Novel Pyridazino[4,3-*b*]indoles with Dual Inhibitory Activity against *Mycobacterium tuberculosis* and Monoamine Oxidase

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Tuberculosis is one of the most common infectious diseases known to man. About 37% of the world's population (about 1.86 billion people) are infected with *Mycobacterium tuberculosis*. According to the World Health Organization, every year approximately 8 million people develop active tuberculosis and almost 2 million of those die from the disease. The incidence of multidrug-resistant tuberculosis (MDR-TB) is increasing. The present drug regimen for treating tuberculosis has been in existence for 30 years. New drugs that will shorten total treatment duration, improve the treatment of MDR-TB, and address latent tuberculosis are the most urgent need of tuberculosis control programs. A new series of synthetic 3-amino-4-arylpyridazino[4,3-*b*]indoles (pyridazinoindoles) were identified as inhibitors of *Mycobacterium tuberculosis*. The design, synthesis, and antimycobacterial activity of these compounds are described. While the most active compounds are still not comparable to the front-line drugs rifampicin and isoniazid, they do show promise. Most of the pyridazinoindoles with appreciable antituberculosis activity also inhibit monoamine oxidase, suggestive of a novel inhibitory effect on mycobacterial redox reactions.

Introduction

Tuberculosis is one of the most important public health problems in Russia, other East European countries, and worldwide. Since 1985 and particularly in the 1990s, a search for new antituberculosis substances has ranked among the priority areas of chemotherapeutic research.[1,2] The highly effective combined therapy regimen of isoniazid (INH), rifampicin (RIF), and pyrazinamide (PZA) forms the basis of the current DOTS (directly observed therapy, short course), which is now being vigorously implemented in FSR (Former Soviet Republic) countries. These three drugs with significantly different chemical structures affect different essential metabolic pathways of *Mycobacterium tuberculosis*.[3] Nevertheless, the emergence of monodrug-resistant and multidrug-resistant strains of *M. tuberculosis* in FSR and elsewhere, even in the course of successful DOTS programs, emphasizes the need for new drug leads of new structural classes and with novel mechanisms of action. Recently, antituberculosis activity was revealed among new types of nitrogen-containing heterocycles,[2,4] alkaloids,[5,6] and amide derivatives of sulfonyl fatty acids with alkyl tails.[7]

Some antituberculosis drugs introduced into therapy have been shown to exert their action by inhibition of specific essential redox enzymes in the tubercle bacillus.[8] For instance, some derivatives of hydrazine, such as INH, are known to destroy catalase−peroxidase enzyme redox metabolic pathways of the pathogen.[9] Also, the dual activity of N,N-containing heterocycles as substrates for *M. tuberculosis* peroxidases and inhibitors of mycobacterial growth is well-known.[10] In the 1960s, Pershin et al. reported that hydrazine derivatives with appreciable antituberculosis activity also inhibited monoamine oxidase (MAO) activity.[11,12] The MAO inhibitory action of INH and particularly iproniazid was described long ago.[13] Iproniazid, initially used as a drug in the treatment of tuberculosis, is a central nervous stimulant because of a mild inhibitory effect on MAO.[13] Several original Russian antidepressants and reversible MAO inhibitors, such as pirlindole and tetrindol, exhibited marked antituberculosis activity.[14] The MAO inhibitor isocarboxazide (marplan), used in psychiatry, and its close analogue 5-methylisoxazole-3-carboxylic acid hydrazide were once employed for treating leprosy caused by *Mycobacterium leprae*.[15]

Pyridazine and fused pyridazine derivatives have long attracted the interest of Russian researchers as antituberculosis agents; antituberculosis compounds were found in the series of 3-pyridazinones[16] and pyridazino[3,4-*b*]quinoxalines.[17] There are other examples.[18−20] Some members of the 3-amino-4-arylpyridazino[4,3-*b*]indole family displayed activity against *M. tuberculosis* H37Rv and *M. fortuitum* with MICs of 23 and 32 μg/mL, respectively,[21] and did not inhibit the growth of Gram-positive or Gram-negative bacteria. Besides those, antituberculosis bicyclic isatin-3-hydrazono derivatives

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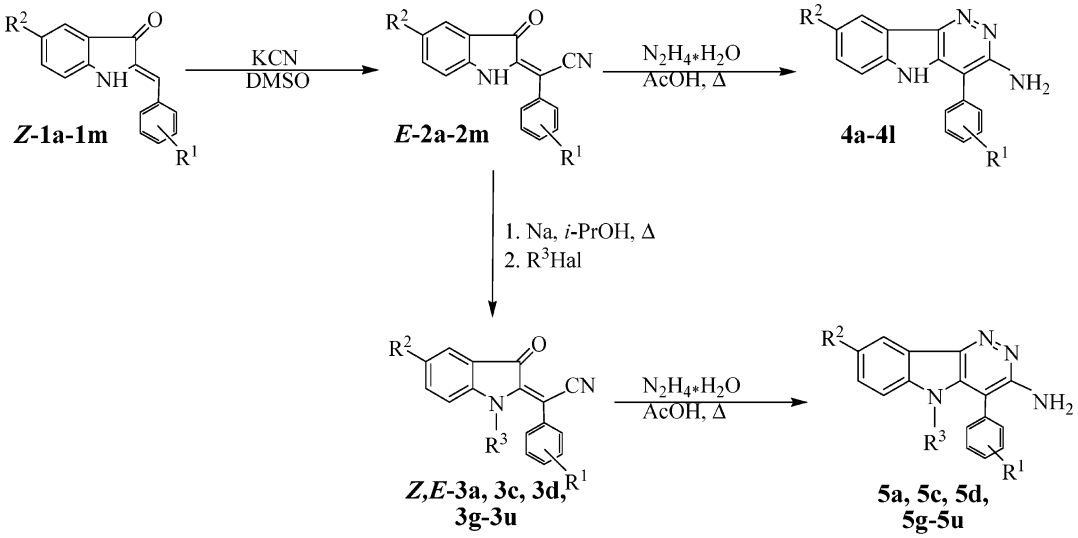
Scheme 1. General Synthetic Methods



