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Synthesis of some pyrrolo[3,4-*d*]pyridazinones and their preliminary anticancer, antimycobacterial and CNS screening

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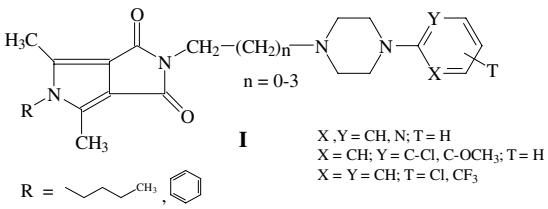
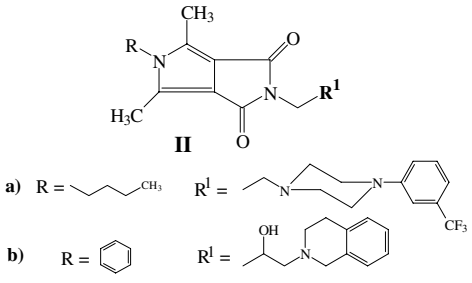
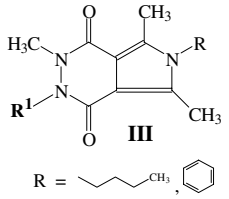
Starting from 1-substituted-2,5-dimethyl-3,4-pyrroledicarboxylic acid anhydrides **1** and *N*-methylhydrazine, 1,2,3,4-tetrahydro-6-substituted-2,5,7-trimethyl-6*H*-pyrrolo[3,4-*d*]pyridazin-1,4-ones **2** were prepared. Reaction of compounds **2** with alkylating agents give 3-*N*- or 4-*O*-alkylated products **3**, **4** or mixtures of these isomers in ratios depending on the alkylating agents. Under preliminary pharmacological screening two of four new pyrrolo[3,4-*d*]pyridazinones were active as cystostatic agents, all the eight compounds displayed moderate activity against *Mycobacterium tuberculosis* and two compounds were active as CNS-depressive agents.

1. Introduction

We have recently described the synthesis and preliminary pharmacological screening of a series of 3,4-pyrroledicarboximide derivatives of general structure **I**. Within this series of compounds some derivatives displayed CNS depressive [1] and analgesic [2, 3] action. The most powerful analgesic effect (1/640 – 1/160 of LD_{50}) was found in compounds with substituents (*o*-Cl, *o*-OCH_3) at the terminal aromatic ring of the side chain. Lack of such a substituent or replacement of the terminal aromatic ring by a heterocyclic one (pyridine, pyrimidine) drastically reduced analgesic activity. Additionally, some 3,4-pyrroledicarboximides **I** and related compounds were selected by the NCI (National Cancer Institute) in Bethesda (USA) for anticancer testing and two of these (**II**), after preliminary screening, were selected for further evaluation against the full panel of human tumor cell lines [4].







The promising results of the pharmacological tests of 3,4-pyrroledicarboximides **I** and **II** encouraged us to prepare a series of cyclic pyrrolehydrazide derivatives of the general structure **III**, possessing the substituent $\mathbf{R^1}$ typical of the basic side chain in compounds **I** or **II**. The proposed cyclic pyrrolehydrazides **III** are pyrrolo[3,4-*d*]pyridazines. A Medline database search indicated that biological properties of pyrrolopyridazines have been described in only a few papers. Meade *et al.* [5, 6] described antiproliferative and antiviral activity of 4-aminopyrrolo[2,3-*d*]pyridazin-7-one nucleosides, Kimura *et al.* and Grundler *et al.* obtained 7-aryloxypyrrolo[2,3-d]pyridazines having antiulcer and antibacterial activity against *Helicobacter pylori* [7–9], Kulagowski *et al.* [10] described derivatives of 4-hydroxy-3-aryl-1*H*-pyrrolo[1,2-*b*]pyridazin-2-one as NMDA and AMPA receptor antagonists, while Ungureanu *et al.* [11] indicated antimicrobial and antifungal activity for derivatives of pyrrolopyridazines.

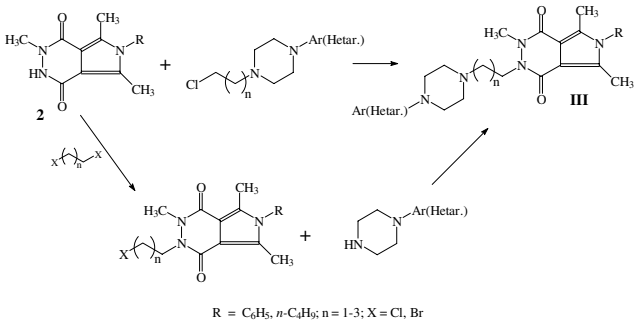
In this context, we planned to screen the representatives of the newly synthesized pyrrolo[3,4-*d*]pyridazinones **III** not only in a series of CNS tests in animal models and for cytotoxic effects, but also for their ability to inhibit growth of *Mycobacterium.*

2. Investigations, results and discussion

2.1. Synthesis of the compounds

The new pyrrolo[3,4-*d*]pyridazinones **2, 3, 4** were prepared by the procedures shown in Schemes 2 and 3 (see below). The five-step synthesis of the starting anhydrides **1a** and **1b** from diethyl α,β-diacetylsuccinate and corresponding amines has been reported earlier [12, 13]. Heating **1** with an excess of *N*-methylhydrazine in toluene gave the 6-substituted-1,2,3,4-tetrahydro-2,5,7-trimethyl-6*H*-pyrrolo[3,2-*d*]pyridazine-1,4-diones **2** (65–70% yield) as evidenced by elementary analyses and spectroscopic data (IR, ^{1}H NMR). Inel *et al.* [14] have recently described the synthesis of 1,2,3,4-tetrahydro-2,5,6,7-tetramethyl-6*H*-pyrrolo[3,2-*d*]pyridazine-1,4-dione from *N*-methylhydrazine and diethyl 1,2,5-trimethylpyrrole-3,4-dicarboxylate in 45% yield, however, the reaction was conducted under extreme conditions (sealed tube, 150 °C, 5 days). It should be noted that the preferential formation of the 1 : 1 cyclized product from anhydrides **1** and an excess of *N*-methylhydrazine in our experiments was not self-evident, because in condensation of, e.g, succinic anhydride with hydrazine, formation of 2 : 1 and 1 : 2 products and polymeric materials has been reported [15].

