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Pyrazolopyrimidines: synthesis, effect on histamine release from rat peritoneal mast cells and cytotoxic activity

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Abstract – A series of 1H-pyrazolo[3,4-d]pyrimidines (**3–6**) substituted at positions 1 (R_1 = Ph, H, *tert*-butyl and ribosetribenzoate), 4 (R_2 = chlorine, nitrogen and oxygen nucleophiles), and 6 (dimethylamino) have been synthesized and their effect on the release of histamine from rat peritoneal mast cells measured. After chemical stimulation, (polymer 48/80), several compounds (i.e. **3b**, **4a**, **4b**, **4d**, **4g**, **5a**), produce inhibition two to three times higher (40–60%) than DSCG but this action is lower after preincubation. **4b** (R_1 = Ph, R_2 = NHCH₂Ph; 50–70% inhibition) and **5a** (R_1 = H, R_2 = OMe; 50–55% inhibition) are the most active ones in both experiments. With ovalbumin as stimulus, several pyrazolopyrimidines show inhibition similar to DSCG, the most active compounds being **6a–d** (IC_{50} = 12–16 μ M; R_1 = ribosetribenzoate, R_2 = methoxy and amino). Compounds **4e** (R_1 = *t*-butyl, R_2 = OMe) and **4g** (R_1 = *t*-butyl, R_2 = piperidino) are inducers of the release of histamine (60 and 150% increase). Compounds **4b** and **4c** showed cytotoxic activity (IC_{50} = 1 μ g/mL) to HT-29 human colon cancer cells. © 2001 Éditions scientifiques et médicales Elsevier SAS

Pyrazolopyrimidines / Histamine / Antihistaminic / Cytotoxicity

1. Introduction

The search for new drugs useful as inhibitors of the release of histamine and other vasoactive mediators, such as leukotrienes and prostaglandins, is an important area of research in relation to the effective treatment of asthma. Disodium cromoglycate (DSCG[®]) (figure 1), and nedocromil sodium [1–4] are two of the most extensively used drugs for this purpose, but a number of nitrogen and sulfur-containing heterocyclic systems have also been explored and found to produce effective oral antiallergics. Representative examples are found within the tetrazole [5–8], thienopyrimidine [9] (i.e. tiiprinast[®]), pyrimidothienotriazine [10, 11], thiophene (i.e. ketotifen[®]) and naphthyridine [12, 13] systems.

We have recently described the action of pyridopyrimidines [14], pyridothienopyrimidines [15], and pyridothienotriazines [16] as inhibitors and inducers of the release of histamine from rat peritoneal mast cells. A preliminary screening suggested us that some pyrazol-fused heterocycles could be potentially interesting

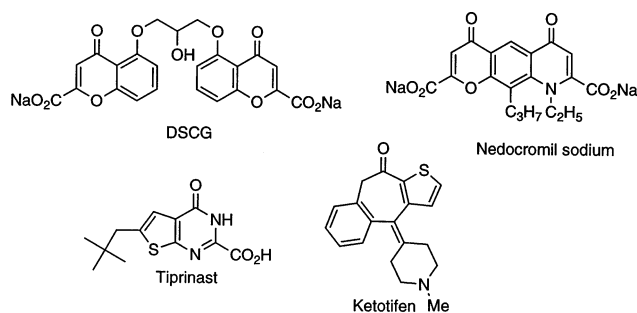
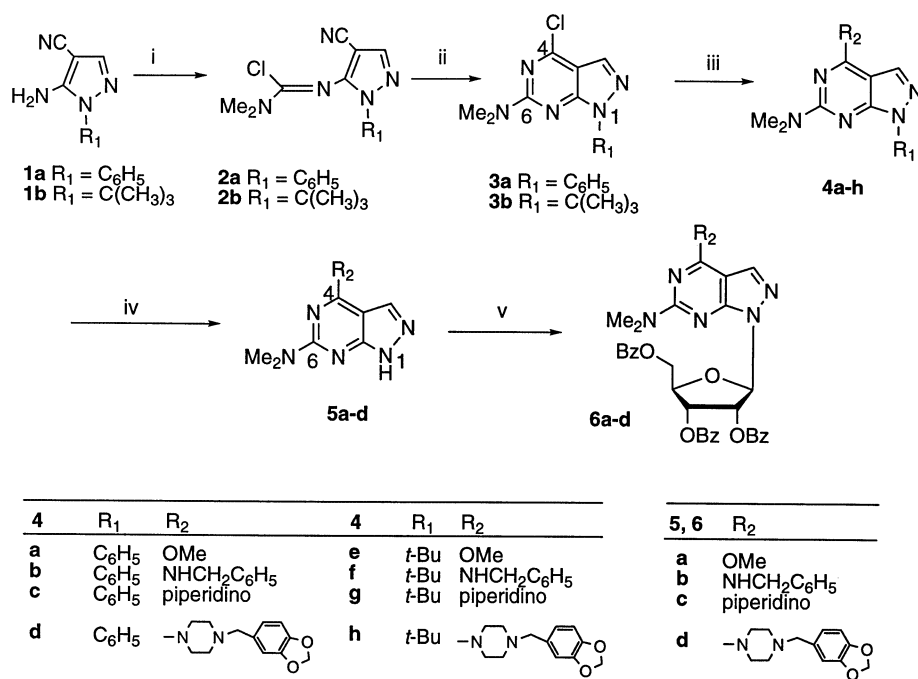


Figure 1.

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Reagents and Conditions: i) Cl₂CNMe₂, 1,2-dichloroethane, reflux; ii) HCl_(g), 1,2-dichloroethane, rt; iii) amine in THF, reflux or NaOMe in MeOH, reflux, iv) HCO₂H, reflux; v) 1-*O*-acetyl-3,4,5-tri-*O*-benzoylribofuranose, SnCl₄, CH₃CN, rt.

Figure 2.

in this process. In this paper, we describe our results on the synthesis and action on the release of histamine of a series of pyrazolopyrimidines (**3–6**) (figure 2).

It is known that the allergic response to a drug can be caused not only by the onset immunological mechanisms (IgE-mediated) but by direct stimulation of the mast cells with the chemical. Therefore, in this study, we investigated the effect of the pyrazolopyrimidines under those two types of stimulus. The amount of histamine released (inhibition and stimulation) by rat peritoneal mast cells was measured after immunological and chemical stimulus using ovalbumin and polymer 48/80, respectively, as inducers. In both experiments, the amount of histamine released was evaluated with and without preincubation of the pyrazolopyrimidine with the stimulus, and the data compared with the amount released by DSCG taken as reference.

In addition, the cytotoxicity of the pyrazolopyrimidines was evaluated *in vitro* for antiproliferative activity against mouse P-388 (lymphoid neoplasm) and human HT-29 (colon carcinoma), A-549 (lung car-

cinoma) and MEL-28 (malignant melanoma) cell lines.

