2)	441 (M^+, 57), 368 (100), 372 (30), 339 (7)

[a]The NMR spectra of **4d** and **9c** were obtained in DMSO-d_6.

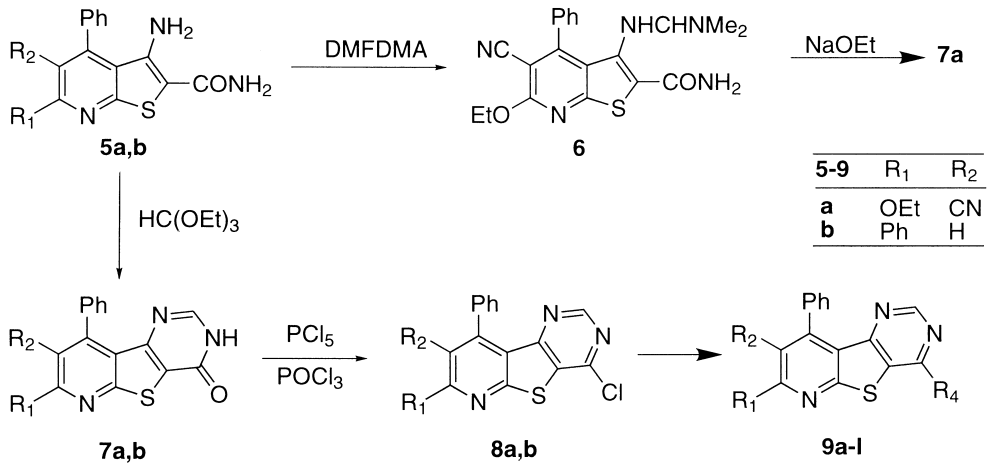
[b]The ^{1}H NMR spectra of compounds **3a, 4a–h, 9a–h**, and **13** exhibited additional signals typical for C_6H_5 [7.40, 7.50–7.46, 7.58 (m, 5H)], and OCH_2CH_3 groups [1.41–1.54 (t, 3H, $J=7.1$ Hz), and 4.57–4.66 (q, 2H, $J=7.1$ Hz)]. Compounds **4i–l** and **9i–l** present also resonances for C_6H_5 [7.42, 7.49–7.59, 7.69 (m, 8H), and 8.02, 8.17–8.08, 8.22 (m, 2H)] and for H-8 [7.65–7.78 (s, 1H)]. Compounds **21a–b** exhibit signals for NMe_2 [3.22 (s, 6H)] and for H-9 [8.56–8.60 (s, 1H)].

[c]The ^{13}C NMR spectra of **3a, 4a–h, 9a–h**, and **13** (compounds **4d** and **9i** are not sufficiently soluble in DMSO-d_6 or $CDCl_3$ to produce good ^{13}C NMR data, showed signals at 14.2–14.4 (CH_3), 64.0–64.6 (CH_2O), 95.1–96.7 (C-8), 100.5–112.3 (C-4a), 114.5–115.0 (CN), 118.5–119.4 (C-9a), 127.7, 128.0–133.4, 134.4 (four peaks for C_6H_5), 154.9–155.7 (C-5a), 155.5–157.4 (C-9b), 157.3–158.6 (C-4), and 163.2–164.6 (two renonances for C-7 and C-9). Compounds **3a, 4a–c, 4e–h** show a resonance for C-2 at 160.2 ppm. In the **9a–h** series C-2 resonates in the range 154.1–154.5 ppm. Compounds **4i–l** and **9i–l** showed signals at 102.7 (C-4a) (**4i–l**) and at 114.0 (C-4a) (**9j–l**), 118.9–119.2 (C-8), 122.8–123.4 (C-9a), 127.3–138.6 (seven absorptions for two C_6H_5 groups), 149.0–149.7 (C-5a), 155.8–157.6 (C-9b), 157.4–158.2 (C-4), 158.1–158.8, and 162.7–163.4 (C-7 and C-9). Compounds **4i–l** showed one resonance at 160.1–160.4 for C-2. Compounds **21a–b** present resonances at 37.1 (NMe), 48.8 (NCH_2), 66.6 (CH_2O), 92.8 (C-8), 100.4 (C-4a), 118.2 (CN), 120.4 (C-9a), 139.5 (C-9), 156.6 (C-5a), 157.6–158.4 (C-9b), 160.4 (C-2), 160.8 (C-7), 165.0 (C-4).

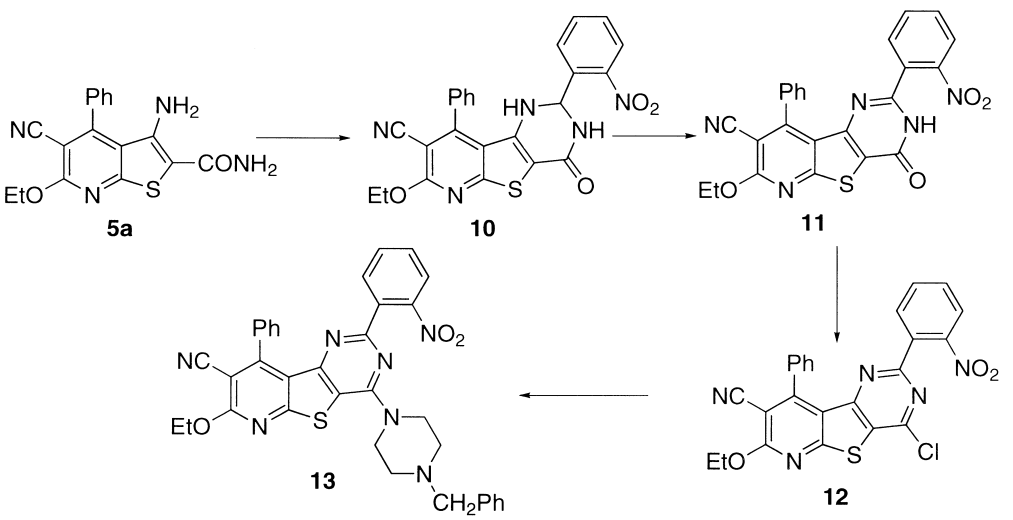
[d]Electron impact MS data (EIMS) is shown for compounds **3a, 4a, 4b, 4f, 4l, 9a–c, 13, 21a**, and **21b**. The IR spectra **21a–b** shows an absorption for the nitrile group at 2900 and 2220 cm^{-1} (CN), while compounds **3a, 4a–h, 9a–h**, and **13** showed the CN band at 2210–2220 cm^{-1}.