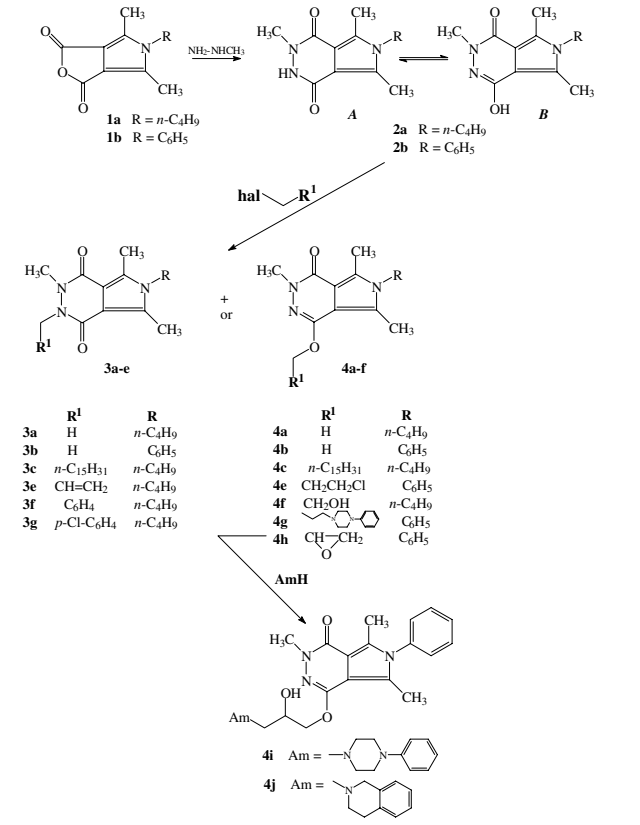
Scheme 1



Initially we planned to synthesize the corresponding 3-*N*-substituted derivatives of pyrrolopyridazinone **2** designed as structural analogues of 3,4-pyrroledicarboximides **1**. Our intention was to synthesize the target compounds using a simple one or two-step method of 3-*N*-alkylation of **2** with 1-chloroalkyl-4-aryl(heteroaryl)piperazines or, alternatively, by alkylation of *N*-aryl(heteroaryl)piperazines using the corresponding 3-*N*-haloalkyl pyrrolopyridazinones as intermediates (Scheme 1).

In order to find the optimum experimental parameters for theses reactions and to evaluate the chemical features of **2**, 3-*N*-methylation of pyrrolopyridazinone **2a** was studied. The control experiments were conducted using classical procedures suitable for the preparations of 2.5 g samples, necessary for preliminary evaluation *in vivo*. Therefore, we examined the methylation of substrate **2a** with ICH_3 in acetonitrile/anhydrous K_2CO_3, ethanol/NaOEt and DMF/NaH. Under these conditions the products were shown to be a mixture of 3-*N*- (**3a**) and 4-*O*-substituted (**4a**) isomers (Scheme 2) which were separated by CC on silica gel. The isomers were obtained with an overall yield of 30–40% with predominance of the expected 3-*N*-methyl derivative **3a**. A similar reaction was observed for the condensation of **2b** with ICH_3/DMF.

Scheme 2



	R^1	R
3a	H	*n*-C_4H_9
3b	H	C_6H_5
3c	*n*-$C_{15}H_{31}$	*n*-C_4H_9
3e	$CH{=}CH_2$	*n*-C_4H_9
3f	C_6H_4	*n*-C_4H_9
3g	*p*-Cl-C_6H_4	*n*-C_4H_9

	R^1	R
4a	H	*n*-C_4H_9
4b	H	C_6H_5
4c	*n*-$C_{15}H_{31}$	*n*-C_4H_9
4e	CH_2CH_2Cl	C_6H_5
4f	CH_2OH	*n*-C_4H_9
4g		C_6H_5
4h		C_6H_5

However, application of the above methods of methylation of **2** using other alkyl halides, i.e. 1-phenyl-4-(3-chloropropyl)piperazine or (2-hydroxyethyl, 3-chloropropyl, 2,3-epoxypropyl) bromides, did not yield the expected 3-*N*-substituted derivatives, but the 4-*O*-isomers **4e–h** (Scheme 2) as the main products.

On alkylation of **2a** with $IC_{16}H_{33}$, the corresponding 3-*N*- and 4-*O*-isomers (**3c** and **4c**) were obtained, in contrast to the methylation of **2** with ICH_3, albeit with the 4-*O*-isomer (**4c**) predominant. Also alkylation of **2b** with the spiro salt **5** (Scheme 3), according to a procedure described in our previous paper [1], gave the corresponding 4-*O*-isomer **4d** as the prevailing product. In contrast, the reaction of **2a** with benzyl and alkenyl (allyl) halides yielded 3-*N*-substituted derivatives (**3e–g**). These results indicate that formation of the 3-*N*- or 4-*O*-isomers is influenced by the nature of the alkylating agent.

The target compounds with the 2-hydroxypropyl central chain (**4i**, **4j**), related to 3,4-pyrroledicaboximide **IIb**, were synthesized by treatment of the 4-*O*-epoxypropyl intermediate **4h** with the corresponding cyclic amines (Scheme 2).

Preferential formation of the 4-*O*-isomers **4** practically prevented our approach to the synthesis of the planned 3-*N*-substituted pyrrrolopyridazinones **3** as analogs of compounds **I** in 2.5 g quantity, necessary for CNS testing in animal models. Therefore, from the series of pyrrolopyridazinones **3** and **4** only one 3-*N*- and one 4-*O*-isomer (**3b** and **4d**) were evaluated in the primary CNS screening, whereas a few more of the compounds **3** and **4** were evaluated as potential antimycobacterial or cytostatic agents. Such preliminary evaluations need samples of only 5 and 20–100 mg, respectively. For example, based on literature data, we introduced the benzyl substituent into compounds prepared for antimycobacterial screening (**3f**, **g**) [16], or we greatly increased the lipophilicity, expressed as log $P_{calc.}$ (the calculated octanol/water partition coefficient; Table 4), of these compounds by introducing the hexadecyl side chain (**3c**, **4c**). This modification was expected to exert a favorable effect as mycobacteria possess a highly lipophilic cell wall.