For the preparation of the title compounds we devised a scheme that allowed for the introduction along the process of diverse substituents at positions 1, 4 and 6 of the heterocycle. In this study we describe the results on compounds having a dimethylamino group fixed at C-6 and explore the effect of modifications on the other two positions ($R_1 = \text{Ph}$, H, *tert*-butyl and ribosetribenzoate) and ($R_2 = \text{nitrogen}$, oxygen, chlorine).

2. Chemistry

The construction of fused pyrimidines from *ortho*-aminonitriles and phosgene iminium salts has been successfully applied to the preparation of many heterocyclic fused systems [17]. For the synthesis of the title pyrazolopyrimidines, we used that strategy starting with *N*-substituted *o*-aminocyanopyrazoles (**1a,b**) [18]. In this way, **1a,b** were easily converted to the corresponding chloroamidines (**2a,b**) by reaction with

phosgene iminium chloride. The pyrazolo[3,4-d]pyrimidines (**3a,b**) were prepared by cyclization of the chloroamidines (**2a,b**). Compounds **3a** and **3b** were converted into a variety of pyrazolo[3,4-d]pyrimidines (**4a–h**) by nucleophilic halide displacement with nucleophiles such as primary and secondary amines and the methoxide ion. In order to prepare the pyrazolo[3,4-d]pyrimidine nucleosides, the *tert*-Bu group of **4e–h** was cleaved with formic acid at 90°C to give **5a–d**, that were subsequently glycosidated by reaction with 1-*O*-acetyl-3,4,5-tri-*O*-benzoylribofuranose in the presence of SnCl₄. All the compounds gave satisfactory elemental analyses and have spectral data (IR, MS, and ¹H and ¹³C NMR) that are consistent with the structures proposed.

3. Pharmacology

The inhibition of the release of histamine by the pyrazolopyrimidines (**3–6**) was measured as described previously [16] using rat mast cells obtained from the pleural and peritoneal cavities after lavages with saline solution, and two different stimuli to provoke the release of histamine; the polymer 48/80 as the chemical stimulus [19] and ovalbumin as the immunologi-

cal stimulus. In the experiments involving the chemical stimulus, mast cells were obtained from standard rats while immunological experiments were carried out with ovalbumin-sensitized rats [14]. 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 these experiments are shown graphically in figures 3–6. The histamine released was calculated as a percentage of the total histamine content of the cells. Figures 3–6 represent the variation of that percentage in comparison with the histamine released by the stimuli controls (48/80 or ovalbumin). For comparative purposes, the histamine released in the presence of the reference compound, the mast cell stabilizer DSCG, has also been included in the plots.

Experiments carried out in the absence of stimuli showed that spontaneous histamine release was never higher than 8%.

The pyrazolopyrimidines were used in concentrations that cover the range from 15 to 40 µM, similar to those of the reference compound DSCG (20 and 40 µM). In order to facilitate the comparison of our

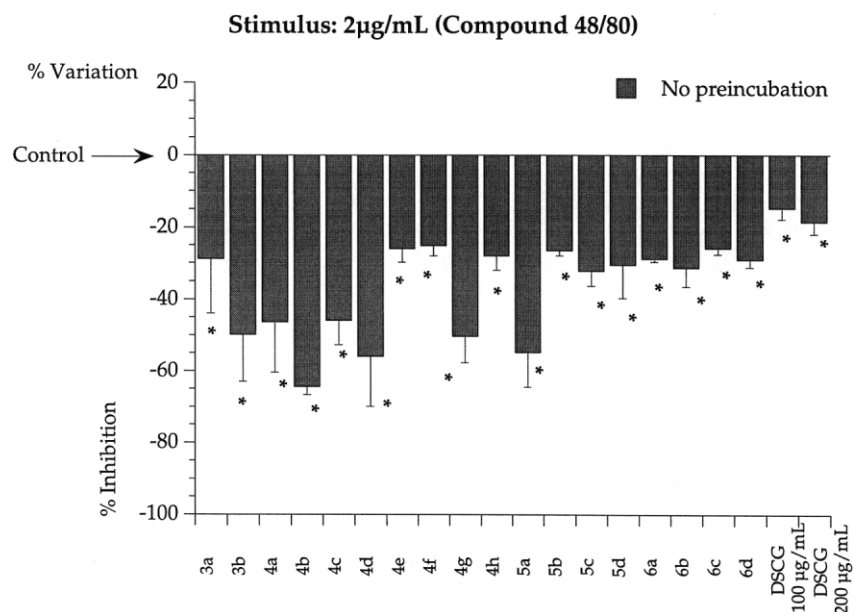


Figure 3. Histamine release in rat peritoneal mast cells stimulated with 2 µg/mL of compound 48/80 in the presence of the pyrazolopyrimidines. The compounds (100 µg/mL), were added to the cells simultaneously with the stimulus (no preincubation). The activity data are normalized to the response of the control experiments (38.76±1.89% in the presence of 2 µg/mL of compound 48/80). The asterisks indicate the statistical significant results.

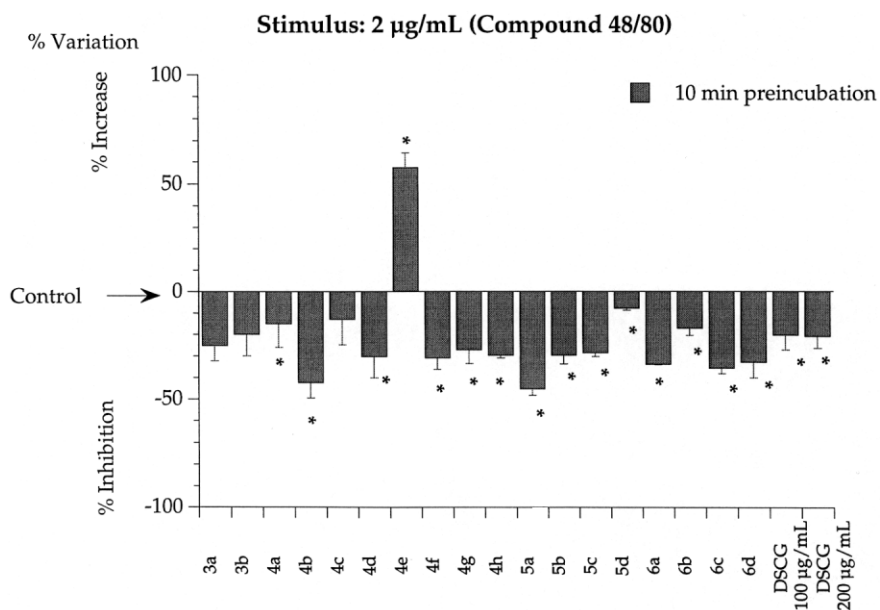


Figure 4. Histamine release in rat peritoneal mast cells stimulated with 2 µg/mL of compound 48/80 in the presence of the pyrazolopyrimidines. The compounds (100 µg/mL), were added to the cells 10 min. before the addition of the stimulus (10 min. preincubation). The activity data are normalized to the response of the control experiments ($39.89 \pm 9.35\%$ in the presence of 2 µg/mL of compound 48/80). The asterisks indicate the statistical significant results.