Cmpd.	R^1	R^2	Cmpd.	R^1	R^2	R^3	Cmpd.	R^1	R^2	R^3
1,2,4 a	H	H	**3,5 a**	H	H	CH_3	**3,5 q**	2-Cl	H	C_2H_5
1,2,4 b	4-F	H	**3,5 c**	4-CH_3	H	CH_3	**3,5 r**	4-*i*-C_3H_7	H	n-C_3H_7
1,2,4 c	4-CH_3	H	**3,5 d**	4-*i*-C_3H_7	H	CH_3	**3,5 s**	4-*i*-C_3H_7	H	$(CH_2)_2N(CH_3)_2$
1,2,4 d	4-*i*-C_3H_7	H	**3,5 g**	2-F	H	CH_3	**3,5 t**	H	H	CH_2Ph
1,2,4 e	4-OCH_3	H	**3,5 h**	2-Cl	H	CH_3	**3,5 u**	2-Cl	H	CH_2Ph
1,2,4 f	4-$N(CH_3)_2$	H	**3,5 j**	3-Br	H	CH_3				
1,2,4 g	2-F	H	**3,5 k**	H	Br	CH_3				
1,2,4 h	2-Cl	H	**3,5 l**	4-*i*-C_3H_7	Br	CH_3				
1,2,4 j	3-Br	H	**3,5 m**	4-*i*-C_3H_7	CH_3	CH_3				
1,2,4 k	H	Br	**3,5 n**	H	H	C_2H_5				
1,2,4 *l*	4-*i*-C_3H_7	Br	**3,5 o**	4-CH_3	H	C_2H_5				
1,2 m	4-*i*-C_3H_7	CH_3	**3,5 p**	4-*i*-C_3H_7	H	C_2H_5				

(hydrazono-1*H*-2-indolinones)[22,23] have some structural features resembling the pyridazinoindoles. Endogenous isatin and some of its analogues, 2-arylidene-3-indolinones (indogenides) and 2-α-cyanoarylmethylene-3-indolinones (cyanoindogenides), in addition to their anti *M. tuberculosis* activity, act as inhibitors of MAO in rat brain mitochondrial fractions.[24] *M. tuberculosis* H37Rv contains a putative flavine-containing MAO, Rv3170,[25] with high homology to mitochrondrial MAO, and so it is possible that the inhibition of mycobacterial growth results from the inhibition of mycobacterial MAO. Accordingly, we speculate that antituberculosis agents will be found among cyclic derivatives of hydrazine, such as fused pyridazines, with MAO inhibitory effects. In continuation of our work on indole-fused compounds,[21,26,27] we choose to search for antituberculosis agents in the new series of 3-amino-4-arylpyridazino-[4,3-*b*]indoles (pyridazinoindoles) that are tricyclic hydrazine derivatives.

The purpose of this study was to synthesize new potent antituberculosis pyridazinoindole analogues and test them for inhibition of the growth of *M. tuberculosis*. We also studied MAO inhibition of the pyridazinoindoles to gain a better understanding of the relationships between antituberculosis activity and MAO inhibition.

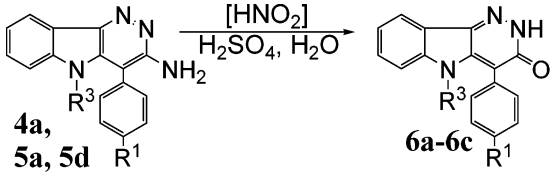
Chemistry

In the synthesis of the 3-amino-4-arylpyridazino[4,3-*b*]indoles, we employed a new reaction involving the transformation of 2-arylidene-3-indolinones (indogenides) **1** to 2-α-cyanoarylmethylene-3-indolinones (cyanoindogenides) **2** under the action of alkali metal cyanides or acetone cyanohydrin in the presence of a base. The reaction proceeds both in protic and aprotic solvents (DMSO, DMF, MeOH)[21] (Scheme 1), and the net result is the replacement of a vinyl hydrogen in the indogenides by a cyano group. The N(1)-unsubstituted indogenides **1** are better substrates than their N(1)-substituted counterparts in this reaction. The starting indogenides **1**, substituted in the 1,2-(substituted phenyl)- and 5-positions, were prepared according to a procedure published earlier.[28]