The final compounds **2**, **3** and **4** were identified by elemental and spectroscopic analyses; their physical data are

Scheme 3

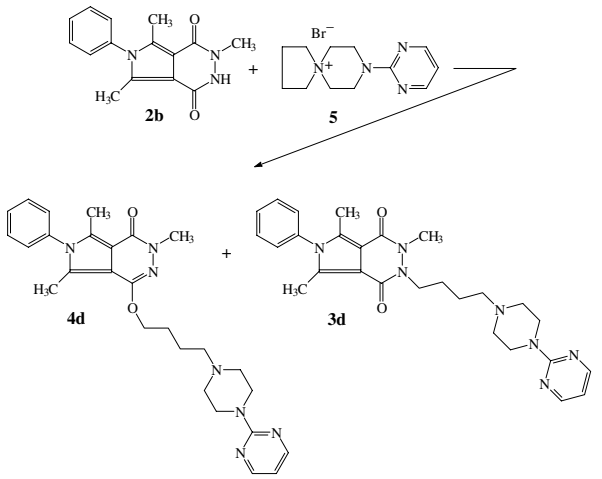
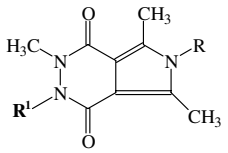
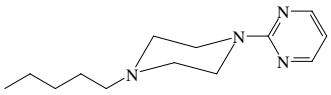


Table 1: Data of pyrrolopyridazinones 2 and 3



Compd.	R	R^1	M.p. (yield %)	Formula, m.w.
2a	*n*-C_4H_9	H	248–250 (65)	$C_{13}H_{19}N_3O_2$ 249.31
2b	C_6H_5	H	279–281 (70)	$C_{15}H_{15}N_3O_2$ 269.30
3a	*n*-C_4H_9	CH_3	114–116 (24–35)	$C_{14}H_{21}N_3O_2$ 263.33
3b	C_6H_5	CH_3	252–254 (33)	$C_{16}H_{17}N_3O_2$ 283.32
3c	*n*-C_4H_9	$CH_2(CH_2)_{14}CH_3$	65–68 (7)	$C_{29}H_{51}N_3O_2$ 473.74
3d	C_6H_5		65–68 (9)	$C_{27}H_{33}N_7O_2$ 487.60
3e	*n*-C_4H_9	$CH_2CH{=}CH_2$	98–100 (31)	$C_{16}H_{23}N_3O_2$ 289.37
3f	*n*-C_4H_9	$CH_2C_6H_5$	134–134 (52)	$C_{20}H_{25}N_3O_2$ 339.43
3g	*n*-C_4H_9	$CH_2C_6H_4$(*p*-Cl)	90–92 (43)	$C_{20}H_{25}N_3O_2$ 373.88

compiled in Tables 1 and 2. The IR and ^{1}H NMR spectra clearly differentiate between 4-*O*-(**4**) and 3-*N*-substituted (**3**) pyrrolo[3,4-*d*]pyridazinones.

The ^{1}H NMR spectra of the isomers **3a** and **4a**, for example, exhibited no D_2O exchangeable protons. The 3-*N*-isomer **3a** shows a plane of symmetry and the signal of the 5- and 7-methyl group of the pyrrole ring appears as a 6H singlet (2.64 ppm). In contrast, in the 4-*O*-isomer **4a** the signals of these methyl groups are separated and observed as two 3H singlets at 2.5 and 2.68 ppm with the singlet of 5-CH_3 shifted to higher field.

The structure of the isomers **3a** and **4a** was also verified on the basis of IR (KBr) data. In the IR spectrum of **3a**, the most intense band was the carbonyl absorption (1640 cm^{-1}). This band was much weaker in the spectrum of the 4-*O*-isomer **4a**; however, this spectrum additionally revealed a characteristic strong ether (C−O−C) absorption (1280 cm^{-1}). These data were also the key elements in differentiating the other 4-*O*-(**4**) and 3-*N*-substituted (**3**) products.

The ^{1}H NMR spectra of the 3-*N*-benzyl derivatives (**3f**, **g**), were an exception, in that the CH_3-protons of the pyrrole ring appeared as two, partially overlapping 3H singlets at 2.64 and 2.67 ppm, respectively, instead of the expected 6H singlet.

These chemical and analytical data suggest that the key intermediates **2** can exist in the tautomeric forms **2A** and **2B** (Scheme 2). The ^{1}H NMR ($CDCl_3$) spectra of **2** showed only one broad signal of the D_2O exchangeable proton, for **2a** at 6.6 ppm and at 8.4 ppm for **2b**. These observations suggest the existence of the pyrrolopyridazinones **2a** and **2b** in chloroform solution in different tautomeric forms. Based on the chemical shifts of the 5- and 7-methyl groups of the pyrrole ring in compounds **3** and **4** (exp. part), it may be concluded that in chloroform solution the 4-oxo tautomer (**2A**) of **2a** is preferred (2.64 ppm − s, 6 H; 5- and 7-CH_3), whereas for **2b** the 4-hydroxy form **2B** is predominant (2.34 ppm, s, 3 H; 5-CH_3 and 2.42 ppm, s, 3 H; 7-CH_3). The hydroxy-oxo tautomeric equilibrium of 1,2,3,4-tetrahydro-6*H*-pyrrolo[3,2-*d*]pyridazine-1,4-diones was recently examined on the basis of UV data [14].

Finally, four of the pyrrolopyridazinones described here (**2a, 4d, 4f, 4i**) were evaluated in a primary anticancer assay at the NCI, eight (**2a, 3c, 3g, 4c, 4d, 4f, 4i, 4j**) were evaluated in collaboration with the TAACF screening program against *Mycobacterium tuberculosis* H_{37}Rv and two (**3b, 4d**) were subjected to preliminary screening to test their CNS activity.