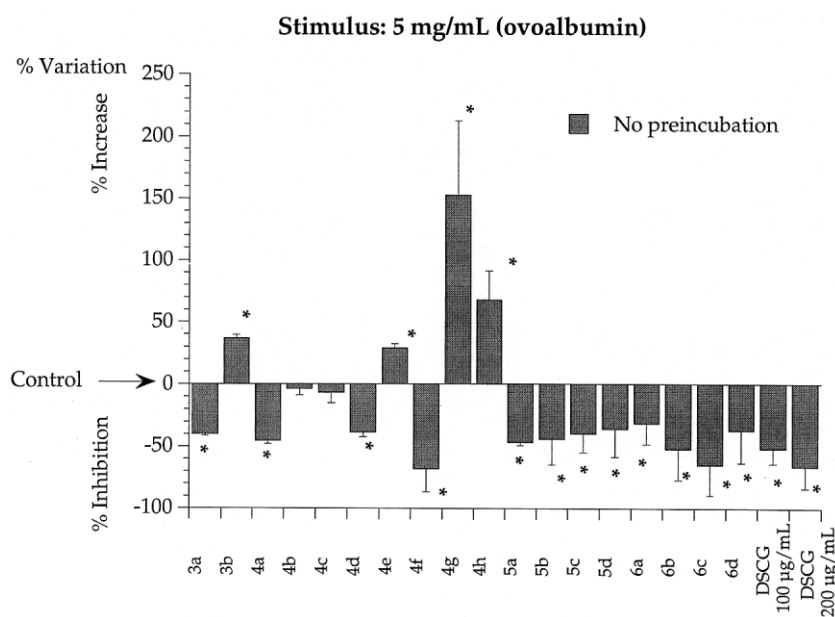


Figure 5. Histamine release in rat peritoneal mast cells stimulated with 5 mg/mL of antigen (ovoalbumin) in the presence of the pyrazolopyrimidines. The compounds (100 µg/mL), were added to the cells simultaneously with the stimulus (no preincubation). The activity data are normalized to the response of the control experiments ($15.05 \pm 3.06\%$ in the presence of 5 mg/mL of antigen). The asterisks indicate the statistical significant results.

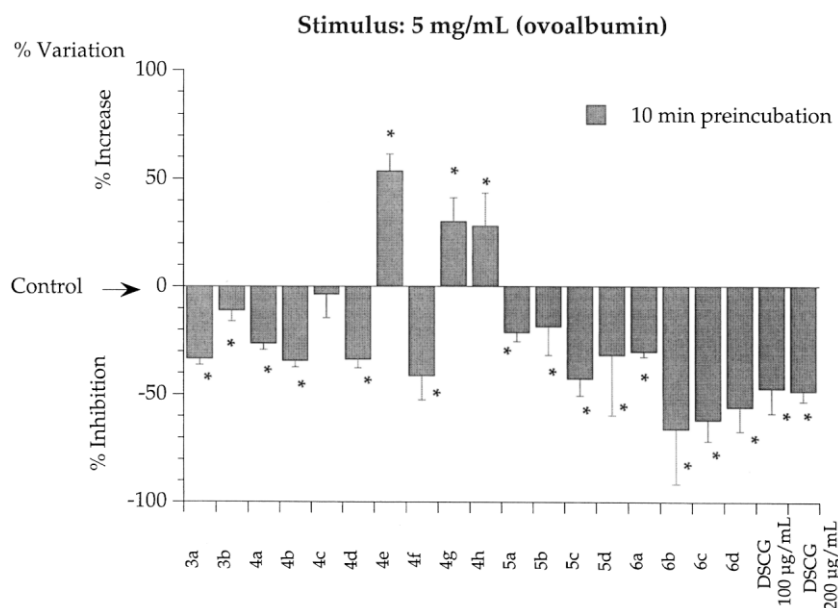


Figure 6. Histamine release in rat peritoneal mast cells stimulated with 5 mg/mL of antigen (ovalbumin) in the presence of the pyrazolopyrimidines. The compounds (100 µg/mL) were added to the cells 10 min. before the addition of the stimulus (10 min. preincubation). The activity data are normalized to the response of the control experiments ($22.53 \pm 3.04\%$ in the presence of 5 mg/mL of antigen). The asterisks indicate the statistical significant results.

results with the literature, the data on *figures 3–6* are expressed in µg/mL. The IC_{50} values were also calculated (µM) and are shown in *table 1*. The cytotoxicity of **3–6** was measured and the results are presented in Section 4.4.

4. Results and discussion

4.1. Histamine release by chemical stimulus

All the pyrazolopyrimidines assayed were found to present inhibitory action when the chemical stimulus polymer 48/80 was added to the rat peritoneal mast cells jointly with the compound (*figure 3*). The inhibition values are between two and three times greater than those obtained with the reference compound DSCG at 100–200 µg/mL. The most active are **3b**, **4b**, **4d**, **4g** and **5a**, that produce about 50–60% less histamine than the reference.

When the cells were preincubated with the compounds before addition of the chemical inducer, (*figure 4*), compound **4e** behaves as a stimulant of the release of histamine while the rest of the compounds are inhibitors although their activity is now reduced to a maximum value of about twice that of DSCG (i.e. **4b** and **6b**).

Overall, under chemical stimulation **4b**, **4d** and **5a** are the most active inhibitors independently of the mode of addition of the stimulus and the heterocycle. Interestingly, compound **4e**, that is an inhibitor when added simultaneously with the stimulus, is an inducer when it is preincubated with the cells before addition of the stimulus.

Table 1. Histamine release inhibition (IC_{50} µM) produced after 10 min. preincubation of the pyrazolopyrimidines with rat peritoneal mast cells immunologically stimulated with ovalbumin (5 mg/mL) ^a

Comp. (preinc.)	IC_{50} (µM)	Comp. (preinc.)	IC_{50} (µM)
DSCG	20–40	5a	51.2
3a	36.3	5b	37.2
4a	36.9	5c	40.6
4b	29.5	5d	26.2
4c	30.8	6a	15.8
4d	21.5	6b	14.0
4f	30.8	6c	14.4
		6d	12.1

^a Five concentrations of pyrazolopyrimidines (0.1, 1, 10, 100 and 500 µg/mL) have been used to calculate the data.

4.2. Histamine release by immunological stimulus

In addition to the experiments carried out with chemical stimulation of the cells, the pyrazolopyrimidines were tested for their action when the release of histamine was induced by an effective immunological stimulus such as the protein ovoalbumin.

Figure 5 shows the results of the tests carried out by simultaneous addition of the immunological stimulus and the pyrazole to the rat peritoneal mast cells. Again, many compounds present an inhibitory activity similar to that of DSCG, but the most interesting results are the surge of **3b**, **4e**, **4h** and principally **4g** (150% more histamine than the reference) as effective stimulants of the release of histamine and the total lack of activity of **4b** and **4c**.