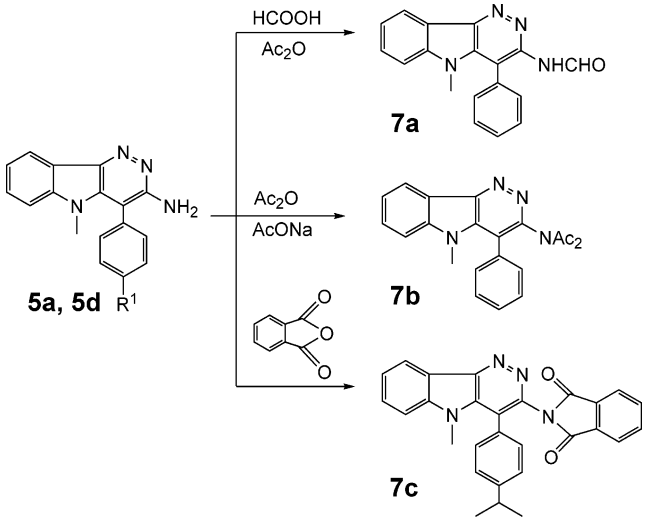
In the present case, the synthesis of cyanoindogenides **2** was carried out in DMSO at 20−30 °C for 3−5 days in the presence of a 3-fold excess of KCN. The yields of the parent cyanoindogenide **2a**, 3- or 4-substituted phenyl analogues **2b**−**f**, and **2j**−**m** were 75−85% under these conditions. Although N(1)-alkylindogenides and 2-substituted phenylindogenides **1g**,**h** also gave cyanoindogenides **3** and **2g**,**h**, respectively, the yields were no greater than 20% under the above reaction conditions. Good yields of N(1)-alkylcyanoindogenides **3** were obtained by alkylation of the corresponding N(1)-unsubstituted cyanoindogenides with alkyl halides in the presence of strong bases such as sodium alkoxides. Then cyanoindogenides **2** and N(1)-alkylcyanoindogenides **3** were converted to pyridazinoindoles **4** and **5** in 60−90% yield by cyclocondensation with hydrazine hydrate in boiling acetic acid.

The synthesis of 3-(2*H*)pyridazinones **6** was carried out via diazotization of pyridazinoindoles **4a**, **5a**, and **5d** followed by hydrolysis of the intermediate diazonium salts with H_2SO_4. The yields of pyridazinones **6** were 80−87% (Scheme 2).

Scheme 2. Synthesis of Compounds **6**



Scheme 3. Synthesis of Compounds **7**



We also tried to obtain N(3)-acylamino-substituted pyridazinoindoles, but this proved to be difficult. The acylation of the primary amino group did not proceed under mild conditions, and the products of N,N-diacylation or mixtures of different products were usually obtained under severe conditions. We did manage to obtain 3-*N*-formylamino-3-*N*,*N*-diacetylamino and 3-*N*-phthaloylamino derivatives **7a**−**c** (Scheme 3).

Estimation of Antimycobacterial Activity of Compounds. Antimycobacterial activity of the pyridazinoindoles **4**−**7** was estimated by their effects on the inhibition of the selective incorporation of radiolabeled 5,6-[^{3}H]-uracil into viable *M. tuberculosis* H37Rv.[29] The assay was based on data demonstrating that parallel estimations of mycobacterial colony-forming units (CFU), conducted as described,[30] and 5,6-[^{3}H]-uracil uptake under identical cultural conditions and dynamics showed correlation coefficients higher than 0.9, thereby validating the usage of the latter, less laborious, and less variable method.[29,31] The results of incorporation of 5,6-[^{3}H]-uracil into growing *M. tuberculosis* are expressed as counts per minute (cpm) ± SD for triplicate cultures. The target compounds (Table 1) and reference drugs RIF and INH were tested at concentrations of 20, 2.0, and 0.2 μg/mL on 25 × 10^4 of *M. tuberculosis* strain H37Rv (Pasteur). The activity of compounds was estimated as percent of inhibition (PI), which was determined as the percent difference between the incorporation of 5,6-[^{3}H]-uracil into growing *M. tuberculosis* in the presence and the absence of the compounds over 42 h of incubation. The MIC_{50} was defined as the lowest concentration of drug resulting in 50% inhibition of [^{3}H]-uracil incorporation into the organism.

Measurement of MAO Inhibition. The inhibitory effects of compounds on activities of MAO A and MAO B from rat liver mitochondria were determined by methods described previously.[32]

Table 1. In Vitro Activity against *M. tuberculosis* H37Rv and MAO Inhibitory Action of Pyridazinoindoles **4**−**7** Bearing Various Substituents in the Ring System