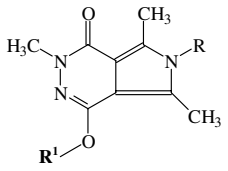
2.2. Biological results

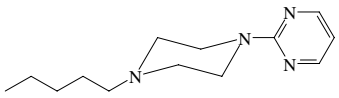
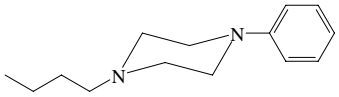
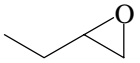
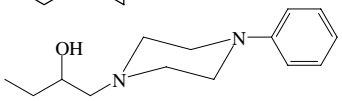
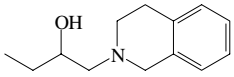
2.2.1. Anticancer activity

The compounds **2a, 4d, 4f, 4i**, selected by the NCI, were tested using a one dose (10^{-4} M) primary anticancer *in vitro* assay against tumor in the 3-cell line panel consisting of MCF7 (breast), NCI-H460 (lung) and SF-268 (CNS) (Table 3).

Only two compounds (**4d** and **4i**) showed marked activity against all three cell types. Compounds **4d** and **4i**, which passed the first criterion of activity, were further evaluated at five concentrations, in 10-fold dilutions (10^{-4}–10^{-8} M), against the panel of 60 human tumor cell lines, belonging to 9 cancer types: leukemia, melanoma and non-small cell lung, colon, CNS, ovarian, renal, prostate, breast cancer [17]. Based on the screening results it may be concluded that the compounds **4d**, **i** generally possess activity against

Table 2: Data of pyrrolopyridazinones 4



Compd.	R	R^1	M.p. (yield %)	Formula, m.w.
4a	*n*-C_4H_9	CH_3	118–121 (9–14)	$C_{14}H_{21}N_3O_2$ 263.33
4b	C_6H_5	CH_3	126–129 (8)	$C_{16}H_{17}N_3O_2$ 283.32
4c	*n*-C_4H_9	$CH_2(CH_2)_{14}CH_3$	80–82 (28)	$C_{29}H_{51}N_3O_2$ 473.74
4d	C_6H_5		79–81 (39)	$C_{27}H_{33}N_7O_2$ 487.60
4e	C_6H_5	$CH_2CH_2CH_2Cl$	126–128 (23)	$C_{18}H_{20}ClN_3O_2$ 345.82
4f	*n*-C_4H_9	CH_2CH_2OH	168–170 (26)	$C_{15}H_{23}N_3O_3$ 293.36
4g	C_6H_5		101–103 (31)	$C_{28}H_{33}N_5O_2$ 471.60
4h	C_6H_5		155–157 (30)	$C_{18}H_{19}N_3O_3$ 325.36
4i	C_6H_5		139–141 (47)	$C_{28}H_{33}N_5O_3$ 487.60
4j	C_6H_5		118–120 (28)	$C_{27}H_{30}N_4O_3$ 458.56

every type of cancer cell line tested, but the anticancer action is weak (GI $\geq 10^4$–10^{-5} M).

2.2.2. *Antimycobacterial activity*

Primary screening of antimycobacterial activity of compounds **2a, 3c, 3g, 4c, 4d, 4f, 4i, 4j** was conducted *in vitro* at the level of 12.5 μg/ml against *Mycobacterium tuberculosis* $H_{37}Rv$. The antimycobacterial activities of the compounds tested are summarized in Table 4. Because none of these compounds demonstrated at least 90% inhibition of *Mycobacterium*, they were not re-tested at a lower concentration.

Table 3: *In vitro* primary anticancer assay of pyrrolopyridazinones at 10^{-4} M concentration

Compd.	Primary anticancer screen (% growth*)			Activity
	Lung cancer NCI-H460	Breast cancer MCF7	CNS cancer SF-268	
4d	−82	−53	−94	Active
4i	−23	−8	−8	Active
4f	89	76	67	Inactive
2a	72	68	74	Inactive

* Negative number indicates cell kill

2.2.3. *CNS activities*

CNS activities of the pyrrolopyridazinones **3b** and **4d** were preliminarily evaluated at the Department of Pharmacology of the Medical Academy at Lublin (Poland), using a procedure previously described [2, 3]. The results of acute toxicity tests showed that the compounds investigated were quite toxic with LD_{50} values, after i.p. administration to mice, of 454 (**3b**) and 784 (**4d**) mg/kg. The tested preparations used at maximum dose (0.1 LD_{50}) had no neurotoxic properties as they did not affect

Table 4: Antibacterial activity *in vitro* of pyrrolopyridazinones against *Mycobacterium tuberculosis* H37Rv (MIC 12.5 μg/ml)

Compd.	Log $P_{calc.}$	% Inhibition (12.5 μg/ml)
4c	8.13	77
3g	3.64	52
4d	3.63	43
4f	1.73	26
3c	7.31	20
2a	1.44	16
4j	4.01	6
4i	4.21	3

motor coordination in the rota-rod test. Both compounds significantly suppressed spontaneous locomotor activity during the 1 h observation period. **3b** was active in this test up to a dose of 1/80 of LD_{50} and **4d** up to a dose of 1/40 of LD_{50}. Compound **3b** showed analgesic activity in the "writhing syndrome" test when administered to mice at a dose of 1/10 of LD_{50}, whereas **4d** was active in this test at a dose of 1/20 of LD_{50}. It was also active in the "hot plate" test at a dose of 1/10 of LD_{50}. **3b** and **4d** slightly reduced the number of head twitch responses induced by 5-HTP in mice (at 1/20 and 1/10 of LD_{50}, respect.). None of the compounds at the highest doses used (0.1 LD_{50}) changed the behaviour of mice in the "four plates" test, antagonized convulsions induced by pentetrazole or lowered pulse rate or arterial blood pressure.

These results of preliminary CNS screening showed that the investigated pyrrolopyridazinones **3b** and **4d** generally depress the central nervous system and are weakly analgetic, whereas they have no anxiolytic and anticonvulsant properties nor affect blood circulation.

3. Experimental

3.1. Chemistry

M.p.'s are uncorrected. ^{1}H NMR spectra were obtained with a Tesla spectrometer [80 MHz, $CDCl_3$, δ (ppm)]. IR spectra were recorded on a Specord-75 IR spectrometer. In the IR (KBr) spectra of isomers **3**, the most intensive bands observed (about 1640 cm^{-1}) were assigned to carbonyl absorption, whereas in the IR spectra of isomers **4**, the ether absorption band (about 1280 cm^{-1}) was very characteristic. Elemental C,H,N analyses were made on a Carlo Erba NA-1500 analyzer. All the results of the C, H, and N determinations were within ±0.4% of the theoretical values. CC were performed on a silica gel column [Kieselgel 60 (70–230 mesh), Merck]. Analytical TLC (R_f) was carried out on Merck silica gel – $60F_{254}$ (Alufolien) and visualized by UV. The $logP_{calc.}$ values (Table 4) were calculated using the ChemPlus program from Hypercube, Inc., IBM PC version, implemented in the HyperChem program package.