In the tests performed with the compound added 10 min. before the ovoalbumin, (preincubation; figure 6), the general pattern of activity is similar to that of the experiment without preincubation: Compounds **4e**, **4g** and **4h** are again inducers of the release of histamine while the rest of the pyrazoles show about half or similar (**6b–d**) activity to that of the reference.

In order to get a clearer comparative picture of the potential interest of these compounds, experiments using five concentrations of inhibitor have been carried and the corresponding IC_{50} values were compared with those of DSCG. The results, expressed in μM , are presented in table 1. The values are in the range 12–50 μM , compounds **6a–d** being the most active of all. This confirms that the pyrazolopyrimidines examined present an inhibitory action under immunological stimulus, similar to that of the reference compound.

Interestingly, preincubation shows a higher response with immunological than with chemical stimulation. This is probably related to the longer time the compound has to modulate the signal used by the stimuli, but in the case of chemical stimulation with compound 48/80 the inhibition is always higher when both drugs are added together. We do not have an explanation to this effect, but a competition to pass through the cellular membrane might explain it, since compound 48/80 requires interaction with G proteins to activate the response [20].

4.3. Structure–activity relationship

From a structural point of view, the results of the chemical stimulated experiments, show no clear-cut pattern of activity outside the lower relative activity of the

compounds **6a–d** with the larger substituent R_1 . In fact, inhibitors similar or slightly better than DSCG are found in all the other three series ($R_1 = \text{Ph}$, H , *tert*-butyl). As for the nature of substituent R_2 , it seems that the activity is mainly associated to two types of substituents: amines (i.e. **4b** and **4d**), and ethers (i.e. **5a**). Preincubation produces a remarkable change in the behaviour of **4e** ($R_1 = \text{tert-butyl}$, $R_2 = \text{OMe}$) that is an inhibitor in the simultaneous addition experiment but becomes a stimulant when the experiment is carried out under preincubation. Curiously, no such change of activity is produced in the other pyrazolopyrimidines that share with **4e** the methoxy group at R_2 (i.e. **4a**, **5a**).

For their part, the results obtained in the immunological stimulated experiments, and the IC_{50} data indicate that those pyrazolopyrimidines bearing the ribosetribenzoate substituent, are slightly more active as inhibitors than the rest of the compounds assayed.

4.4. Cytotoxic activity

Pyrazolopyrimidines have been tested in vitro for their cytotoxic activity against four standard tumoral cell lines P-388 (lymphoid neoplasm) and human A-549 (lung carcinoma), HT-29 (colon carcinoma) and MEL-28 (malignant melanoma) cell lines. Only two products, **4b** and **4c**, showed a sufficiently strong activity to deserve to be mentioned. ($IC_{50} = 1 \mu g/mL$ towards HT-29 colon carcinoma cells). The other pyrazolopyrimidines, were not cytotoxic at concentrations lower than 5–10 $\mu g/mL$.

5. Conclusions

In conclusion, several compounds containing the pyrazolopyrimidine heterocyclic system are inhibitors of the liberation of histamine, similar to DSCG, but unfortunately no clear structure-activity pattern emerges from that data apart from the higher activity (50–70%) of those carrying the ribosetribenzoate substituent (**6a–d**) in the immunological experiments and that of those with smaller ones (i.e. **4b**, **4d** and **5a**) when the cells are stimulated chemically. In addition, some compounds are found to stimulate the release of histamine in the range 50–150%. Particularly interesting is **4e** that stimulates the release of histamine (35–60%), in both chemical and immunological experiments. Compounds, **4g** and **4h** (30–150%) are also stimulants but under immunological stimulation only.

The in vitro cytotoxicity of these heterocycles is low in terms of activity (IC_{50} of 10 mg/mL or more) and only **4b** and **4c** show some action with $IC_{50} = 1 \mu\text{g/mL}$ towards human colon cancer cell line HT-29 only.

6. Experimental

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. Orthophtalaldehyde for the spectrofluorimetric measurements was purchased from Merck and the *Bordetella pertussis* used in the sensitization of the rats was obtained from Wako. Eagle's minimum essential medium, Earle's balanced salts, nonessential aminoacids, (EMEM/nea), and L-glutamine were purchased from Biosciences and fetal calf serum (FCS) and trypsin from Seromed.

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 described previously [21]. 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 $300 \pm 5 \text{ mOsm/kg H}_2\text{O}$. 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 $1.5\text{--}2 \times 10^6$ 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 as described.

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 $1\text{--}1.5 \times 10^5$ 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 min. 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 $1000 \times g_{\text{max}}$ 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). 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 assays

Histamine release was measured spectrofluorometrically [22] both in the pellet and in the supernatants with and without preincubation (10 min.) of each drug (100 $\mu\text{g/mL}$ in the single dose experiments) with the cells before addition of the stimulus. The fluorophore obtained by reaction in alkaline medium of the 0.1% solution of orthophtalaldehyde with the histamine released, is stabilized by acidification and remains useful for measurement for about 2 h at room temperature. In the experiments involving the chemical stimulus, the mast cells were obtained from standard rats while the experiments using immunological stimulus were carried out with the ovalbumin-sensitized rats. The results are expressed as a percentage of histamine released with respect to the total histamine content. Calculations were done subtracting 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. All the experiments were repeated at least three times in duplicate and the data was analyzed using the Student's *t*-test for unpaired data. A probability level of 0.05 or less was used for statistical significance and the results are expressed as mean \pm S.E.M. Five concentrations of inhibitor (0.1, 1, 10, 100 and 500 $\mu\text{g/mL}$) were used in order to obtain the IC_{50} values.

6.1.5. Antineoplastic assays

In vitro antitumor assays were carried out by an adaptation of the method described by Bergeron et al. [23] and the activity measured 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). The cells were maintained in logarithmic growth in EMEM/nea, 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% CO₂ and 98% H₂O 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 IC₅₀ 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 reported uncorrected. IR spectra were recorded on potassium bromide disks on a Perkin–Elmer 783 spectrophotometer. ¹H and ¹³C NMR spectra were obtained on a Bruker AC200F instrument at r.t. MS were obtained on 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 Elemental Analyses General Service of the University of La Coruña.

6.2.1. Preparation of 5-[chloro(dimethylamino)-methyleneamino]-4-cyanopyrazoles (**2a–b**)

6.2.1.1. General procedure

A solution of **1a** or **1b** [17] (8 mmol) and phosgenium chloride (12 mmol) in 1,2-dichloroethane (30 mL) was refluxed for 2 h. The solvent was removed under

reduced pressure and the residue was purified by flash chromatography to yield the corresponding amide chloride.