	percent inhibition of *M. tuberculosis*				inhibition of MAO activity	
compd	PI[a] at 20 μg/mL	PI[a] at 2.0 μg/mL	PI[a] at 0.2 μg/mL	MIC_{50}[b] (μg/mL)	IC_{50} MAO A (μM)	IC_{50} MAO B (μM)
4a	28.5	25.5	2.3		NI[c]	NI[c]
4b	35.1	0.0	0.0		NI[c]	NI[c]
4c	40.5	0.0	0.0	16.49	31.6	158
4d	61.9	32.9	8.7	12.63	>1000	>1000
4e	30.2	0.0	0.0	14.86		
4f	66.0	10.0	5.2		79.4	~1000
4g	40.3	0.0	0.0		631	NI[c]
4h	35.0	0.0	0.0		316	NI[c]
4j	32.0	0.0	0.0			
4k	91.4	44.3	9.2	4.17	63.1	25.1
4l	88.5	56.3	7.1	1.77	63.0	79.0
5a	40.2	25.5	2.3		28.2	~1000
5c	62.3	0.0	0.0			
5d	88.0	63.6	21.3	1.42	14.1	316
5g	45.3	0.0	0.0		NI[c]	41.6
5h	3.1	0.0	0.0		~1000	~1000
5j	60.1	0.0	0.0		63.1	100
5k	54.2	0.0	0.0			
5l	60.7	0.0	0.0	17.12		
5m	40.5	0.0	0.0			
5n	40.3	0.0	0.0		NI[c]	72
5o	30.1	0.0	0.0		NI[c]	11
5p	94.3	10.5	0.0	10.48	100	15.8
5q	15.9	0.0	0.0		>1000	100
5r	20.0	0.0	0.0			
5s	20.4	0.0	0.0		1000	NI[c]
5t	20.7	0.0	0.0			
5u	50.8	0.0	0.0		17.8	35.4
6a	0					
6b	0					
6c	0					
7a	0					
7b	0					
7c	0					
RIF	94.3	91.7	54.8	0.10		
INH	68.7	59.1	49.7	0.25		

[a] PI: percent of inhibition based on the difference in incorporation of [^{3}H]-uracil into growing *M. tuberculosis* (25 × 10^4 CFU) over 42 h at the 20, 2.0, and 0.2 μg/mL concentration of the compounds compared to the nontreated cultures. [b] MIC_{50}: concentration of compounds resulting in 50% reduction in [^{3}H]-uracil incorporation compared to the nontreated cultures. [c] NI: no inhibition observed at the highest concentration tested (1000 μM).

Results

We designed and tested a large congeneric series of 3-, 4-, 5-, and 8-substituted pyridazino[4, 3-*b*]indole derivatives (34 compounds) in order to improve the antituberculosis activity of previously synthesized pyridazinoindoles **4a**, **4b**, **4d**, **4e**, **4g**, **5a** and to gain an understanding of their structure−activity relationships in the context of anti-*M. tuberculosis* and anti-MAO activities (Table 1).

The compounds tested for antituberculosis activity were divided into four series. Series A comprised N(5)-

unsubstituted compounds **4a**−**j**, derived from the parent structure **4a** with various substituents in positions 2−4 of the phenyl ring, and 8-bromosubstituted compounds **4k**,**l**. Series B comprised N(5)-alkylated compounds, both monosubstituted **5a**,**n**,**t** and disubstituted **5c**−**k**,**o**−**s**,**u** and trisubstituted pyridazinoindoles **5l**,**m** with variations in positions 4, 5, and 8 of the ring system. Series C and D involved N(5)-unsubstituted 3-(2*H*)-pyridazinone **6a** and its mono- and disubstituted analogues **6b** and **6c**, and also 5-methyl-3-*N*-acylamino-substituted pyridazinoindoles **7a**−**c**, respectively.

Antimycobacterial activity of the pyridazinoindoles **4**−**7** was estimated from their effects on the inhibition of incorporation of radiolabeled 5,6-[^{3}H]-uracil into viable *M. tuberculosis* H37Rv. The results (Table 1) demonstrate a reduction in 5,6-[^{3}H]-uracil uptake in the range of 20−94% at 20 μg/mL for most of the compounds tested compared to about 94% and 69% for RIF and INH, respectively. Nine of the pyridazinoindoles (**4d**, **4f**, **4k**, **4l**, **5c**, **5d**, **5j**, **5l**, **5p**) exhibited the greatest antituberculosis activity with PI ≥ 60% at 20 μg/mL. Only compounds **4l** and **5d** at 2.0 μg/mL were as active as INH at the same concentration in impairing [^{3}H]-uracil incorporation. However, at 0.2 μg/mL, all pyridazinoindoles were poorly active, whereas RIF and INH inhibited [^{3}H]-uracil incorporation by about 50%. Compounds **5d** and **4l** had MIC_{50} values of 1.42 and 1.77 μg/mL, respectively, compared to MIC_{50} values of 0.10 and 0.25 μg/mL for RIF and INH, respectively. Thus, they were the most active pyridazinoindoles tested. These two did not inhibit the growth of *Staphylococcus aureus* or *Escherichia coli* even at 10-fold higher concentrations.