Compound **13** was obtained from **5a** by the sequence shown in Scheme 3. **5a** was reacted with *o*-nitrobenzaldehyde in refluxing toluene containing a catalytic amount of *p*-toluensulfonic acid, with azeotropic removal of water, to give **10** in 81% yield. Refluxing compound **10** with DDQ in THF for a few hours afforded pyridothienopyrimidone **11**, [EIMS $m/z = 427$ ($M^+ - OCN$) and 292 ($M^+ - OCNRHC$)]. Reaction of **10** with $POCl_3$ yielded the expected 4-chloropyridothienopyrimidine derivative **12**, which gave **13** on reaction with 4-benzylpiperazine. The ^1H NMR spectrum of pyridothienopyrimidine **10** showed signals at $\delta = 6.25$ for H-2, and two other signals at $\delta = 5.13$ and $\delta = 8.26$, which could be exchanged with deuterium oxide, assigned to the N-bonded protons at positions 1 and 3, respectively. The ^{13}C NMR spectrum of **10** showed one signal at $\delta = 63.2$ corresponding to the carbon at position 2 of the newly formed pyrimidine ring. The EIMS of pyridothienopyrimidone **11** showed the expected molecular ion and other fragments at $m/z = 427$ ($M^+ - OCN$) and 292 ($M^+ - OCNRHC$).

For the preparation of pyridothienopyrimidines **21a–b** we started from the aminocyanopyridine **14**.[17] This compound was first converted into the thienopyridine **19** and then cyclized to give the pyridothienopyrimidines using the phosgene iminium chloride method discussed above. The whole synthetic process is represented in Scheme 4. All compounds gave elemental analyses and spectra consistent with the assigned structures.



Scheme 2.



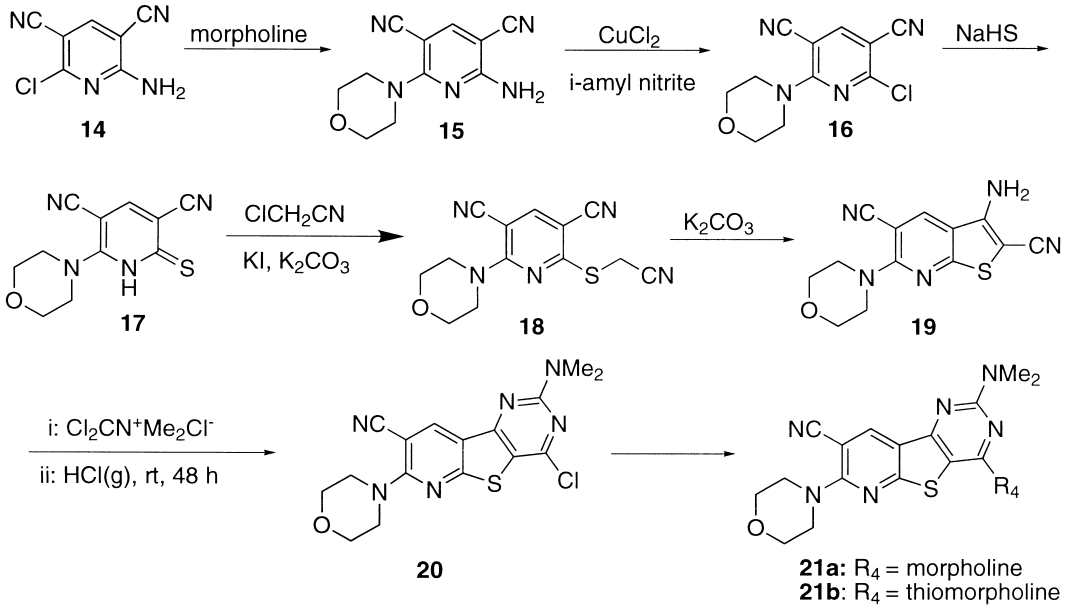
Scheme 3.

Biological Results

Immunological stimulus

Initial information about the ability of the pyridothienopyrimidines to inhibit the release of histamine from rat mast cells was obtained by screening the activity using a single dose (100 μg/mL) of compound. In the experiments performed with preincubation (compound added to the cells 10 min before than the stimulus, Fig. 1), the inhibitory action of **4k, 9i, 9k, 9l**, and **21a** is of around 40%, compound **21a** being the most active showing a 60% inhibitory activity.

When the experiments were carried out with simultaneous addition of the compound and the stimulus (i.e. without preincubation, Figure 2), the inhibitory pattern



Scheme 4.

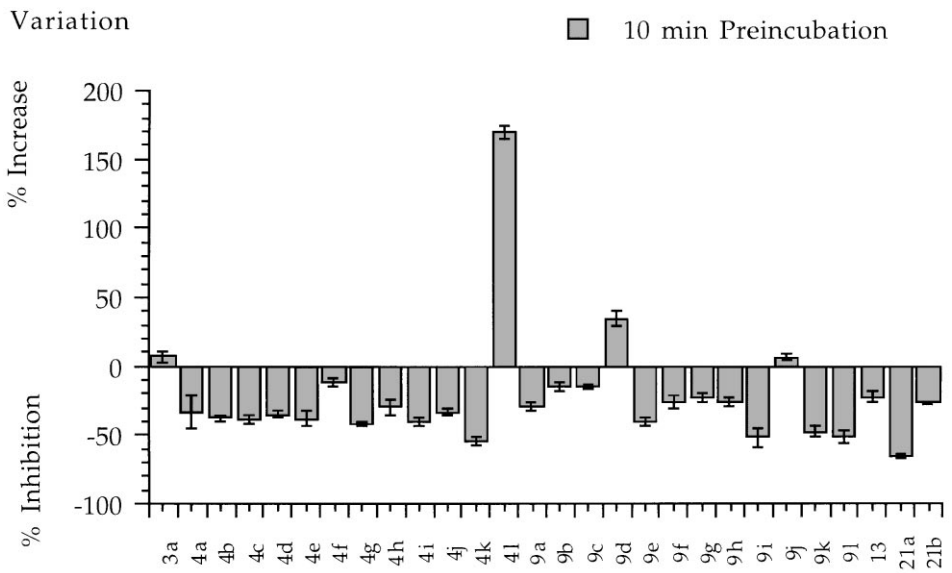


Figure 1. Histamine release in mast cells stimulated with 3.5 mg/mL of antigen (ovoalbumin) in the presence of pyridothienopyrimidines. The compounds were added to the cells 10 min before the addition of the stimulus (10 min preincubation). The activity data are normalized to the response of the control experiments (29.63 ± 1.59% in the presence of 5 mg/mL of antigen).

is very similar to that in the previous figure, with a consistent inhibition of around 40% for several compounds. Nevertheless, **4h, 4i, 9a**, and **21a** show a higher inhibitory activity (60%) and values of 90% inhibition are obtained with **9b** and **9l**. From those experiments, pyridothienopyrimidines **9l** and **21a** emerge as especially interesting since they show consistent inhibition of the immunological stimulus and a low desensitization.

In contrast to the inhibitory activity found in those heterocycles, two compounds, **9d** (40–60%) and **4l** (170–220%) have been shown to be activators of the exocytosis in rat mast cells.

Non-immunological stimulus

The pyridothienopyrimidines were also tested when the release of histamine was induced by the chemical inducer compound 48/80.[18]

The results of single dose experiments (at 100 μg/mL) are presented in Figures 3 and 4 and show that compounds **4c, 4i, 4k**, and **9l** produce a 50–60% inhibition when preincubated with the stimulus while in the experiments carried out with simultaneous addition only four compounds (**4c, 4e, 4k**, and **9l**) show some inhibitory action (20–30%). Overall, **4c, 4k**, and **9l** are active in both conditions **9l** being the most effective inhibitor with inhibition values of 30% (simultaneous addition) and 60% (preincubated), respectively. Figure 3 indicates the presence of several histamine releasers too. The most active one in the experiments with preincubation is **9e** (100%). In the assays performed with simultaneous addition of the stimulus (Fig. 4) there is only one good inducer in the whole series and this is again **9e** (170%), which therefore is a good releaser both with and without preincubation.