6.2.2. 5-[Chloro(dimethylamino)-methyleneamino]-4-cyano-1-phenyl-1H-pyrazole (**2a**)

Using CH₂Cl₂/hexanes (2:1) as eluent. Yield 80%; m.p. 105–107°C. IR (cm⁻¹): 2220 (CN); 1640. ¹H NMR (CDCl₃): δ = 3.14 (s, 6H, NMe₂); 7.32–7.59 (m, 5H, C₆H₅); 7.75 (s, 1H, H-3). ¹³C NMR (CDCl₃): δ = 40.2; 83.7; 114.2; 123.3; 127.3; 128.5; 138.0; 141.3; 143.0; 150.0. MS (EI, *m/z*, %): 275 (*M*⁺+2, 30); 273 (*M*⁺, 82). Anal. Calc. C₁₃H₁₂N₅Cl: C, 57.04; H, 4.42; N, 25.59. Found: C, 56.89; H, 4.59; N, 25.68%.

6.2.3. 1-tert-butyl-5-[Chloro(dimethylamino)-methyleneamino]-4-cyano-1H-pyrazole (**2b**)

Using hexanes/AcOEt (3:1) as eluent. Yield. 80%; m.p. 76–78°C. IR (cm⁻¹): 2220 (CN); 1640. ¹H NMR (CDCl₃): δ = 1.50 (s, 9H, C(CH₃)₃); 3.19 (s, 6H, NMe₂); 7.48 (s, 1H, H-3). ¹³C NMR (CDCl₃): δ = 28.8; 40.2; 60.4; 83.7; 114.6; 138.3; 142.1; 150.3. MS (EI, *m/z*, %): 255 (*M*⁺+2, 13); 253 (*M*⁺, 51). Anal. Calc. C₁₁H₁₆N₅Cl: C, 52.07; H, 6.36; N, 27.60. Found: C, 52.30; H, 6.18; N, 27.81%.

6.2.4. Preparation of 4-chloropyrazolo[3,4-d]pyrimidines (**3a–b**)

6.2.4.1. General procedure

A stream of dry hydrogen chloride was passed through a solution of **1a** or **1b** in 1,2-dichloroethane for 1 h, the solution was stirred at r.t. for 3 days. The solvent was removed under reduced pressure and the residue was purified by flash chromatography to yield **3a–b**.

6.2.5. 4-Chloro-6-dimethylamino-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**3a**)

Using CH₂Cl₂/hexanes (1:1) as eluent. Yield 96%; m.p. 124–126°C. IR (cm⁻¹): 2900; 1540. ¹H NMR (CDCl₃): δ = 3.28 (s, 6H, NMe₂); 7.25–8.27 (m, 5H, C₆H₅); 7.98 (s, 1H, H-3). ¹³C NMR (CDCl₃): δ = 37.6; 107.4; 120.2; 125.7; 128.9; 133.8; 139.0; 154.3; 155.6; 160.5. MS (EI, *m/z*, %): 275 (*M*⁺+2, 36); 273 (*M*⁺, 100). Anal. Calc. C₁₃H₁₂N₅Cl: C, 57.04; H, 4.42; N, 25.59. Found: C, 57.30; H, 4.17; N, 25.70%.

6.2.6. 1-tert-butyl-4-Chloro-6-dimethylamino-1H-pyrazolo[3,4-d]pyrimidine (**3b**)

Using CH₂Cl₂/hexanes (4:1) as eluent. Yield 74%; m.p. 110–111°C. IR (cm⁻¹): 2970; 1610. ¹H NMR (CDCl₃):

$\delta = 1.75$ (s, 9H, $\text{C}(\text{CH}_3)_3$); 3.23 (s, 6H, NMe_2); 7.75 (s, 1H, H-3). ^{13}C NMR (CDCl_3): $\delta = 28.7$; 37.5; 59.7; 107.4; 130.6; 153.9; 155.3; 159.4. MS (EI, m/z , %): 255 ($M^+ + 2$, 16); 253 (M^+ , 45). Anal. Calc. $\text{C}_{11}\text{H}_{16}\text{N}_5\text{Cl}$: C, 52.07; H, 6.36; N, 27.60. Found: C, 51.89; H, 6.52; N, 27.78%.

6.2.7. Preparation of 6-dimethylamino-4-methoxy-1H-pyrazolo[3,4-d]pyrimidines (**4a–e**)

6.2.7.1. General procedure

To a solution of sodium methoxide (0.05 g of Na, 2 mmol) in methanol (5 mL) was added **3a** or **3b** (0.5 mmol). The solution was refluxed until the starting material had disappeared (TLC) (1–4 h). The solvent was removed under reduced pressure and water (10 mL) was added to the residue. The solution was neutralized (2 N HCl) and the solid formed was filtered and recrystallized to yield **4a,e**.

6.2.8. 6-Dimethylamino-4-methoxy-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**4a**)

Recrystallized from EtOH. Yield 67%; m.p. 126–128°C. IR (cm^{-1}): 2900; 1640. ^1H NMR (CDCl_3): $\delta = 3.27$ (s, 6H, NMe_2); 4.07 (s, 3H, CH_3); 7.21–8.33 (m, 5H, C_6H_5); 7.93 (s, 1H, H-3). ^{13}C NMR (CDCl_3): $\delta = 37.3$; 53.3; 97.1; 120.2; 125.2; 133; 139.9; 157.4; 161.4; 163.6. MS (EI, m/z , %): 269 (M^+ , 19). Anal. Calc. $\text{C}_{14}\text{H}_{15}\text{N}_5\text{O}$: C, 62.44; H, 5.61; N, 26.01. Found: C, 62.67; H, 5.80; N, 25.82%.

6.2.9. 1-tert-butyl-6-Dimethylamino-4-methoxy-1H-pyrazolo[3,4-d]pyrimidine (**4e**)

Recrystallized from EtOH. Yield 90%; m.p. 84–86°C. IR (cm^{-1}): 2980; 1610. ^1H NMR (CDCl_3): $\delta = 1.75$ (s, 9H, $\text{C}(\text{CH}_3)_3$); 3.23 (s, 6H, NMe_2); 4.04 (s, 3 H, CH_3); 7.71 (s, 1H, H-3). ^{13}C NMR (CDCl_3): $\delta = 28.7$; 37.2; 53.2; 59.1; 97.0; 129.5; 157.4; 160.5; 163.5. MS (EI, m/z , %): 249 (M^+ , 50). Anal. Calc. $\text{C}_{12}\text{H}_{19}\text{N}_5\text{O}$: C, 57.81; H, 7.68; N, 28.09. Found: C, 58.02; H, 7.80; N, 27.88%.

6.2.10. Preparation of 6-dimethylamino-1H-pyrazolo[3,4-d]pyrimidines (**4b–d** and **4f–h**)

6.2.10.1. General procedure

A solution of **3a** or **3b** (0.5 mmol) and the appropriate amine (2 mmol) in THF (5 mL) was refluxed until the starting material had disappeared (TLC) (1.5–8 h). The solid was filtered and recrystallized or was purified by flash chromatography.