In series A, the parent pyridazinoindole **4a** and its analogues **4b**, **4c**, **4e**, **4g**−**j** substituted in positions 2, 3, and 4 of the 4-phenyl ring displayed only weak antituberculosis activity. The replacement of the para hydrogen with an isopropyl or dimethylamino substituent or the insertion of bromine into position 8 led to the more active compounds **4d**, **4f**, **4k**, **4l**. In series B, N(5)-alkylated derivatives **5c**, **5d**, **5j**, **5l**, and **5p** exhibited the greatest potency. Irrespective of the substituted aryl, an N(5)-alkyl (methyl, ethyl) substituent at the ring nitrogen was highly favorable for antituberculosis activity (compare **4a** and **5a**, **4c** and **5c**, **4d** and **5d**, **4d** and **5p**). In the 4-(4-isopropyl)phenyl series represented by **5p**, increasing the bulk of the indole substituent to *n*-propyl (**5r**), dimethylaminoethyl (**5s**), or benzyl (**5t**) was detrimental to antimycobacterial activity. The replacement of hydrogen in position 8 with bromine was beneficial in series A (compare **4a** and **4k**, **4d** and **4l**) but had a variable effect in series B (compare **5a** and **5k**, **5d** and **5l**). Trisubstituted compounds **5l** and **5m** showed modest levels of antituberculosis activity. Conversion of the 3-amino group to a hydroxyl, as in the pyridazinones **6** of series C, or N-acylation of the 3-amino group, as in compounds **7** of series D, destroyed antituberculosis activity. Overall, members of the 4-(4-isopropyl)phenyl series with either an N(5)-methyl (**5d**) or an 8-bromo (**4l**) substituent exhibited the greatest antituberculosis activity. Thus, the level of antituberculosis activity depends to a large extent on the nature and the specific combination of substituents in the 4-, 5-, and 8-positions. These results suggest the involvement of steric and electronic factors in influencing antituberculosis activity of compounds **4**−**7**. Furthermore, lipophilicity may also play a role on the effect of the compounds tested.

Twenty pyridazinoindoles were tested as inhibitors of rat liver mitochondrial MAO A and/or MAO B (Table 1).[32,33] Two pyridazinoindoles (**4a** and **4b**) did not inhibit MAO A or MAO B even at the highest concentration tested (1000 μM). Six other analogues (**4g**, **4h**, **5g**, **5n**, **5o**, and **5s**) were active against only one of the isoforms. Nearly half of all compounds studied had IC_{50} values in the range of 10−80 μM with respect to MAO A and/or MAO B. These IC_{50} values were higher than those of known MAO inhibitors (e.g., IC_{50} values for MAO inhibition by fused indoles such as pirlindole and tetrindol are 0.2 and 0.05 μM, respectively[32,33]). Within the present series, compounds **5d** and **5p** were among the most potent and selective inhibitors of MAO A and MAO B, respectively. Compound **5u** was rather potent and appeared to be a nonselective inhibitor of both MAO A and MAO B with IC_{50} values of 17.8 and 35.4 μM, respectively. Among the nine compounds (see above) with the greatest antituberculosis activity (PI ≥ 60% at 20 μg/mL), seven (**4d**, **4f**, **4k**, **4l**, **5d**, **5j**, **5p**) were tested for MAO A inhibition, and for six of them (**4f**, **4k**, **4l**, **5d**, **5j**, **5p**), the IC_{50} values with respect to MAO A were ≤100 μM. It is noteworthy that the two most active compounds against *M. tuberculosis*, **5d** (PI = 88% at 20 μg/mL; PI = 63.6% at 2.0 μg/mL) and **4l** (PI = 88.5% at 20 μg/mL; PI = 56.3% at 2.0 μg/mL), had MAO A IC_{50} inhibitory values of 14.1 and 63 μM, respectively; i.e., they are effective MAO inhibitors (Table 1).

Discussion

We have long considered that the relationship between the antituberculosis and the MAO inhibitory action of such compounds as pyridazinoindoles is not fortuitous in that simultaneous manifestation of antipsychotic, anti-MAO, and antituberculosis activities is typical for some classes of N,N-containing compounds and fused indoles.[11−14,32,33] However, there is no consistent correlation between inhibition of *M. tuberculosis* and MAO activity. In the case of the bromo-substituted pyridazinoindoles such as **4l** and **5l**, **4k** and **5k**, we conclude that the insertion of a bromo substituent changes not only electronic effects but also topographical features of the structures. The insertion of a 4-isopropyl group in the aryl substituent of pyridazinoindoles sometimes increases both antibacterial and MAO inhibitory activities. For example, the insertion of this group into compound **5a** converted it into **5d**, resulting in an increase in percent inhibition of mycobacterial growth from 40.2% (at 20 μg/mL) to 88%, and the IC_{50} value for MAO A inhibition decreased from 28.2 to14.1 μM. In the context of the influence of the ortho effect (*o*-chloro-substituted phenyl; i.e., pyridazinoindoles **5h**, **5q**, **5u**), as a rule, the insertion of the *o*-chloro substituent decreases the value of the percent inhibition of mycobacterial growth and increases the IC_{50} value for MAO inhibition. This ortho effect is noticeable in the case of the sterically hindered *o*-aryl-substituted pyridazinoindoles that also have *N*-alkyl substituents. For example, compound **5a** inhibits [^{3}H]-uracil incorporation by 40.2% (at 20 μg/mL) and has an IC_{50} value for MAO

A inhibition of 28.2 μM, whereas the comparable figures for the Cl-containing analogue **5h** are 3.1% and 1000 μM. However, there is an exception in that the corresponding figures for the Cl-containing compound, **5u**, are 50.8% and 17.8 μM, respectively.