3.1.1. 1,2,3,4-Tetrahydro-6-substituted-2,5,7-trimethyl-6H-pyrrolo[3,4-d]-pyridazine-1,4-diones **2a** *and* **2b**

A mixture of 0.01 mol of the corresponding 1-substituted-2,5-dimethyl-3,4-pyrroledicarboxylic acid anhydride (**1a** or **1b**) and 1 ml of *N*-methylhydrazine in 50 ml of toluene was refluxed with stirring for 4 h. After cooling the product was filtered off and purified by crystallization from ethanol to give **2a** or **2b** (Table 1).

2a: ^{1}H NMR: 0.85–1.85 (m, 7 H; $CH_2CH_2CH_3$), 2.64 (s, 6 H; 5,7-CH_3), 3.5 (s, 3 H; 2-N–CH_3), 3.89 (t, 2 H; 6-N–CH_2, J = 7.5 Hz,), broad absorption centered at 6.6 [1H, (D_2O exchangeable); IR (KBr): 3100–2500 cm^{-1}].

2b: ^{1}H NMR: 2.34 (s, 3 H; 5-CH_3), 2.42 (s, 3 H; 7-CH_3), 3.56 (s, 3 H; 2-N–CH_3), 7.47–7.62 (m, 5 H; ArH), broad absorption centered at 8.4 [1H, (D_2O exchangeable); IR (KBr): 3100–2500 cm^{-1}].

3.1.2. 1,2,3,4-Tetrahydro-6-n-butyl-2,3,5,7-tetramethyl-6H-pyrrolo[3,4-d]-pyridazine-1,4-dione **(3a)** *and 1,2-dihydro-6-n-butyl-2,5,7-trimethyl-4-methoxy-6H-pyrrolo[3,4-d]pyridazine-1-one* **(4a)**

Method A: 0.17 g of NaH (56–58% suspension in mineral oil) were added in a few portions to a solution of 1.0 g (4 mmol) of pyrrolopyridazinone **2a** in anh. DMF (20 ml). After stirring at room temperature for 1 h, 1 ml of ICH_3 was added, and stirring was continued at 40 °C for 5 h. The mixture was then poured into cold water. The precipitate was filtered off and chromatographed (CC; ethyl acetate). The fractions containing the product of $R_f = 0.65$ yielded **4a** (0.1 g; Table 2), whereas fractions of $R_f = 0.27$ gave 0.25 g of **3a** (Table 1). These products were further purified by crystallization from cyclohexane.

3a: ^{1}H NMR: 0.9–1.8 (m, 7 H; $CH_3CH_2CH_2$), 2.64 (s, 6 H; 5,7-CH_3), 3.54 (s, 6 H; 2,3-N–CH_3), 3.9 (t, 2 H; 6-N–CH_2, J = 7.2 Hz).

4a: ^{1}H NMR: 0.9–1.8 (m, 7 H; $CH_3CH_2CH_2$), 2.5 (s, 3 H; 5-CH_3), 2.68 (s, 3 H; 7-CH_3), 3.58 (s, 3 H; 2-N–CH_3), 3.88 (m, 5 H; 6-N–CH_2 and 4-O–CH_3).

Method B: A mixture of 1.0 g (4 mmol) of pyrrolopyridazinone **2a**, 0.6 g of anh. K_2CO_3 and 1.5 ml of ICH_3 in acetonitrile (50 ml) was stirred at 40 °C for 15 h. After filtration the solvent was distilled off and the residue was chromatographed (CC; ethyl acetate). The fractions containing the product of $R_f = 0.65$ yielded 0.15 g of **4a** (Table 2), whereas fractions of $R_f = 0.27$ gave 0.37 g of **3a** (Table 1).

Method C: To a solution of sodium ethoxide, prepared from 0.09 g of Na and 50 ml of anh. ethanol, 1.0 g (4 mmol) of pyrrolopyridazinone **2a** and 1.5 ml of ICH_3 were added. The solution was stirred at 40 °C for 15 h, evaporated and then the resulting residue was chromatographed (CC; ethyl acetate). The fractions containing the compound of $R_f = 0.65$ were combined and evaporated to provide 0.05 g of **4a** (Table 2), whereas fractions of $R_f = 0.27$ yielded 0.4 g of **3a** (Table 1).

3.1.3. 1,2,3,4-Tetrahydro-2,3,5,7-tetramethyl-6-phenyl-6H-pyrrolo[3,4-d]-pyridazine-1,4-dione **(3b)** *and 1,2-dihydro-4-methoxy-2,5,7-trimethyl-6-phenyl-6H-pyrrolo[3,4d]pyridazine-1-one* **(4b)**

The isomers **3b** (0.35 g, $R_f = 0.3$) and **4b** (0.1 g, $R_f = 0.8$) were prepared from 1.0 g (3.7 mmol) of **2b** and 1.5 ml of ICH_3 analogously to method C.

3b: ^{1}H NMR: 2.4 (s, 6 H; 5,7-CH_3), 3.59 (s, 6 H; 2,3-N–CH_3), 7.14–7.3 (m, 2 H; ArH), 7.47–7.57 (m, 3 H; ArH).

4b: ^{1}H NMR: 2.28 (s, 3 H; 5-CH_3), 2.44 (s, 3 H; 7-CH_3), 3.61 (s, 3 H; 2-N–CH_3), 3.9 (s, 3 H; 4-O–CH_3), 7.11–7.29 (m, 2 H; ArH), 7.44–7.56 (m, 3 H; ArH).