The activity of the pyridothienopyrimidines was also tested using the antineoplastic drugs adryamicin and vinorelbine, recently reported to induce histamine release,[19] as chemical inducers. The results (not shown) obtained with a selection of pyridothienopyrimidines (**4b, 9a, 9c, 9g, 9h**, and **13**) indicated that the amount of histamine released after simultaneous addition of adryamicin was practically the same as in the absence of the chemical. Experiments carried out with preincubation of the cells with the pyridothienopyrimidines before addition of the antineoplastic drug, showed a small increase in the inhibition values that reached up to 30% in some cases. Similar tests with vinorelbine as histamine releaser did not show any clear inhibitory action either with or without preincubation. In summary, no clear correlation was observed between the results of the experiments using adryamicin, vinorelbine and compound 48/80 as chemical releasers of histamine.

In order to get a better picture of the ability of these heterocycles to inhibit the release of histamine, and to allow a direct comparison of their potency with that of known antiallergics as cromoglycate (DSCG) and ketotifen,[20] experiments using five concentrations of inhibitor were carried out. Table 3 shows the results

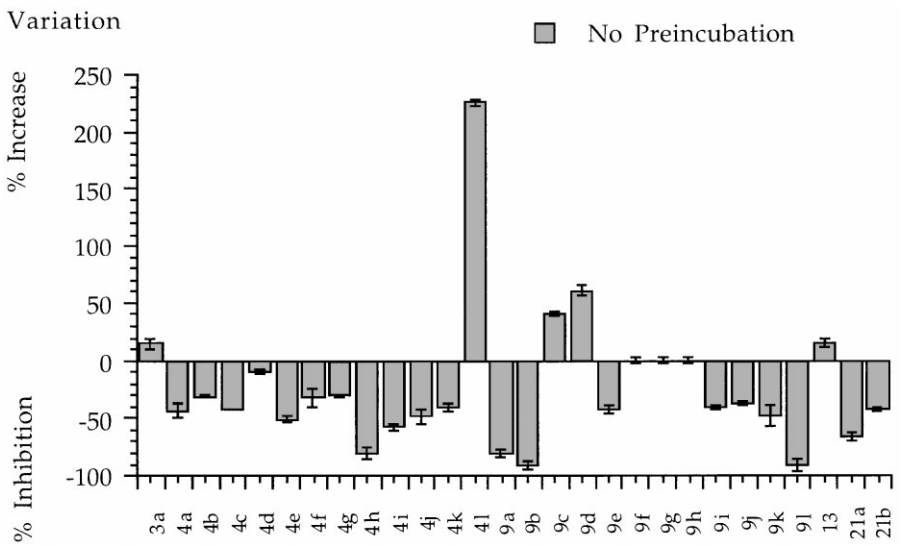


Figure 2. Histamine release in mast rat cells stimulated with 3.5 mg/mL of antigen (ovoalbumin) in the presence of pyridothienopyrimidines. The compounds were added to the cells simultaneously with the stimulus (no preincubation). The activity data are normalized to the response of the control experiments (21.47 ± 2.4% in the presence of 5 mg/mL of antigen).

expressed as IC_{50} values, for a selection of pyridothienopyrimidines. The figures are in the range 2–25 μM well below those of cromoglycate (DSCG) and similar to ketotifen in the immunologically stimulated assays. In the chemical stimulated experiments, the selected pyridothienopyrimidines are clearly much more active than both references. These results prove that these heterocycles are up to 10–100 times more active than the standards and confirm their potential interest.

Antitumor activity

The pyridothienopyrimidines shown in Table 1 have been tested in vitro for their antitumor activity against four standard tumor cell lines. The IC_{50} figures presented in Table 4 show that four pyridothienopyrimidines can be considered to be active in these assays. These are **4d** and **9d** (IC_{50}: 2.5–0.5 μg/mL) and **9i** and **4l**, which show IC_{50} values of 0.12–0.25 μg/mL. Structurally,

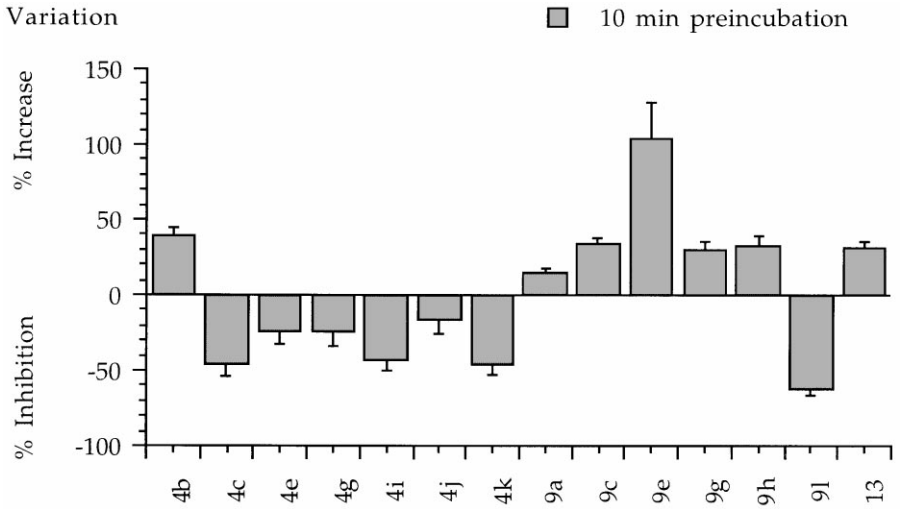


Figure 3. Histamine release in mast rat cells stimulated with 2 μg/mL of compound 48/80 in the presence of pyridothienopyrimidines. The compounds were added to the cells simultaneously with the stimulus (no preincubation). The activity data are normalized to the response of the control experiments (32.0 ± 4.7% in the presence of 2 μg/mL of compound 48/80.