6.2.11. 4-Benzylamino-6-dimethylamino-1-phenylpyrazolo[3,4-d]pyrimidine (**4b**)

Recrystallized from EtOH. Yield 60%; m.p. 118–120°C. IR (cm^{-1}): 3300 (NH); 2900; 1640. ^1H NMR (CDCl_3): $\delta = 3.25$ (s, 6H, NMe_2); 4.80 (d, 2H, $J = 5.4$ Hz, CH_2); 5.5 (m, 1H, NH); 7.20–7.52, 8.29–8.35 (m, 10 H, C_6H_5); 7.78 (s, 1H, H-3). ^{13}C NMR (CDCl_3): $\delta = 37.2$; 45.0; 95.7; 120.2; 125.0; 128.7; 127.4; 127.7; 138.7; 132.1; 139.9; 156.6; 157.5; 161.1. MS (EI, m/z , %): 344 (M^+ , 52). Anal. Calc. $\text{C}_{20}\text{H}_{20}\text{N}_6$: C, 69.75; H, 5.85; N, 24.40. Found: C, 69.87; H, 6.02; N, 24.11%.

6.2.12. 6-Dimethylamino-1-phenyl-4-piperidino-1H-pyrazolo[3,4-d]pyrimidine (**4c**)

Recrystallized from EtOH. Yield 68%; m.p. 108–110°C. IR (cm^{-1}): 2900; 1560. ^1H NMR (CDCl_3): $\delta = 1.72$ (m, 6H, CH_2); 3.23 (s, 6H, NMe_2); 3.86–3.89 (m, 4H, NCH_2); 7.24–7.52, 8.33–8.37 (m, 5H, C_6H_5); 7.91 (s, 1H, H-3). ^{13}C NMR (CDCl_3): $\delta = 24.5$; 25.6; 37.1; 46.4; 95.6; 120.5; 124.9; 128.6; 134.5; 140; 156.8; 157.5; 161.1. MS (EI, m/z , %): 322 (M^+ , 100). Anal. Calc. $\text{C}_{18}\text{H}_{22}\text{N}_6$: C, 67.06; H, 6.88; N, 26.06. Found: C, 67.22; H, 7.02; N, 25.76%.

6.2.13. 6-Dimethylamino-1-phenyl-4-[4-(3,4-methylene-dioxybenzyl)piperazino]-1H-pyrazolo[3,4-d]pyrimidine (**4d**)

Recrystallized from EtOH. Yield 64%; m.p. 158–160°C. IR (cm^{-1}): $\nu = 2920$; 1560. ^1H NMR (CDCl_3): $\delta = 2.55$ –2.60 (m, 4H, NCH_2); 3.22 (s, 6H, NMe_2); 3.48 (s, 2H, CH_2); 3.91–3.96 (m, 4H, NCH_2); 5.97 (s, 2H, OCH_2O); 6.78–6.91 (m, 3H, $\text{C}_6\text{H}_3\text{CH}_2\text{O}_2$); 7.51–8.33 (m, 5H, C_6H_5); 7.88 (s, 1H, H-3). ^{13}C NMR (CDCl_3): $\delta = 37.1$; 45.2; 52.6; 62.7; 95.7; 100.9; 107.8; 109.4; 120.6; 122.2; 125.1; 128.6; 131.5; 134.3; 139.9; 146.7; 147.7; 157.1; 157.5; 161. MS (EI, m/z , %): 457 (M^+ , 12). Anal. Calc. $\text{C}_{25}\text{H}_{27}\text{N}_7\text{O}_2$: C, 65.63; H, 5.95; N, 21.43. Found: C, 65.58; H, 6.12; N, 21.18%.

6.2.14. 4-Benzylamino-1-tert-butyl-6-dimethylamino-1H-pyrazolo[3,4-d]pyrimidine (**4f**)

Using CH_2Cl_2 as eluent. Yield 84%; m.p. 190–192°C. IR (cm^{-1}): 3300 (NH); 1640. ^1H NMR (CDCl_3): $\delta = 1.76$ (s, 9H, $\text{C}(\text{CH}_3)_3$); 3.20 (s, 6H, NMe_2); 4.78 (d, 2H, $J = 5.86$ Hz, CH_2); 5.36 (s, 1H, NH); 7.32–7.41 (m, 5H, C_6H_5); 7.56 (s, 1H, H-3). ^{13}C NMR (CDCl_3): $\delta = 28.7$; 37.1; 44.9; 58.9; 95.9; 126.9; 127.3; 127.7; 128.6; 139.0; 128.6; 156.0; 160.6. MS (EI, m/z , %): 324 (M^+ , 91). Anal. Calc. $\text{C}_{18}\text{H}_{24}\text{N}_6$: C, 66.64; H, 7.46; N, 25.90. Found: C, 66.89; H, 7.32; N, 25.79%.

6.2.15. 1-*tert*-butyl-6-Dimethylamino-4-piperidino-1*H*-pyrazolo[3,4-*d*]pyrimidine (**4g**)

Recrystallized from EtOH. Yield 90%; m.p. 86–88°C. IR (cm⁻¹): ν = 2970; 1610. ¹H NMR (CDCl₃): δ = 1.62–1.75 (m, 15H, C(CH₃)₃, CH₂); 3.17 (s, 6H, NMe₂); 3.82–3.85 (m, 4H, NCH₂); 7.66 (s, 1H, H-3). ¹³C NMR (CDCl₃): δ = 24.6; 25.6; 28.5; 37.0; 46.4; 58.7; 95.9; 130.6; 156.9; 160.0. MS (EI, *m/z*, %): 302 (*M*⁺, 19). Anal. Calc. C₁₆H₂₆N₆: C, 63.54; H, 8.67; N, 27.79. Found: C, 63.66; H, 8.84; N, 27.50%.

6.2.16. 1-*tert*-butyl-6-Dimethylamino-4-[4-(3,4-methylenedioxybenzyl)piperazino]-1*H*-pyrazolo[3,4-*d*]pyrimidine (**4h**)

Using hexanes/AcOEt (4:1) as eluent. Yield 80%; m.p. 78–80°C. IR (cm⁻¹): ν = 2980; 1600. ¹H NMR (CDCl₃): δ = 1.72 (s, 9H, C(CH₃)₃); 2.04–2.83 (m, 4H, NCH₂); 3.12 (s, 6H, NMe₂); 3.32 (s, 2H, CH₂); 3.46–4.07 (m, 4H, NCH₂); 5.85 (s, 2H, OCH₂O); 6.67–6.82 (m, 3H, C₆H₃CH₂O₂); 7.61 (s, 1H, H-3). ¹³C NMR (CDCl₃): δ = 28.2; 36.7; 44.7; 52.2 (NCH₂); 58.4; 62.3; 95.7; 100.5; 107.5; 109.0; 121.8; 130.3; 131.2; 146.7; 147.7; 156.7; 159.5. MS (EI, *m/z*, %): 437 (*M*⁺, 24). Anal. Calc. C₂₃H₃₁N₇O₂: C, 63.14; H, 7.14; N, 22.41. Found: C, 62.88; H, 7.28; N, 22.64%.