3.1.4. 1,2,3,4-Tetrahydro-6-n-butyl-3-n-hexadecyl-2,5,7-trimethyl-2H-pyrrolo[3,4-d]pyridazine-1,4-dione **(3c)** *and 1,2-dihydro-6-n-butyl-4-n-hexadecyloxy-2,5,7-trimethyl-6H-pyrrolo[3,4-d]pyridazine-1-one* **(4c)**

A mixture of 1.0 g (4 mmol) of pyrrolopyridazinone **2a**, 0.6 g of anh. K_2CO_3 and 1,41 g (4 mmol) of 1-iodohexadecane in acetonitrile (50 ml) was refluxed with stirring for 10 h. After filtration the solvent was distilled off and the resulting residue was chromatographed (CC; ethyl acetate). The fractions containing the product of $R_f = 0.73$ yielded 0.15 g of **3c** (Table 1), whereas fractions of $R_f = 0.6$ gave 0.6 g of **4c** (Table 2).

3c: ^{1}H NMR: 0.9–1.7 (m, 38 H; CH), 2.64 (s, 6 H; 5,7-CH_3), 3.46 (s, 3 H; 2-N–CH_3), 3.8–4.15 (m, 4 H; 3,6 N–CH_2).

4c: ^{1}H NMR: 0.9–1.8 (m, 38 H; CH), 2.52 (s, 3 H; 5-CH_3), 2.69 (s, 3 H; 7-CH_3), 3.57 (s, 3 H; 2-N–CH_3), 3.91 (m, 2 H; 6-N–CH_2), 4.2 (m, 2 H; 4-O–CH_2).

3.1.5. 1,2,3,4-Tetrahydro-3-{4-[4-(2-pyrimidinyl)piperazin-1-yl]butyl}-2,5,7-trimethyl-6-phenyl-6H-pyrrolo[3,4-d]pyridazine-1,4-dione **(3d)** *and 1,2-dihydro-4-{4-[4-(2-pyrimidinyl)piperazin-1-yl]butoxy}-2,5,7-trimethyl-6-phenyl-6H-pyrrolo[3,4-d]pyridazine-1-one* **(4d)**

A mixture of 2.7 g (0.01 mol) of pyrrolopyridazinone **2b**, 1.4 g of anh. K_2CO_3 and 3.8 g (0.011 mol) of 8-(2-pyrimidinyl)-8-aza-5-azoniaspiro[4,5]decane bromide (**5**) [1] in xylene (80 ml) was refluxed with stirring for 20 h. After filtration and evaporation the mixture of **3d** and **4d** was treated with 30 ml of ethyl acetate. Undissolved product was filtered off and crystallized from ethyl acetate to give 1.8 g of pure **4d** (Table 2). Both filtrates of ethyl acetate were evaporated, and the residue was chromatographed (CC; ethyl acetate). The fractions containing the product of $R_f = 0.11$ yielded 0.45 g of **3d** (Table 1), while fractions of $R_f = 0.38$ gave an additionaly 0.3 g of **4d**.

3d: ^{1}H NMR: 1.57–1.84 (m, 4 H; CH_2CH_2), 2.39 (s, 6 H; 5,7-CH_3), 2.53–2.8 [m, 6 H; $N(CH_2)_3$], 3.53 (s, 3 H; 2-N–CH_3), 3.77–4.21 [m, 6 H; $N(CH_2)_2$ + 3-N–CH_2], 6.52 (t, 1 H; 5-H pyrimidine, J = 4.8 Hz), 7.15–7.31 (m, 2 H; ArH), 7.48–7.62 (m, 3 H; ArH), 8.31 (d, 2 H; 4,6-H pyrimidine, J = 4.8 Hz).

4d: ^{1}H NMR: 1.6–1.95 (m, 4 H; CH_2CH_2), 2.29 (s, 3 H; 5-CH_3), 2.45 (s, 3 H; 7-CH_3), 2.5 [m, 6 H; $N(CH_2)_3$], 3.59 (s, 3 H; 2-N-CH_3), 3.75–3.95 [m, 4 H; $N(CH_2)_2$], 4.17–4.4 (m, 2 H; 4-OCH_2), 6.48 (t, 1 H; 5-H pyrimidine, *J = 4.8 Hz*), 7.15–7.37 (m, 2 H; ArH), 7.5–7.7 (m, 3 H; ArH), 8.3 (d, 2 H; 4,6-H pyrimidine, J = 5.4 Hz).

3.1.6. 1,2,3,4-Tetrahydro-3-allyl-6-n-butyl-2,5,7-trimethyl-6H-pyrrolo[3,4-d]-pyridazine-1,4-dione **(3e)**

A mixture of 1 g (4 mmol) of pyrrolopyridazinone **2a**, 0.7 g of anh. K_2CO_3 and 0.52 ml (6 mmol) of allyl bromide in acetonitrile (50 ml) was refluxed with stirring for 5 h. After filtration the solvent was distilled off and the residue was chromatographed [CC; ethyl acetate/cyclohexane (1 : 1)]. The fractions containing the product of $R_f = 0.25$ yielded 0.4 g of **3e** (Table 1).

1H NMR: 0.85–1.85 (m, 7 H; $CH_3CH_2CH_2$), 2.64 (s, 6 H; 5,7-CH_3), 3.48 (s, 3 H; 2-N–CH_3), 3.88 (t, 2 H; 6-N–CH_2, J = 7.2 Hz), 4.61–6.23 (m, 5 H; $CH_2CH{=}CH_2$).

3.1.7. 1,2,3,4-Tetrahydro-3-benzyl-6-n-butyl-2,5,7-trimethyl-6H-pyrrolo-[3,4-d]pyridazine-1,4-dione **(3f)**

1.0 g (4 mmol) of pyrrolopyridazinone **2a**, 0.7 g of anh. K_2CO_3 and 0.48 ml (4 mmol) of benzyl bromide: see 3.1.6. – After filtration the solvent was distilled off and the resulting residue was crystallized from cyclohexane to give pure **3f** (0.75 g; Table 1).

1H NMR: 0.88–1.87 (m, 7 H; $CH_3CH_2CH_2$), 2.64 (s, 3 H; CH_3), 2.68 (s, 3 H; CH_3), 3.39 (s, 3 H; 2-N–CH_3), 3.9 (t, 2 H; 6-N–CH_2, J = 7.2 Hz),

5.29 (s, 2 H; CH_2Ar), 7.09–7.37 (m, 5 H; ArH).