Accordingly, the hypothesis that the antituberculosis activity of pyridazinoindoles is due to inhibition of bacterial MAO must be treated with caution. Although the presence of the gene for an MAO homologue has been reported in the *M. tuberculosis* genome,[25] a functioning MAO requires substantiation. Such a mycobacterial MAO may participate in the biochemical synthesis of hydrogen peroxide playing a role in the response of mycobacteria to oxidative stress and thus be a potential target for new drug development with the pyridazinoindoles described herein as the initial lead compounds. However, the most promising compounds arising from this work will first require extensive testing as inhibitors of mycobacterial growth in vitro and in the mouse model of tuberculosis, in conjunction with toxicity testing prior to extensive study of structural−functional relationships.

Tuberculosis (TB) case notifications have been increasing in the newly independent states of the former Soviet Union and parts of Russia.[34,35] Human immunodeficiency virus (HIV) associated TB is widespread in the sub-Saharan African countries hardest hit by acquired immunodeficiency syndrome (AIDS),[36] and there has been a disturbing increase in the number of TB cases caused by organisms resistant to the two most important drugs, isoniazid and rifampicin; a survey of 72 countries suggested that the multidrug-resistant TB (MDR-TB) problem is more widespread than previously thought and is likely worsening.[37] MDR-TB appears to be especially serious in the Russian Federation, where it has spread in prisons and throughout the entire population.[38] No novel compounds to treat TB have been introduced into clinical practice in the past 30 years. Incremental improvements have been made by modifying the rifampin structure to generate long-lasting rifamycins: rifapentine, rifabutin, and rifalazid.[34] Several quinolones are part of the second-line regimen applied to the management of MDR-TB.[34] However, these are modest alternative regimens in light of the dimensions of TB and HIV/TB and the threat of MDR-TB. There is an urgent need for new chemotherapy that will allow a shortening of the total duration of effective treatment, improve the treatment of MDR-TB, and provide an antidote to latent TB infection. Further experiments will tell whether the pyrazinoindoles conform to some of these specifications.

Conclusions

Development of novel classes of compounds capable of shortening the duration of effective treatment of tuberculosis, treating isoniazid- and rifampin-resistant tuberculosis (defined as MDR-TB), and addressing latent tuberculosis is a major priority of global tuberculosis control programs. There was evidence in the earlier Russian literature that cyclic derivatives of hydrazine, such as fused pyridazines, could inhibit the growth of *M. tuberculosis*. We chose to search for such activity among the newer series of pyrazino[4,3-*b*]-indoles (pyrazinoindoles), tricyclic hydrazine derivatives. Overall, members of the 4-(4-isoprenyl)phenyl series with either an N(5)-methyl or an 8-bromo substituent exhibited the greatest effect on *M. tuberculosis* based on inhibition of 5,6-[^{3}H]-uracil incorporation. A partial correlation between inhibition of bacterial growth and monoamine oxidase activity suggests that the pyrazinoindole may act through inhibition of mycobacterial redox reactions.

Experimental Section

Chemistry. Melting points are uncorrected. ^{1}H NMR (400 MHz) data were measured in DMSO-d_6, and chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane as internal standard. Mass spectra were measured on a VG-7070E spectrometer (EI, 70 eV). Elemental analyses were performed at the laboratory for microanalysis of A. N. Nesmeyanov Institute of Organoelement Compounds, Moscow. TLC was performed on Silufol UV-254 plates. Anhydrous solvents were purified by standard procedures.

Key Intermediates: 2-Arylidene-1,2-dihydro-3*H*-indol-3-ones (1). 2-Arylidene-1,2-dihydro-3*H*-indol-3-ones (**1**) were prepared as described[27] (Table S1 in Supporting Information). A product sufficiently pure to be used as such for the next step was generated. Recrystallization was achieved from methanol/water (2:1), ethyl acetate, and acetonitrile.

Procedure for General Cyanation of 2-Arylidene-1,2-dihydro-3*H*-indol-3-ones (1) To Give 2-(α-cyanoarylmethylene)-1,2-dihydro-3*H*-indol-3-ones (2) (Table S2 in Supporting Information). A mixture of **1** (10 mmol) and potassium cyanide (1.95 g, 30 mmol) in DMSO (15 mL) was stirred at room temperature for 3−5 days. The course of the reaction was followed to completion by TLC in 15% ethyl acetate/hexane. The green reaction mixture was quenched with a saturated NaCl solution (50 mL). A solution with an orange color resulted, and the product precipitated within 10−12 h to give an orange residue. This final product **2** was filtered and washed with water. Recrystallization was possible from ethanol, 2-propanol, or 2-propanol/water (2:1). MS, *m/z*: **2a**, 246 [M]$^+$; **2b**, 264 [M]$^+$; **2d**, 288 [M]$^+$; **2e**, 276 [M]$^+$; **2g**, 264 [M]$^+$.

Procedure for General N-Alkylation of 2-(α-cyanoarylmethylene)-1,2-dihydro-3*H*-indol-3-ones: 1-Alkyl-2-(α-cyanoarylmethylene)-1,2-dihydro-3*H*-indol-3-ones (3) (Table S2 in Supporting Information). To a solution of sodium ethanolate prepared from sodium (0.25 g, 11 mmol) and ethanol (50 mL) was added the appropriate cyanoindogenide **2** (10 mmol) in one portion, and the mixture was stirred at room temperature for 30 min. Then alkyl halide (25 mmol) was added to the dark-blue solution. The resulting mixture was stirred for 5−8 h. The reaction was determined to be complete by TLC in 15% ethyl acetate/hexane. The reaction mixture then was diluted with water (50 mL), and the residue was filtered and washed with ethanol/water (2:1). A product sufficiently pure to be used as such for the next step was generated. Recrystallization is possible from ethanol, 2-propanol, or 2-propanol/water (2:1). MS, *m/z*: **3a**, 260 [M]$^+$.