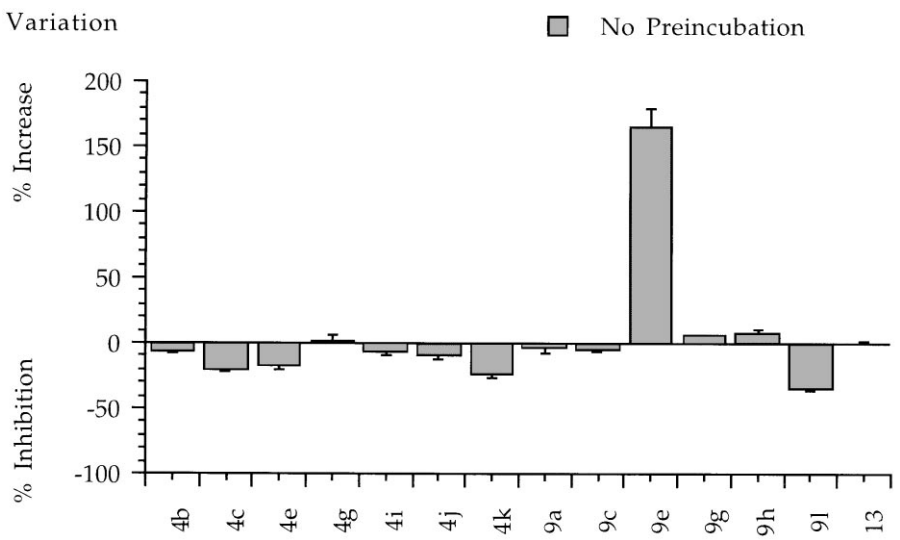


Figure 4. Histamine release in mast rat cells stimulated with 2 μg/mL of compound 48/80 in the presence of pyridothienopyrimidines. The compounds were added to the cells 10 min before the addition of the stimulus (10 min preincubation). The activity data are normalized to the response of the control experiments (35.0 ± 3.5% in the presence of 2 μg/mL of compound 48/80).

Table 3. Histamine release inhibition (IC_{50} μM) of selected pyridothienopyrimidines

Immunological stimulation (5 mg/mL)		Chemical stimulation (2 μg/mL)		Immunological stimulation (5 mg/mL)		Chemical stimulation (2 μg/mL)	
Compd (Preinc.)	IC_{50} (μM)	Compd (Preinc.)	IC_{50} (μM)	Compd (No preinc.)	IC_{50} (μM)	Compd (No preinc.)	IC_{50} (μM)
DSCG[a]	400	DSCG[a]	200	**4h**	4 ± 0.73	**4c**	6 ± 1.1
Ketotifen[a]	10	Ketotifen[a]	100	**4i**	6 ± 1.2	**4e**	2 ± 0.4
4k	10 ± 1.2	**4c**	10 ± 1.0	**9a**	20 ± 0.8	**4k**	5 ± 1.0
9i	25 ± 2.5	**4i**	20 ± 0.9	**9b**	25 ± 2.4	**9l**	15 ± 1.7
9k	14 ± 1.3	**4k**	14 ± 1.3	**9l**	9 ± 1.3		
9l	20 ± 1.7	**9l**	25 ± 1.7	**21a**	20 ± 3.2		
21a	25 ± 3.5						

[a]See ref. 20.

the last two compounds have significantly different combinations of R_1, R_2, and R_3 but have in common the piperazine substituent (R), suggesting that this unit plays an important role in their action. It is interesting to note that **4l** and **9d** are the stronger stimulants for the liberation of histamine found in the series of pyridothienopyrimidines studied.

Conclusions

In conclusion, we have presented efficient synthetic procedures for the preparation of pyridothienopyrimidines of interest as antiallergics. Their activity has been evaluated with rat mast cells and the assays have been carried out under different conditions and with different types of stimulus. Under immunological stimulus with ovoalbumin (Figs 1 and 2, Table 3), several pyridothienopyrimidines are stronger inhibitors of the release of histamine than DSCG (up to 100 times more potent) and of the same order of potency as Ketotifen,[20] although only two compounds, **9l** and **21a** are active with and without preincubation. In the assays using compound 48/80 as chemical inducer (Figs 3 and 4, Table 3), there are also several inhibitors, 10–100 times more potent than DSCG and Ketotifen.[20] Three of them, **4c, 4k**, and **9l**, are active with and without preincubation.

Table 4. Antitumoral activity of selected pyridothienopirimidines expressed in IC_{50}

Compd	IC_{50} (μg/mL)			
	P-388	A-549	HT-29	MEL
4d	2.5	2.5	2.5	2.5
4l	0.25	0.25	0.25	0.50
9d	0.5	1	2	1
9i	0.12	0.25	0.25	0.25

Overall, the data shown in Table 3 demonstrates that pyridothienopyrimidines are worth considering for their potential antiallergic activity. Compound **9l** is particularly interesting because it is the only one that conserves its strong inhibitory activity (IC_{50} = 9–25 μM) in all the conditions tested (chemical and immunological stimulus, with/without preincubation).

From the structure–activity point of view, our results indicate that the modification of the substituents on the pyridothienopyrimidine skeleton is not as effective in improving the inhibitory action as similar changes in the cyanopyridines and pyridopyrimidine series,[12a] and this may explain why so many pyridothienopyrimidines show some inhibition. Nevertheless, some trends can be distinguished and for example, comparison of the inhibitors **9b** and **9l** with the inactive **9g** and **9j** may illustrate the influence of R_1 and R_2. Similarly, the good inhibitory action of **9a** is lost in its *N*-dimethyl derivative **4b**.

The fact that some inhibitory action is observed in virtually all cases examined (Figs 1–4), the absence of any apparent link between the chemical and the immunologically stimulated experiments, prevents the formulation of any further conclusion. Similarly, comparison of the results obtained with compound 48/80[18] and the drugs adryamicin and vinorelbine indicate that no apparent correspondence exists between those three chemical releasers.

A completely different picture is seen if our attention is focussed on the heterocycles acting as inducers of the release of histamine. This action is found to be very selective among the compounds studied. Indeed, only one pyridothienopyrimidine (**4l**) acts as a releaser of histamine under immunological conditions (170–220%), and another one (**9e**, 100–170%) in the chemically driven experiments. This effect is observed in both pre-

incubated and non-preincubated experiments, because, as expected, is independent of the stimulus itself. Both structures share the same R substituent (R = piperidine) but comparison with other members of the series, and particularly with the inactive compounds **4d** and **9i**, suggests that the combination of substituents represented by **4l** is necessary to obtain the maximum activity.

The discovery of strong histamine releasers among the compounds investigated is interesting because, although inducers have no clinical interest so far, knowledge of their structures may provide an aid to the prediction[21] of unwanted side effects.

Some pyridothienopyrimidines[11] and pyridopyrimidines[22] have been reported to be antineoplastic. In our tests, only four compounds were found to be active, so cytotoxicity would not pose a problem in the eventual use of these compounds as antiallergics. However, it is worth noting that two of the stronger stimulants, **4l** and **9d**, are among the more cytotoxic pyridothienopyrimidines.

Experimental

Chemistry

All reagents used were commercial grade chemicals from freshly opened containers. The histamine releaser compound 48/80, is a condensation product of *N*-methyl-*p*-methoxy-phenethylamine, was obtained from Sigma Chemical Co. Melting points were determined using a Büchi 510 apparatus and are uncorrected. IR spectra were recorded as potassium bromide disks using a Perkin-Elmer 783 spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained using a Bruker AC 200F instrument at room temperature. Mass spectra were obtained using a VG QUATTRO spectrometer. The silica gel 60 HF254 + 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 Servicio General de Analisis Elemental at the University of La Coruña.

Histamine release and neoplastic assays[12b-d]

Rat mast cells and human and mouse tumoral cells were prepared and incubated as previously described.[12b] Histamine release was measured spectrofluorometrically[12c] both in the pellet and in the supernatants with and without preincubation (10 min) of each drug (100 μg/mL in the single dose experiments) with the cells before addition of the stimulus. All the experiments were repeated at least three times in duplicate and the data was analyzed using the Student's *t*-test for umpaired data. A probability level of 0.05 or less was used for statistical significance and the results are expressed as mean ± SEM. Five concentrations of inhibitor (0.1, 1, 10, 100, and 500 μg/mL) were used in order to obtain the IC_{50} values.