3.1.8. 1,2,3,4-Tetrahydro-3-p-chlorobenzyl-6-n-butyl-2,5,7-trimethyl-6H-pyrrolo[3,4-d]pyridazine-1,4-dione **(3g)**

A solution of sodium ethoxide, prepared from 0.09 g of Na and 50 ml of anhydrous ethanol, 1.0 g (4 mmol) of pyrrolopyridazinone **2a** and 0.65 g (4 mmol) of 4-chlorobenzyl chloride was refluxed for 5 h and then evaporated. The residue was chromatographed (CC, ethyl acetate). The fractions containing the compound of $R_f = 0.75$ were combined and evaporated to give 0.65 g of **3g** (Table 1).
1H NMR: 0.88–1.77 (m, 7 H; $CH_3CH_2CH_2$), 2.64 (s, 3 H; CH_3), 2.67 (s, 3 H; CH_3), 3.33 (s, 3 H; 2-N−CH_3), 3.9 (t, 2 H; 6-N−CH_2, J = 7.2 Hz), 5.23 (s, 2 H; CH_2Ar), 7.07–7.32 (m, 4 H; ArH).

3.1.9. 1,2-Dihydro-2,5,7-trimethyl-6-phenyl-4-chloropropoxy-6H-pyrrolo-[3,4-d]pyridazine-1-one **(4e)**

A mixture of 1.0 g (3.7 mmol) of pyrrolopyridazinone **2b**, 0.6 g of anh. K_2CO_3 and 1.2 ml (12 mmol) of 1-bromo-3-chloropropane in acetonitrile (50 ml) was refluxed with stirring for 10 h. After filtration the solvent was distilled off and the resulting residue was chromatographed (CC; ethyl acetate). The fractions containing the product of $R_f = 0.7$ yielded 0.35 g of **4e** (Table 2).
4e: ^{1}H-NMR: 2.2 (m, 2 H; $CH_2CH_2CH_2$), 2.27 (s, 3 H; 5-CH_3), 2.45 (s, 3 H; 7-CH_3), 3.6 (s, CH_3, 2-N−CH_3), 3.73 (t, 2 H; 4-OCH_2, J = 6 Hz), 4.42 (t, 2 H; CH_2Cl, J = 6 Hz), 7.11–7.27 (m, 2 H; ArH), 7.45–7.57 (m, 3 H; ArH).

3.1.10. 1,2-Dihydro-6-n-butyl-4-(2-hydroxyethoxy)-2,5,7-trimethyl-6H-pyrrolo[3,4-d]pyridazine-1-one **(4f)**

A mixture of 1 g (4 mmol) of pyrrolopyridazinone **2a**, 0.7 g of anh. K_2CO_3 and 0.43 ml (6 mmol) of 2-bromoethanol in acetonitrile (50 ml) was refluxed with stirring for 10 h. After filtration the solvent was distilled off and the resulting residue was chromatographed (CC; ethyl acetate). The fractions containing the product of $R_f = 0.4$ yielded 0.3 g of **4f** (Table 2).
4f: ^{1}H NMR: 0.88–1.66 (m, 7 H; $CH_3CH_2CH_2$), 2.52 (s, 3 H; 5-CH_3), 2.68 (s, 3 H; 7-CH_3), 3.55 (s, 3 H; 2-N−CH_3), 3.8–4.0 (m, 4 H; 6-N−CH_2, 4-O−CH_2), 4.37–4.47 (m, 2 H; O−CH_2). The position of the O<u>H</u> signal was not established. − IR (KBr): 3370 (OH).

3.1.11. 1,2-Dihydro-2,5,7-trimethyl-6-phenyl-4-[3-(4-phenylpiperazin-1-yl)-propoxy]-6H-pyrrolo[3,4-d]pyridazine-1-one **(4g)**

To a solution of sodium ethoxide, prepared from 0.09 of Na and 50 ml of anhydrous ethanol, 1.0 g (3.7 mmol) of pyrrolopyridazinone **2b** and 0.95 g (4 mmol) of 1-phenyl-4-(3-chloropropyl)piperazine [18] were added. The solution was refluxed with stirring for 10 h, then it was evaporated. The residue was dissolved in chloroform, filtered, and the solvent was distilled off. The residue was chromatographed (CC; ethyl acetate). The fractions containing the compound of $R_f = 0.47$ were combined and evaporated to give 0.5 g of **4g** (Table 2).
^{1}H NMR: 1.96–2.14 (m, 2 H; $CH_2CH_2CH_2$), 2.29 (s, 3 H; 5-CH_3), 2.44 (s, 3 H; 7-CH_3), 2.53–2.77 [m, 6 H; $N(CH_2)_3$], 3.17–3.28 [m, 4 H; Ar−$N(CH_2)_2$], 3.6 (s, 3 H; 2-N−CH_3), 4.33 (t, 2 H; 4-O−CH_2, J = 6.6 Hz), 6.75–7.58 (m, 10 H; ArH)

3.1.12 1,2-Dihydro-2,5,7-trimethyl-6-phenyl-4-(2,3-epoxypropoxy)-6H-pyrrolo[3,4-d]pyridazine-1-one **(4h)**

0.18 g of NaH (56–58% suspension in mineral oil) were added in portions to a solution of 1.0 g (3.7 mmol) of pyrrolopyridazinone **2b** in anh. DMF (5 ml). After stirring at room temperature for 1 h, 0.4 ml (4.7 mmol) of epibromohydrin were added, and stirring was continued at room temperature for 24 h. The reaction mixture was then poured into cold water (50 ml) and the precipitate was filtered off and purified by crystallization from *n*-heptane to give 0.4 g of pure product **4h** (Table 2).
^{1}H NMR: 2.3 (s, 3 H; 5-CH_3), 2.44 (s, 3 H; 7-CH_3), 2.7–2.97 (m, 2 H; oxiran-CH_2), 3.3–3.5 (m, 1 H; oxiran-CH), 3.58 (s, 3 H; 2-CH_3), 4.16 (dd, 1 H; 4-O−CH_2; $J_1 = 12$ Hz, $J_2 = 6$ Hz), 4.6 (dd, 1 H; 4-O−CH_2; $J_1 = 12$ Hz, $J_2 = 3$ Hz), 7.07–7.3 (m, 2 H; ArH), 7.46–7.6 (m, 3 H; ArH).