6.2.17. Preparation of 6-dimethylamino-1*H*-pyrazolo[3,4-*d*]pyrimidines (**5a–d**)

6.2.17.1. General procedure

A solution of **4e–h** (6 mmol) in formic acid (2.5 mL) was heated at 90°C until the starting material had disappeared (TLC). The solvent was removed under reduced pressure and the residue was recrystallized or purified by flash chromatography to yield **5a–d**.

6.2.18. 6-Dimethylamino-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (**5a**)

Recrystallized from EtOH. Yield 70%; m.p. 224–226°C. IR (cm⁻¹): 3100 (NH); 1610. ¹H NMR (DMSO): δ = 3.14 (s, 6H, NMe₂); 4.00 (s, 3H, CH₃); 7.80 (s, 1H, H-3); 12.9 (s, 1H, NH). ¹³C NMR (DMSO): δ = 36.9; 53.0; 94.7; 131.6; 159; 160.7; 162.8. MS (EI, *m/z*, %): 193 (*M*⁺, 100). Anal. Calc. C₈H₁₁N₅O: C, 49.73; H, 5.74; N, 36.25. Found: C, 50.06; H, 5.96; N, 36.00%.

6.2.19. 4-Benzylamino-6-dimethylamino-1*H*-pyrazolo[3,4-*d*]pyrimidine (**5b**)

Using hexanes/AcOEt (2:1) as eluent. Yield 40%; m.p. 248–250°C. IR (cm⁻¹): 3360 (NH); 3160 (NH); 1600;

1520. ¹H NMR (CDCl₃): δ = 3.22 (s, 6H, NMe₂); 4.80 (d, 2H, *J* = 5.37 Hz, CH₂); 5.40 (s, 1H, NH); 7.37–7.39 (m, 5H, C₆H₅); 7.68 (s, 1H, H-3). ¹³C NMR (CDCl₃): δ = 37.1; 44.9; 95.9; 126.9; 127.3; 127.7; 128.6; 139; 128.6; 156.0; 160.6. MS (EI, *m/z*, %): 268 (*M*⁺, 53). Anal. Calc. C₁₄H₁₆N₆: C, 62.67; H, 6.01; N, 31.32. Found: C, 62.90; H, 5.92; N, 31.18%.

6.2.20. 6-Dimethylamino-4-piperidino-1*H*-pyrazolo[3,4-*d*]pyrimidine (**5c**)

Using AcOEt as eluent. Yield 94%; m.p. 225–227°C. IR (cm⁻¹): 3240 (NH); 2970; 1560. ¹H NMR (CDCl₃): δ = 1.71 (br s, 6H, CH₂); 3.28 (s, 6H, NMe₂); 3.86–3.89 (m, 4H, NCH₂); 7.79 (s, 1H, H-3). ¹³C NMR (CDCl₃): δ = 24.6; 25.6; 37.7; 46.4; 94.4; 133.9; 156.6; 158.8; 160.8. MS (EI, *m/z*, %): 246 (*M*⁺, 100). Anal. Calc. C₁₂H₁₈N₆: C, 58.51; H, 7.37; N, 34.12. Found: C, 58.34; H, 7.28; N, 34.38%.

6.2.21. 6-Dimethylamino-4-[4-(3,4-methylene-dioxybenzyl)piperazino]-1*H*-pyrazolo[3,4-*d*]pyrimidine (**5d**)

Using CH₂Cl₂/MeOH (26:1) as eluent. Yield 70%; m.p. 168–170°C. IR (cm⁻¹): 2980; 1600. ¹H NMR (CDCl₃): δ = 2.47–2.87 (m, 4H, NCH₂); 3.21 (s, 6H, NMe₂); 3.60 (s, 2H, CH₂); 3.95–4.00 (m, 4H, NCH₂); 5.95 (s, 2H, OCH₂O); 6.76–6.87 (m, 3H, C₆H₃); 7.75 (s, 1H, H-3); 8.00 (br s, 1H, NH). ¹³C NMR (CDCl₃): δ = 37.8; 44.6; 51.9; 62.1; 94.4; 101.0; 108.0; 109.7; 122.8; 129.5; 133.7; 147.1; 147.7; 156.3; 156.6; 158.8. MS (EI, *m/z*, %): 381 (*M*⁺, 12). Anal. Calc. C₁₉H₂₃N₇O₂: C, 59.83; H, 6.08; N, 25.71. Found: C, 60.05; H, 5.87; N, 25.90%.

6.2.22. Preparation of nucleosides (**6a–d**)

6.2.22.1. General procedure

To a solution of 1-*O*-acetyl-3,4,5-tri-*O*-benzoylribofuranose (0.5 mmol) in dry acetonitrile (15 mL) SnCl₄ (1 mmol) was added. The solution was stirred under inert atmosphere at r.t. for 15 min. and then **5a–d** (0.5 mmol) was added and stirring was continued until starting material had disappeared (TLC). The solvent was evaporated and a saturated solution of NaHCO₃ was added. The solution was extracted with AcOEt (3515 mL) and dried (Na₂SO₄). After evaporation of the solvent, the residue was purified by flash chromatography.

6.2.23. 1-(2',3',5'-O-Tribenzoyl-d-ribofuranosyl)-6-dimethylamino-4-methoxy-1H-pyrazolo[3,4-d]-pyrimidine (6a)

Using CH₂Cl₂/AcOEt (10:1) as eluent. Yield 50%; m.p. 152–154°C. $[\alpha]_D = -77.1$. IR (cm⁻¹): 2960; 1760 (CO); 1640; 1560. ¹H NMR (CDCl₃): $\delta = 3.27$ (s, 6H); 4.01 (s, 3 H); 4.66–4.88 (m, 3H), 6.21 (d, $J = 0.88$ Hz, 1H); 6.31 (m, 2H); 7.28–8.05 (m, 16H). ¹³C NMR (CDCl₃): $\delta = 37.5$; 53.3; 64.1; 71.8; 75.9; 80.6; 93.0; 98.6; 123.7; 128.3; 128.5; 128.8; 129.4; 132.9; 133.3; 133.6; 129.7; 161.1; 164.5; 164.9; 165.0; 166.2. MS (FAB, m/z , %): 638 [(MH)⁺, 19]. Anal. Calc. C₃₄H₃₁N₅O₈: C, 64.04; H, 4.90; N, 10.98. Found: C, 63.88; H, 5.10; N, 11.17%.