General Ring Closure Procedure: 3-Amino-4-aryl-5*H*-(or 5-alkyl)pyridazino[4,3-*b*]indoles (4 and 5) (Table S3 in Supporting Information). Method A. To a hot solution of hydrazine acetate prepared from 80% hydrazine hydrate (224 mmol, 14 mL) and acetic acid (30 mL) was added the appropriate cyanoindogenide **2** or **3** (3.5 mmol) in one portion, and the reaction mixture was heated to gentle reflux for 5 h. After cooling, the reaction mixture was poured into ice/water (100 mL). The final product was filtered and washed with water. Recrystallization is possible from ethanol, 2-propanol, or *N*,*N*-dimethylformamide/methanol (1:5). Method A was used for the preparation of the following compounds: **4a**,**d**,**e**,**j** and **5a**,**h**,**l**,**o**,**q**,**r**,**t**. MS, *m/z*: **4a**, 260 [M]$^+$; **4b**, 278 [M]$^+$; **4d**, 302 [M]$^+$; **4e**, 290 [M]$^+$; **4g**, 278 [M]$^+$; **5a**, 274 [M]$^+$.

3-Amino-4-aryl-5*H*-(or 5-alkyl)pyridazino[4,3-*b*]indoles hydrochlorides (4 and 5) (Table S3 in Supporting Information). Method B. The 3-amino-4-aryl-5*H*-(or 5-alkyl)-

pyridazino[4,3-*b*]indoles were prepared in the same manner as indicated above. After cooling, the reaction mixture was poured into ice/water (100 mL) and finally rendered acidic with 20% HCl. The final product was filtered and washed with cold water. Recrystallization is possible from ethanol or acetonitrile or *N*,*N*-dimethylformamide/methanol (2:5). Method B was used for the preparation of the following compounds: **4b**,**c**,**f**−**h**,**k**,**l** and **5c**,**d**,**g**,**j**,**k**,**m**,**n**,**p**,**r**,**u**.

General Diazotization Procedure: 2,5-Dihydro-4-aryl-3*H*-pyridazino[4,3-*b*]indol-3-ones (6) (Table S3 in Supporting Information). Sodium nitrite (150 mg, 2.17 mmol) was added at 0 °C with vigorous stirring to concentrated H_2SO_4 (2.5 mL) followed by addition of the suspension or solution of the appropriate amine **4** or **5** (1.92 mmol) in acetic acid (10 mL). The reaction mixture was stirred for 1 h at 20 °C followed by addition of water (75 mL) until precipitation ceased and was allowed to stand 5 h at room temperature. The final product was filtered and washed three times with water. The analytical sample was prepared after recrystallization from *N*,*N*-dimethylformamide/methanol (2:5).

3-*N*-Formylamino-5-methyl-4-phenylpyridazino[4,3-*b*]-indole (7a) (Table S3 in Supporting Information). The mixture of 1-methyl-3-amino-2-phenylpyridazino[4,3-*b*]indole **5a** (137 mg, 0.5 mmol), 98% formic acid (10 mL), and acetic anhydride (1 mL) was refluxed for 3−5 h. After removal of the liquids under reduced pressure, the same amounts of formic acid and acetic anhydride were added and the mixture was refluxed and triturated as above. This procedure was repeated three times. After removal of the liquids under reduced pressure, the residue was purified by silica gel column chromatography (chloroform/acetone (10:1)) to afford **7a** (48 mg, 50%) as a white powder and recovered starting material **5a** (50 mg). The analytical sample was prepared by recrystallization from chloroform/pentane (2:5).

3-*N*,*N*-Diacetylamino-1-methyl-4-phenylpyridazino-[4,3-*b*]indole (7b) (Table S3 in Supporting Information). The mixture of 1-methyl-3-amino-2-phenylpyridazino[4,3-*b*]-indole **5a** (140 mg, 0.51 mmol) and sodium acetate (4.6 mg, 0.056 mmol) was refluxed in acetic anhydride (2 mL) for 10 min and after cooling was diluted with 10 mL of water. The final product was filtered and washed three times with water. The analytical sample was prepared by recrystallization from 2-propanol.

5-Methyl-3-phthaloylamino-4-(4-isopropylphenyl)pyridazino[4,3-*b*]indole (7c) (Table S3 in Supporting Information). The finely ground mixture of hydrochloride **5d** (51 mg, 0.145 mmol) and phthalic anhydride (80 mg, 0.54 mmol) was heated in an open flask at 150−160 °C for 1 h. Then the excess sublimated phthalic anhydride was removed mechanically. The residue was dissolved in chloroform and was purified by silica gel column chromatography (chloroform/acetone (10:1)) to afford **7c** (31 mg, 74%). The analytical sample was prepared by recrystallization from chloroform/pentane (2:5).