The antineoplasic activity was measured[12d] as IC_{50} against 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).

3-Chlorodimethylaminomethylenamino-2-cyanothieno[2,3-*b*]-pyridine: General procedure (2a,b). A solution of **1a** or **1b** (1.3 mmol) and phosgene iminium salt (0.26 g, 1.6 mmol) in 1,2-dichloroethane (20 mL) was refluxed for 45 min. The solvent was removed under reduced pressure and the resulting solid was purified by flash chromatography using CH_2Cl_2/hexane (2/1) as eluent.

3-Chlorodimethylaminomethylenamino-2,5-dicyano-6-ethoxy-4-phenylthieno[2,3-*b*]pyridine (2a). Yield 78%; mp 220–222 °C; $C_{20}H_{16}N_5OClS$ calcd C, 58.61; H, 3.93; N, 17.09. found: C, 58.67; H, 4.07; N, 17.17; ^{1}H NMR ($CDCl_3$) δ 1.50 (t, 3H, J = 7.1 Hz, CH_3), 2.70 (s, 6H, NMe_2), 4.61 (q, 2H, J = 7.1 Hz, CH_2), 7.27–7.55 (m, 5H, C_6H_5); ^{13}C NMR ($CDCl_3$) δ 14.4 (CH_3), 39.6 (NMe_2), 64.4 (CH_2), 90.9 (C-2), 96.6 (C-5), 113.8 (CN), 113.9 (CN), 119.2 (C-3a), 127.5, 128.4, 128.9, 133.6 (C_6H_5), 139.8, 151.1, 154.2, 162.1, 162.6; MS (EI, *m*/*z*, %): 411 ($M^+ + 2$, 33), 409 (M^+, 85), 374 (100), 346 (40), 289 (31); IR (KBr, cm^{-1}): 2220 (CN), 2200 (CN), 1640, 1550, 1430.

3-Chlorodimethylaminomethylenamino-2-cyano-4,6-phenylthieno[2,3-*b*]pyridine (2b). Yield 95%; mp 153–155 °C; $C_{23}H_{17}N_4ClS$ calcd C, 66.26; H, 4.11; N, 13.44. found: C, 66.07; H, 4.01; N, 13.58; ^{1}H NMR ($CDCl_3$) δ 2.76 (s, 6H, NMe_2), 7.34–7.55 (m, 8H, C_6H_5), 7.65 (s, 1H, H-5), 8.09–8.15 (m, 2H, C_6H_5); ^{13}C NMR ($CDCl_3$) δ 39.7 (NMe_2), 93.3 (C-2), 114.5 (CN), 119.2 (C-5), 123.0 (C-3a), 127.3, 127.4, 127.9, 128.9, 129.1, 129.8, 137.8, 138.0 (C_6H_5), 140.0 (C–Cl), 148.3 (C-3), 150.9, 157.3, 161.6 (C-4, C-6, C-7a); MS (EI, *m*/*z*, %): 418 ($M^+ + 2$, 37), 416 (M^+, 94), 381 (100), 365 (20), 336 (28), 324 (46); IR (KBr, cm^{-1}): 2200 (CN), 1640, 1560, 1320.

4-Chloro-2-dimethylaminopyrido[3′,2′:4,5]thieno[3,2-*d*]-pyrimidine: General procedure (3a,b). A stream of dry hydrogen chloride was passed through a solution of **2a** or **2b** (0.43 mmol) in 1,2-dichloroethane (10 mL) for 3 h. After that time, the reaction mixture was allowed to stand overnight at room temperature, the solvent was

removed under reduced pressure and the resulting solid was purified by flash chromatography eluting with CH_2Cl_2/Hexane (2/1) to yield **3a** (Tables 1 and 2) or **3b**.

4-Chloro-7,9-diphenylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine (3b). Yield 83%; mp 237–239 °C; $C_{23}H_{17}N_4ClS$ calcd C, 66.26; H, 4.11; N, 13.44. found: C, 66.34; H, 4.19; N, 13.51; ^{1}H NMR ($CDCl_3$) δ 2.94 (s, 6H, NMe_2), 7.48–7.65 (m, 8H, C_6H_5), 7.75 (s, 1H, H-8), 8.16–8.21 (m, 2H, C_6H_5); ^{13}C NMR ($CDCl_3$) δ 37.1 (NMe_2), 119.2 (C-8), 122.9 (C-9a), 127.5, 127.7, 128.4, 128.9, 129.6, 130.0, 137.4, 138.1 (C_6H_5), 150.2, 154.3, 158.3, 158.7, 160.3, 165.2; MS (EI, *m*/*z*, %): 418 ($M^+ + 2$, 40), 416 (M^+, 100), 401 (37), 387 (86), 372 (32); IR (KBr, cm^{-1}): 1590, 1530, 1480, 1410.

8-Cyano-4,7-diethoxy-2-dimethylaminopyrido[3′,2′:4,5]-thieno[3,2-*d*]pyrimidine (4a). A solution of **3a** (0.15 g, 0.37 mmol) in ethanol (20 mL) containing NaOEt (0.08 g of Na, 3.7 mmol) was refluxed for 1 h. The solid was filtered off and recrystallized from ethanol/acetone to yield **4a** (Tables 1 and 2).

2-Dimethylaminopyrido[3′,2′:4,5]thieno[3,2-d]pyrimidine-4-substituted (4b–l): General procedure. A solution of **3a** or **3b** (0.37 mmol) and the appropriate amine (0.44 mmol) in ethanol (10 mL) was refluxed until the starting material had disappeared (TLC). The solid was filtered off and recrystallized or was purified by flash chromatography (Tables 1 and 2).

5-Cyano-6-ethoxy-3-dimethylaminomethylenamino-4-phenylthieno[2,3-*b*]pyridine-2-carboxamide (6). A solution containing **5a** (1.0 g, 3.0 mmol) and DMFDMA (0.54 g, 4.5 mmol) in DMF (5 mL) was stirred at 80 °C for 15 min. Ethanol (20 mL) was added and the solid was filtered off and recrystallized from ethanol/acetone to yield **6** (1.03 g, 89%): mp 242 °C; $C_{20}H_{19}N_5O_2S$ calcd C, 61.05; H, 4.87; N, 17.80. found: C, 60.89; H, 4.99; N, 17.74; ^{1}H NMR ($CDCl_3$) δ 1.47 (t, 3H, $J = 7.1$ Hz, CH_3), 3.12 (s, 3H, NMe_2), 3.12 (s, 3H, NMe_2), 4.59 (q, 2H, $J = 7.1$ Hz, CH_2), 7.45–7.59 (m, 5H, C_6H_5), 8.53 (s, 1H, CH = N); ^{13}C NMR ($CDCl_3$) δ 14.3 (CH_3), 35.3 (NMe_2), 41.3 (NMe_2), 63.9 (CH_2), 94.4 (C-5), 105.6 (C-2), 114.7 (CN), 117.6 (C-3a), 128.1, 129.1, 130.1, 133.2 (C_6H_5), 146.1, 153.1, 158.9, 162.2, 163.7, 174.5 (CO); MS (EI, *m*/*z*, %): 393 (M^+, 28), 349 (100), 321 (59), 294 (9); IR (KBr, cm^{-1}): 3460 (NH), 3300 (NH), 2220 (CN), 1630 (CO), 1570, 1330.

Pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4(3H)-ones (7a,b): General procedure. Method A: A solution of **6** (0.58 g, 1.48 mmol) and an excess of NaOEt in ethanol (25 mL) was refluxed for 30 min. The solvent was removed under reduced pressure and water (40 mL) was added. The solution was neutralized with 2 N HCl and the resulting solid was filtered off to yield **7a** (0.42 g, 82%). **Method B**: A solution of **5a** or **5b** (3.00 mmol) and a catalytic amount of PTSA in $HC(OEt)_3$ (15 mL) was refluxed until the starting material had disappeared (TLC). The solid was filtered off and the crude product was used in the next step without further purification.

8-Cyano-7-ethoxy-9-phenylpyrido[3′,2′:4,5]thieno[3,2-*d*]-pyrimidin-4(3*H*)-one (7a). Yield 98%; mp > 300 °C; $C_{18}H_{12}N_4O_2S$ calcd C, 62.06; H, 3.47; N, 16.08; found: C, 62.20; H, 3.55; N, 16.25; ^{1}H NMR (DMSO-d_6) δ 1.43 (t, 3H, $J = 7.1$ Hz, CH_3), 4.61 (q, 2H, $J = 7.1$ Hz, CH_2), 7.49–7.67 (m, 5H, C_6H_5), 8.03 (s, 1H, H-2), 12.89 (br s, 1H, exchangeable with D_2O, NH). MS (EI, *m*/*z*, %): 348 (M^+, 73), 319 (100), 306 (19), 292 (23), 264 (20), 236 (32), 165 (31); IR (KBr, cm^{-1}): 3240 (NH), 2220 (CN), 1680 (CO), 1330, 1230.

7,9-Diphenylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (7b). Yield 93%; mp > 300 °C; $C_{21}H_{13}N_3OS$ calcd C, 70.97; H, 3.69; N, 11.82; found: C, 71.07; H, 3.57; N, 11.75; ^{1}H NMR (DMSO-d_6) δ 7.47–7.70 (m, 8H, C_6H_5), 7.98 (s, 1H, H-8), 8.11 (s, 1H, H-2), 8.26–8.30 (m, 2H, C_6H_5), 12.85 (br s, 1H, exchangeable with D_2O, NH); ^{13}C NMR (DMSO-d_6) δ 119.6 (C-8), 122.7 (C-9a), 123.5 (C-4a), 127.4, 127.6, 128.7, 129.0, 130.0, 130.1, 136.7, 137.2 (C_6H_5), 146.5 (C-2), 149.4, 157.4, 162.8 (C-5a, C-7, C-9), 151.4 (C-9b), 156.7 (CO); MS (EI, *m*/*z*, %): 355 (M^+, 100), 299 (23), 255 (16), 227 (16); IR (KBr, cm^{-1}): 1660 (CO), 1540, 1400.

4-Chloropyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine (8a,b). A stirred solution of **7a** or **7b** (2.37 mmol) and phosphorous pentachloride (3.55 mmol) in phosphorous oxychloride (6 mL) was refluxed until starting material had disappeared (TLC). The solvent was removed under reduced pressure and the resulting solid was purified by flash chromatography using CH_2Cl_2/hexanes as eluent (3:2 for **8a** and 2:1 for **8b**).

4-Chloro-8-cyano-7-ethoxy-9-phenylpyrido[3′,2′:4,5]thieno-[3,2-*d*]pyrimidine (8a). Yield 85%; mp 202–204 °C; $C_{18}H_{11}N_4OClS$ calcd C, 58.94; H, 3.02; N, 15.27; found: C, 59.14; H, 3.20; N, 15.09; ^{1}H NMR ($CDCl_3$) δ 1.56 (t, 3H, $J = 7.1$ Hz, CH_3), 4.72 (q, 2H, $J = 7.1$ Hz, CH_2), 7.48–7.61 (m, 5H, C_6H_5), 8.77 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$) δ 14.2 (CH_3), 65.1 (CH_2), 97.4 (C-8), 113.9 (CN), 118.7 (C-9a), 128.3, 129.1, 130.3, 132.6 (C_6H_5), 154.3 (C-5a), 154.7 (C-2), 156.6, 156.8, 164.8, 166.0. MS (EI, *m*/*z*, %): 368 ($M^+ + 2$, 38), 366 (M^+, 100), 337 (99), 324 (35); IR (KBr, cm^{-1}): 2220 (CN), 1540, 1320, 1030.

4-Chloro-7,9-diphenylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine (8b). Yield 68%; mp 199–201 °C; $C_{21}H_{12}N_3ClS$ calcd C, 67.47; H, 3.24; N, 11.24; found: C, 67.22; H, 3.41; N,

11.06; ^{1}H NMR ($CDCl_3$) δ 7.45–7.66 (m, 8H, C_6H_5), 7.80 (s, 1H, H-8), 8.11–8.16 (m, 2H, C_6H_5), 8.81 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$) δ 120.0 (C-8), 122.4 (C-9a), 127.5, 128.0, 128.9, 129.1, 129.5, 130.3, 136.5, 137.4 (C_6H_5), 150.6, 153.9 (C-2), 154.4, 157.0, 159.4, 164.3; MS (EI, *m*/*z*, %): 375 ($M^+ + 2$, 16), 373 (M^+, 44), 372 (100), 337 (8), 309 (8); IR (KBr, cm^{-1}): 1560, 1540, 1490, 1420.

Pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine-4-substituted (9a–l): General procedure. A solution of **8a** or **8b** (0.27 mmol) and the appropriate amine (0.32 mmol) in ethanol (10 mL) was refluxed until the starting material had disappeared (TLC). The solvent was removed under reduced pressure and the resulting solid was purified by flash chromatography or, alternatively, filtered off and recrystallized to yield (**9a–l**) (Tables 1 and 2).