6.2.24. 1-(2',3',5'-O-Tribenzoyl-d-ribofuranosyl)-4-benzylamino-6-dimethylamino-1H-pyrazolo[3,4-d]-pyrimidine (6b)

Using CH₂Cl₂/AcOEt (4:1) as eluent. Yield 43%; m.p. 96–98°C. $[\alpha]_D = -52.82$. IR (cm⁻¹): 3380 (NH); 1760 (CO); 1640; 1540. ¹H NMR (CDCl₃): $\delta = 3.20$ (s, 6H); 4.58–4.86 (m, 5H); 5.80 (br s, 1H); 6.16 (s, 1H); 6.24–6.31 (m, 2H); 7.29–8.05 (m, 21H). ¹³C NMR (CDCl₃): $\delta = 37.5$; 44.7; 60.4; 64.3; 72.0; 75.9; 80.4; 92.8; 98.3; 122.9; 127.3; 128.6; 128.8; 129.1; 129.4; 129.5; 129.6; 129.7; 133.1; 133.2; 133.4; 133.6; 135.3; 138.7; 157.1; 161.8; 163.5; 165.0; 165.1; 165.5; 166.2; 169.8; 171.2. MS (FAB): 713 [(MH)⁺, 40]. Anal. Calc. C₄₀H₃₆N₆O₇: C, 67.40; H, 5.09; N, 11.79. Found: C, 67.66; H, 4.86; N, 11.95%.

6.2.25. 1-(2',3',5'-O-Tribenzoyl-d-ribofuranosyl)-6-dimethylamino-4-piperidino-1H-pyrazolo[3,4-d]-pyrimidine (6c)

Using CH₂Cl₂/AcOEt (100:1) as eluent. Yield 60%; m.p. 96–98°C. $[\alpha]_D = -62.3$. IR (cm⁻¹): 2940; 1750 (CO); 1580; 1520. ¹H NMR (CDCl₃): $\delta = 1.64$ –2.00 (m, 6H); 3.22 (s, 6H); 3.70–3.75 (m, 4 H); 4.67–4.86 (m, 3H); 6.19 (s, 1H); 6.33 (d, 2H, $J = 4.88$ Hz); 7.31–8.03 (m, 16H). ¹³C NMR (CDCl₃): $\delta = 24.4$; 25.6; 37.4; 46.4; 64.4; 72.1; 76.0; 80.6; 92.8; 97.8; 129.7; 124.7; 127.9; 128.4; 128.5; 128.8; 128.9; 129.5; 133.1; 133.4; 133.5; 133.6; 147.5; 157.4; 161.5; 164.7; 164.9; 165.2; 166.2. MS (FAB, m/z , %): 691 [(MH)⁺, 14]. Anal. Calc. C₃₈H₃₈N₆O₇: C, 66.07; H, 5.54; N, 12.17. Found: C, 65.88; H, 5.70; N, 12.01%.

6.2.26. 1-(2',3',5'-O-Tribenzoyl-d-ribofuranosyl)-6-dimethylamino-4-[4-(3,4-methylenedioxybenzyl)-piperazino]-1H-pyrazolo[3,4-d]pyrimidine (6d)

Using CH₂Cl₂/AcOEt (10:1) as eluent. Yield 5%; m.p.

100–102°C. $[\alpha]_D = -50.87$. IR (cm⁻¹): 2960; 1730 (CO); 1600; 1530. ¹H NMR (CDCl₃): $\delta = 2.47$ –2.52 (m, 4H); 3.21 (s, 6H); 3.46 (s, 2H); 3.74–3.86 (m, 4H); 4.66–4.87 (m, 3H); 5.96 (s, 2H); 6.18 (d, $J = 1.96$ Hz); 6.47 (m, 2H); 6.77–6.89 (m, 3H); 7.30–8.04 (m, 16H). ¹³C NMR (CDCl₃): $\delta = 37.4$; 45.0; 52.5; 62.6; 64.3; 71.9; 75.9; 92.8; 92.8; 97.7; 100.9; 107.9; 109.5; 122.3; 124.2; 128.3; 128.4; 128.9; 129.4; 129.7; 129.8; 131.3; 133.1; 133.4; 133.6; 146.8; 147.7; 157.6; 161.3; 164.6; 165.0; 165.2; 166.0. MS (FAB): 826 [(MH)⁺, 98]. Anal. Calc. C₄₅H₄₃N₇O₉: C, 65.44; H, 5.25; N, 11.87. Found: C, 65.19; H, 5.42; N, 11.99%.

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References

- [1] 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.
- [2] Assem E., Ritcher A., Immunol. Lond. 21 (1971) 729–730.
- [3] Foreman J., Garland L., Br. Med. J. 1 (1976) 820–821.
- [4] Lal S., Malhotra S., Gribben D., Hoddre D., Thorax 39 (1984) 809.
- [5] Unangst P.C., Brown R.E., Herzig D.J., J. Med. Chem. 23 (1980) 1251–1255.
- [6] Klaubert D.H., Sellstedt J.H., Ginosso C.J., Bell S.C., Capetola S.J., J. Med. Chem. 24 (1981) 748–752.
- [7] Phillip A., Jirkousky I., Martel R.R., J. Med. Chem. 23 (1980) 1372–1376.
- [8] Schwender C.F., Sunday B.R., Kerbleski J.J., Herzig D.J., J. Med. Chem. 23 (1980) 964–972.
- [9] Madding G.D., Thompson M.D., J. Heterocyc. Chem. 24 (1987) 581–586.
- [10] Youssefeyeh 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.
- [11] Khandwala A., Van Inwegen R., Coutts S., Dally-Meade V., Youssefeyeh R.D., Int. J. Immunopharmacol. 5 (1983) 491–502.
- [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] Sherwood J., Sehring S., Rizzo C., Chapman R.W., Siegel M.I., Egan R.W., J. Pharmacol. Exp. Ther. 247 (1988) 997–1003.
- [14] Quintela J.M., Peinador C., Botana L., Estevez M., Riguera R., Biorganic Med. Chem. 5 (1997) 1543–1553.

- [15] Quintela J.M., Peinador C., Veiga C., González L., Botana L.M., Alfonso A., Riguera R., *Biorg. Med. Chem.* 6 (1998) 1911–1925.
- [16] Quintela J.M., Peinador C., Veiga C., Botana L.M., Alfonso A., Riguera R., *Eur. J. Med. Chem.* 33 (1998) 887–897.
- [17] Quintela J.M., Peinador C., Moreira M.J., Alvarez-Sarandés R., *Recent Res. Dev. Org. Chem.* 2 (1998) 409–418.
- [18] Barald P.G., Cacciari B., Spalluto G., Pineda de las Infantas y Villatoro M.J., Zocchi C., Dionisotti S., Ongini E., *J. Med. Chem.* 39 (1996) 1164–1171.
- [19] Scharemborg A.M., Kinet J.P., *Chem. Immunol.* 61 (1995) 72–87.
- [20] Mousli M., Bronner C., Bockaert J., Rouot B., Landry Y., *Immunol. Lett.* 25 (1990) 355–357.
- [21] Estevez M.D., Vieytes M.R., Louzao M.C., Botana L.M., *Biochem. pharmacol.* 47 (1994) 591–593.
- [22] Shore P.A., The chemical determination of histamine, in: Glick D. (Ed.), *Analysis of biogenic amines and their related enzymes*, Wiley Interscience, Amsterdam, 1971, pp. 89–92.
- [23] 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.