3.1.13. 1,2-Dihydro-2,5,7-trimethyl-6-phenyl-4-[2-hydroxy-3-(4-phenylpiperazin-1-yl)propoxy]-6H-pyrrolo[3,4-d]pyridazine-1-one **(4i)**

A solution of 1 g (3.1 mmol) of **4h** and 0.55 g (3.5 mmol) of N-phenylpiperazine in absol. ethanol (15 ml) was stirred at room temperature for 15 h. The precipitate was filtered off and purified by crystallization from cyclohexane to give 0.7 g of **4i** (Table 2).
^{1}H NMR: 1.9 [br, 1 H; OH (D_2O exchangeable)], 2.31 (s, 3 H; 5-CH_3), 2.43 (s, 3 H; 7-CH_3), 2.54–2.97 [m, 6 H; $N(CH_2)_3$], 3.13–3.25 [m, 4 H; $ArN(CH_2)_2$], 3.59 (s, 3 H; 2-CH_3), 3.99–4.37 (m, 3 H; OCH_2CH), 6.74–7.55 (m, 10 H; ArH). − IR (KBr): 3330 (OH).

3.1.14. 1,2-Dihydro-2,5,7-trimethyl-6-phenyl-4-[2-hydroxy-3-(1,2,3,4-tetrahydroisoquinolin-1-yl)propoxy]-6H-pyrrolo[3,4-d]pyridazine-1-one **(4j)**

A solution of 1 g (3.1 mmol) of **4h** and 0.45 g (3.5 mmol) of 1,2,3,4-tetrahydroisoquinoline in absol. ethanol (15 ml) was stirred at room temperature for 15 h. The solvent was distilled off and the residue was chromatographed (CC; ethyl acetate). The fractions containing the compound of $R_f = 0.25$ were combined and evaporated to give 0.35 g of **4j** (Table 2).
^{1}H NMR: 2.31 (s, 3 H; 5-CH_3), 2.44 (s, 3 H; 7-CH_3), 2.65–2.95 (m, 6 H; $CH_2NCH_2CH_2Ar$), 3.4 [br, 1 H; OH (D_2O exchangeable)], 3.59 (s, 3 H; 2-N-CH_3), 3.7–3.85 (m, 2 H; NCH_2Ar), 4.1–4.5 (m, 3 H; OCH_2CH), 7.0–7.35 (m, 6 H; ArH), 7.5–7.7 (m, 3 H; ArH). − IR (KBr): 3300 (OH).

3.2. Pharmacological experiments

3.2.1. Primary anticancer assay

Compounds were evaluated in an *in vitro* model in the 3-cell line panel, one dose assay. Each cell line [MCF7 (breast), NCI-H460 (lung) and SF-268 (CNS)] was inoculated and preincubated on a microtiter plate. Test agents were then added at a single concentration (10^{-4} M) and the culture incubated for 48 h. End-point determinations were made with sulforhodamine B, a protein-binding dye. Results for each test agent are reported as % of growth of the treated cells when compared to untreated control cells. Compounds which reduce the growth of any one of the cell lines to 32% or less (negative numbers indicate cell kill) are passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range [17].

3.2.2. Evaluation of antimycobacterial activity

Antimycobacterial activity of compounds was assayed by U.S. Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), NIAID/NIH Contract No. N01-AI-45246
Primary screening was conduced at 12.5 μg/ml against *Mycobacterium tuberculosis* $H_{37}Rv$ in BACTEC 12B medium using the BACTEC 460-radiometric system.

References

1 Malinka, W.; Tatarczynska, E.: Farmaco **48**, 933 (1993)
2 Malinka, W.; Sieklucka-Dziuba, M.; Rajtar, G.; Rubaj, A.; Kleinrok, Z.: Farmaco **54**, 390 (1999)
3 Malinka, W.; Sieklucka-Dziuba, M.; Rejdak, R.; Rejdak, K.; Kleinrok, Z.: Pharmazie **50**, 9 (2000)
4 Data obtained from the NCI for compounds coded as NSC 713752-Z and NSC 713750-X
5 Meade, E. A.; Wotring, L. L.; Drach, J. C.; Townsend, L. B.: J. Med. Chem. **36**, 3834 (1993)
6 Meade, E. A.; Wotring, L. L.; Drach, J. C.; Townsend, L. B.: J. Med. Chem. **40**, 794 (1997)
7 Kimura, T.; Fujihara, Y.; Shibakawa, N.; Fuijwara, H.; Itoh, E.; Matsunobu, K.; Tabata, K.; Yasuda, H.: PCT Int. Appl. WO 95 19,980; C.A. **124**, 8849h (1996)
8 Grundler, G.; Senn-Bilfinger, J.; Schaefer, H.; Rainer, G.; Klemm, K.; Simon, W. A.; Schudt, C.; Riedel, R.; Postius, S.: PCT Int. Appl. WO 92 06,979; C.A. **117**, 111632m (1992)
9 Grundler, G.; Rainer, G.; Schaefer, H.; Senn-Bilfinger, J.; Klemm, K.; Simon, W. A.; Riedel, R.; Postius, S.; Sturm, E.; Huber, R.; David, M: PCT Int. Appl. WO 93 08,190; C.A. **119**, 180803w (1993)
10 Kulagowski, J. J.; Lesson, P. D.: Brit. UK Pat. Appl. GB 2,265,372; C.A. **120**, 134504x (1994)
11 Ungureanu, M.; Mangalagiu, I.; Grasu, G.; Petrovanu, M.: Ann. Pharm. Franc. **55**, 69 (1997)
12 Nightingale, D. V.; Gallagher, J. A.: J. Org. Chem. **24**, 501 (1959)
13 Malinka, W.; Bodalski, T.: Polish J. Chem. **68**, 297 (1994)
14 Inel, S.; Jones, A.; Ogretir, C.: Tetrahedron **40**, 3979 (1984)
15 Feuer, H.; Bachman, G. B.; White, E. H.: J. Am. Chem. Soc. **73**, 4716 (1951)
16 Mahboobi, S.; Grothus, G.; Meindl, W.: Arch. Pharm. **327**, 105 (1994)
17 Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff et. al.: J. Natl. Cancer Inst. **83**, 757 (1991)
18 Howard, K.; Steward, W.; Conray, E.; Denton, J.: J. Org. Chem. **57**, 1484 (1953)

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