8-Cyano-7-ethoxy-9-phenyl-2-(2-nitrophenyl)-4-oxo-1,2,3,4 tetrahydropyrido[3′,2′:4,5]thieno[3,2-*d*] pyrimidine (10). A catalytic amount of PTSA was added to a solution of **5a** (0.3 g, 0.87 mmol) and 2-nitrobenzaldehyde (0.14 g, 0.95 mmol) in toluene (30 mL). The reaction mixture was refluxed for 2 h and the water formed was continuously removed by means of a Dean–Stark trap. The desired product was filtered off and recrystallized from ethanol/acetone to yield **10** (0.33 g, 81%): mp 291–293 °C; $C_{24}H_{17}N_5O_4S$ calcd C, 61.14; H, 3.63; N, 14.85; found: C, 61.31; H, 3.75; N, 14.64; ^{1}H NMR (DMSO-d_6) δ 1.37 (t, 3H, $J = 7.1$ Hz, CH_3), 4.52 (q, 2H, $J = 7.1$ Hz, CH_2), 5.13 (d, 1H, exchangeable with D_2O, $J = 3.5$ Hz, NH), 6.25 (t, 1H, $J = 3.4$ Hz, H-2), 7.28–7.79 (m, 9H, C_6H_5 and C_6H_4), 8.26 (d, 1H, exchangeable with D_2O, $J = 3.5$ Hz, NH); ^{13}C NMR (DMSO-d_6) δ 14.1 (CH_3), 63.2 (C-2), 64.1 (CH_2O), 95.1, 103.3, 114.2 (CN), 116.8, 125.4, 127.9, 128.2, 128.4, 128.8, 129.1, 130.0, 130.3, 132.2, 134.0, 135.2, 143.1, 146.9, 153.5, 161.1, 162.0, 163.4; MS (EI, *m*/*z*, %): 471 (M^+, 80), 349 (100), 322 (69), 294 (49); IR (KBr, cm^{-1}): 3410 (NH), 3170 (NH), 2220 (CN), 1650 (CO).

8-Cyano-7-ethoxy-9-phenyl-2-(2-nitrophenyl)-4-oxo-3,4-dihydropyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine (11). A solution of **10** (0.20 g, 0.45 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (0.15 g, 0.67 mmol) in tetrahydrofuran (10 mL) was refluxed for 3 h. After cooling, the precipitate obtained was filtered off and the crude solid **11** was used in the next step without further purification. Compound **11**, (0.15 g, 70%): mp > 300 °C; $C_{24}H_{13}N_5O_4S$ calcd C, 61.40; H, 3.22; N, 14.92; found: C, 61.56; H, 3.14; N, 14.99; ^{1}H NMR (DMSO-d_6) δ 1.43 (t, 3H, $J = 7.1$ Hz, CH_3), 4.60 (q, 2H, $J = 7.1$ Hz, CH_2), 7.34–8.14 (m, 9H, C_6H_5 and C_6H_4), 13.21 (br s, 1H, exchangeable with D_2O, NH). MS (EI, *m*/*z*, %): 469 (M^+, 61), 292 (69), 264 (42); IR (KBr, cm^{-1}): 2220 (CN), 1640 (CO).

4-Chloro-8-cyano-7-ethoxy-9-phenyl-2-(2-nitrophenyl)-pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine (12). A stirred solution of **11** (0.14 g, 0.29 mmol) and phosphorous pentachloride (0.06 g, 0.29 mmol) in phosphorous oxychloride (5 mL) was refluxed for 15 h. The more volatile components of the reaction mixture were evaporated under reduced pressure, and ice (10 g) was added. The resulting solid was filtered off and recrystallized from ethanol/acetone to yield **12** (0.13 g, 90%): mp 241–243 °C; $C_{24}H_{13}N_5O_3ClS$ calcd C, 59.08; H, 2.89; N, 14.35; found: C, 59.09; H, 2.75; N, 14.50; ^{1}H NMR (DMSO-d_6) δ 1.46 (t, 3H, $J = 7.1$ Hz, CH_3), 4.63 (q, 2H, $J = 7.1$ Hz, CH_2), 7.52–7.93 (m, 9H, C_6H_5 and C_6H_4); ^{13}C NMR ($CDCl_3$) δ 14.3 (CH_3), 65.2 (CH_2), 97.3 (C-8), 114.0 (CN), 118.7 (C-9a), 127.5 (C-4a), 124.0, 128.3, 128.8, 129.9, 130.6, 131.2, 131.7, 131.8, 132.5, 149.5 (C_6H_4NO2 and C_6H_5), 154.1, 156.5, 156.8, 159.2, 164.8, 166.2 MS (EI, *m*/*z*, %): 489 ($M^+ + 2$, 5), 487 (M^+, 13), 470 (100), 440 (59), 411 (36), 221 (8); IR (KBr, cm^{-1}): 2220 (CN), 1540, 1340, 1310.

4-(4-Benzylpiperazino)-8-cyano-7-ethoxy-9-phenyl-2-(2-nitrophenyl)-pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine (13). A solution of **12** (0.10 g, 0.22 mmol) and 4-benzylpiperazine (0.08 g, 0.44 mmol) in ethanol (7 mL) was refluxed for 3 h. The solid was filtered off and recrystallized from ethanol/dichloromethane (Tables 1 and 2).

2-Amino-3,5-dicyano-6-morpholinopyridine (15). A solution of **14**[17] (0.18 g, 1.12 mmol) and morpholine (0.12 g, 1.34 mmol) in ethanol/THF (1/3, 15 mL) was refluxed until the starting material had disappeared (TLC). The solid was filtered off and recrystallized from ethanol to yield **15** (0.14 g, 54%): mp 198–200 °C; $C_{11}H_{11}N_5O$ calcd C, 57.63; H, 4.84; N, 30.55; found: C, 57.72; H, 4.68; N, 30.47; ^{1}H NMR ($CDCl_3$) δ 3.78 (m, 4H, NCH_2), 3.88 (m, 4H, CH_2O), 5.36 (br s, 2H, exchangeable with D_2O, NH_2), 7.77 (s, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 47.3 (NCH_2), 66.5 (CH_2O), 81.4, 82.6 (C-3, C-5), 115.8 (CN), 117.8 (CN), 149.5 (C-4), 159.1, 159.4 (C-2, C-6); MS (EI, *m*/*z*, %): 229 (M^+, 32), 172 (38), 144 (100), 117 (51), 89 (47); IR (KBr, cm^{-1}): 3400, 3315, 3210 (NH), 2210 (CN), 1650, 1600, 1540, 1480.

2-Chloro-3,5-dicyano-6-morpholinopyridine (16). To a stirred suspension of anhydrous $CuCl_2$ (4.4 g, 32.8 mmol) in dry CH_3CN (100 mL), containing isoamyl nitrite (3.8 g, 32.8 mmol), was added compound **15** (5.0 g, 21.8 mmol). The reaction mixture was heated at 65 °C for 2.5 h. After cooling, the reaction mixture was poured into 20% HCl (200 mL) and extracted with CH_2Cl_2 (3×50 mL). The organic layers were combined and evaporated under reduced pressure and the resulting solid was purified by flash chromatography using CH_2Cl_2/AcOEt (35/1) as eluent to yield **16** (2.9 g, 53%): mp 186–188 °C; $C_{11}H_9N_4ClO$ calcd C, 53.13; H, 3.65;

N, 22.53; found: C, 53.35; H, 3.76; N, 22.57; ^{1}H NMR ($CDCl_3$) δ 3.82 (m, 4H, NCH_2), 4.02 (m, 4H, CH_2O), 7.96 (s, 1H, H-4), ^{13}C NMR ($CDCl_3$) δ 47.5 (NCH_2), 66.4 (CH_2O), 89.9, 98.1 (C-3, C-5), 114.1 (CN), 115.9 (CN), 149.9 (C-4), 154.8, 157.2 (C-2, C-6); MS (EI, *m*/*z*, %): 250 ($M^+ + 2$, 13), 248 (M^+, 39), 217 (35), 205 (65), 191 (66), 163 (100), 128 (44); IR (KBr, cm^{-1}): 2220 (CN), 1600, 1535, 1410, 1275.

3,5-Dicyano-6-morpholinopyridine-2-(1*H*)-thione (17). A mixture of **16** (2.4 g, 9.8 mmol) and $NaHS \times H_2O$ (2.4 g) in ethanol (100 mL) was stirred at room temperature for 1.5 h. The solvent was removed under reduced pressure. After addition of water (50 mL) the solution was acidified with 2 N HCl and **17** deposited as a solid that was filtered off and used in the next step without further purification. Compound **17** (2.3 g, 9.5 mmol): mp 228–230 °C; $C_{11}H_{10}N_4OS$ calcd C, 53.65; H, 4.09; N, 22.75; found: C, 53.47; H, 4.33; N, 22.59; ^{1}H NMR (acetone-d_6) δ 3.66 (br s, 1H, exchangeable with D_2O), 3.88 (m, 8H, NCH_2CH_2O), 8.05 (s, 1H, H-4); MS (EI, *m*/*z*, %): 246 (M^+, 49), 215 (34), 189 (82), 161 (100), 134 (46), 128 (17); IR (KBr, cm^{-1}): 2210 (CN), 1590, 1495, 1280.

3,5-Dicyano-2-cyanomethylthio-6-morpholinopyridine (18). A mixture of **17** (0.1 g, 0.4 mmol), chloroacetonitrile (40 mg, 0.5 mmol), potassium carbonate (70 mg, 0.5 mmol), and a catalytic amount of KI in acetone (15 mL) was stirred at room temperature for 4 h. The insoluble solid was removed by filtration and washed with acetone. The filtrate and the washings were combined and the solvent was evaporated. The resulting solid was recrystallized from acetone to yield **18** (75 mg, 0.3 mmol): mp 176–178 °C; $C_{13}H_{11}N_5OS$ calcd C, 54.73; H, 3.89; N, 24.55; found: C, 54.62; H, 3.78; N, 24.42; ^{1}H NMR ($CDCl_3$) δ 3.87 (m, 6H, NCH_2 and SCH_2), 4.05 (m, 4H, CH_2O), 7.88 (s, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 16.1 (SCH_2), 47.7 (NCH_2), 66.5 (CH_2O), 88.5, 95.3 (C-3, C-5), 113.9 (CN), 115.4 (CN), 116.4 (CN), 148.4 (C-4), 157.5, 162.3 (C-2, C-6). MS (EI, *m*/*z*, %): 285 (M^+, 46), 245 (100), 226 (61), 173 (25), 160 (20); IR (KBr, cm^{-1}): 2240 (CN), 2215 (CN), 1580, 1535, 1400, 1340.

5-Amino-3,6-dicyano-2-morpholinothieno[2,3-*b*]pyridine (19). Method A. A mixture of **18** (2.0 g, 7.0 mmol) and potassium carbonate (1.2 g, 8.4 mmol) was stirred at room temperature for 1 h. The solid was filtered off and recrystallized from ethanol to yield **19** (1.7 g, 88%): mp 294–296 °C; $C_{13}H_{11}N_5OS$ calcd C, 54.73; H, 3.89; N, 24.55; found: C, 54.65; H, 3.76; N, 24.45; ^{1}H NMR (DMSO-d_6) δ 3.69 (m, 8H, NCH_2CH_2O), 7.28 (br s, 2H, exchangeable with D_2O, NH_2), 8.75 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6) δ 48.3 (NCH_2), 65.8 (CH_2O), 68.5 (C-6), 92.2 (C-3), 115.8 (CN), 116.5 (CN), 117.9 (C-4a), 140.1 (C-4), 150.1 (C-5), 158.8, 162.6 (C-2, C-7a); MS (EI, *m*/*z*, %): 285 (M^+, 95), 270 (15), 254 (33), 240 (34), 228 (80), 200 (100), 173 (59); IR (KBr, cm^{-1}): 3400, 3340, 3220 (NH), 2210 (CN), 2190 (CN), 1655, 1595, 1565, 1555, 1510, 1435.

Method B (from compound 17). A mixture of **17** (2.3 g, 9.3 mmol), chloroacetonitrile (0.8 g, 11.2 mmol), potassium carbonate (1.5 g, 11.2 mmol), and a catalytic amount of KI in acetone (50 mL) was stirred at room temperature for 4 h. The solvent was removed under reduced pressure and the crude solid obtained was stirred at room temperature for 1 h with a solution of potassium carbonate (1.5 g, 11.2 mmol) in ethanol (50 mL). The solid was filtered off and recrystallized from ethanol to yield **19** (2.6 g, 99%).

4-Chloro-8-cyano-2-dimethylamino-7-morpholinopyrido-[3′,2′:4,5]thieno[3,2-*d*]pyrimidine (20). A solution of **19** (2.3 g, 8.1 mmol) and phosgene iminium salt (2.0 g, 12.2 mmol) in 1,2-dichloroethane (20 mL) was refluxed for 6 h. After cooling, a stream of dry hydrogen chloride was passed through the reaction mixture for 30 min, and the stirring continued at room temperature for 48 h. The resulting solid was filtered off and recrystallized from ethanol/acetone to yield **20** (1.6 g, 53%): mp 225–227 °C; $C_{16}H_{15}N_6OClS$ calcd C, 51.26; H,4.03; N, 22.42; found: C, 51.37; H, 3.95; N, 22.49; ^{1}H NMR ($CDCl_3$) δ 3.25 (s, 6H, NMe_2), 3.89 (m, 8H, NCH_2CH_2O), 8.48 (s, 1H, H-9); ^{13}C NMR ($CDCl_3$) δ 37.4 (NMe_2), 48.5 (NCH_2), 66.6 (CH_2O), 92.2 (C-8), 114.4 (C-4a), 117.9 (CN), 119.2 (C-9a), 140.0 (C-9), 153.7 (C-4), 157.4 (C-5a), 159.9 (C-9b), 160.8 (C-7), 167.0 (C-2); MS (EI, *m*/*z*, %): 374 (M^+, 92), 359 (15), 344 (6), 330 (11), 317 (62), 287 (29), 238 (24); IR (KBr, cm^{-1}): 2970, 2210 (CN), 1600, 1580, 1550, 1490.

8-Cyano-2-dimethylamino-7-morpholinopyrido[3′,2′:4,5]-thieno[3,2-*d*]pyrimidine-4-substituted (21a,b). A solution of **20** (0.15 g, 0.27 mmol) and the appropriate amine (0.32 mmol) in ethanol/THF (1/3, 10 mL) was refluxed until the starting material had disappeared (TLC). The solid was filtered off and recrystallized from ethanol/acetone to yield **21a,b** (Tables 1 and 2).

Acknowledgements

We acknowledge financial support from Rhone-Poulenc-España. We are also grateful to CICYT (Grants MAR95-1933-CO2-O2 and PM95 0135) and to the Xunta de Galicia (XUGA-20908B97).

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