Methods for Evaluation of Inhibitory Activities of Compounds. Portions (5 mg) of each compound were dissolved in 120 μL of DMSO and then diluted in complete medium, RPMI 1640, with 5 mM HEPES, 2 mM L-glutamine, and 2% fetal bovine serum (FBS) (Sigma) to obtain concentrations of 800, 80, 8.0, and 0.8 μg/mL. The final compound concentrations were 20, 2.0, and 0.2 μg/mL. *M. tuberculosis* H37Rv (Pasteur) from the Central TB Research Institute Culture Museum, Moscow, as frozen samples were thawed, added to 5 mL of Dubos broth (Difco, Detroit, MI), and incubated for 1 week at 37 °C. The mycobacterial suspension (0.5 mL) was diluted in 20 mL of fresh, warm Dubos medium and further cultured for 1 week. The resulting suspension was washed three times at 3000*g*, 4 °C, with 0.02% EDTA-PBS (Ca^{2+}- and Mg^{2+}-free) solution, resuspended in RPMI-1640 medium supplemented with 2% FBS, 5 mM HEPES, 2 mM l-glutamine (Sigma), and filtered through a 4 μm pore size filter (Millipore Corporation, Badford, MA) to remove clumps. To estimate CFU content in the filtrate, 10 μL from each 5-fold serial dilution was plated on Dubos agar (Difco Laboratories, Detroit, MI), and the total number of microcolonies in spots was calculated under an inverted microscope (CK-2, Olympus, Osaka, Japan) after culturing for 3 days at 37 °C. The bulk of filtered cultures were stored at +4 °C. Earlier, it was found that no change in the CFU content occurred during this period.[31]

Antimycobacterial activity of compounds was estimated by growth inhibition of *M. tuberculosis*, as measured by selective incorporation of radiolabeled 5,6-[^{3}H]-uracil into bacterial cells as described.[29,30] Briefly, on the basis of CFU results, cultures were diluted so that the number of *M. tuberculosis* was 50×10^4, 25×10^4, 12.5×10^4, and 6.25×10^4 CFU in 100 μL of RPMI 1640 medium with 5mM Hepes, 2mM L-glutamine, and 2% FBS. Different concentrations (100 μL) of *M. tuberculosis* were added to each well of 96-well tissue culture microplates (Costar-Corning, Badhoevedorp, The Netherlands). The compounds in the three concentrations of 20, 2.0, and 0.2 μg/mL (100 μL of each) were added. Each concentration of *M. tuberculosis* and the compound was studied in triplicate. After 24 h, 1 μCi of 5,6-[^{3}H]-uracil (Isotop, St. Petersburg, Russia) was added to every well and incubated for 18 h in a CO_2 incubator at 37 °C. The harvests were collected onto microporous filters (Skatron Filter Mat 11731, dimensions of 102 mm × 256 mm) using a cell harvester (Skatron, Oslo, Norway). After numerous washings with water and drying in an oven for 30 min at 80 °C, the filters were placed in vials with scintillant and β-radiation was registered with the Wallac 1409 scintillant register (Wallac, Turku, Finland). Incorporation of 5,6-[^{3}H]-uracil into growing *M. tuberculosis* was measured in counts per minutes (cpm). Results are expressed as mean cpm ± SD for triplicate cultures. Parallel estimations of mycobacterial CFU counts and 5,6-[^{3}H]-uracil uptake under identical cultural conditions and dynamics provided coefficients of correlation higher than 0.9, validating the usage of this less laborious and variable technique.[29] The activity of compounds was estimated as percent inhibition, which was determined on the basis of the percent difference in incorporation of 5,6-[^{3}H]-uracil into growing *M. tuberculosis* over 42 h in the presence and the absence of the compounds. The MIC_{50} value was defined as the concentration of compounds resulting in 50% reduction in the cpm on that plate compared to those on a plate free of the drug at the same dilution of *M. tuberculosis* suspension.

Methods of Evaluation of MAO Inhibitory Activity. Rat liver mitochondria isolated by the conventional method of differential centrifugation were used as a source of MAO-A and MAO-B.[32] Isolated mitochondria were washed and suspended in 50 mM phosphate buffer, pH 7.4, and stored at −20 °C. MAO activity was assayed radiometrically by accumulation of ^{14}C-labeled aldehydes formed during enzymatic deamination of ^{14}C-labeled amines.[32] [^{14}C]-5-Hydroxytryptamine creatinine sulfate (0.1 mM) and [^{14}C]-phenylethylamine (10 μM) were used as substrates of MAO-A and MAO-B, respectively. The enzyme activities were determined after preincubation of mitochondria with inhibitors for 60 min at 37 °C using concentrations in the range 10^{-8}−10^{-3} (or 10^{-4}) M. Protein content was determined by the Lowry method[39] using bovine serum albumin as the standard.

Statistics. To evaluate the statistical value of differences between averages, the Student's *T*-test was used and the BIOSTAT program (Stenten A. Glanz, 1993; translation by "Praktika", Moscow, 1998) was applied.

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a measurement of growth inhibition. We also thank Dr. Konstantin B. Majorov for his help in establishing this method and Mrs. Lyudmila Axenova for assistance during assay on MAO inhibition.

Supporting Information Available: Chemical data on the indogenides **1** (Table S1), cyanoindogenides **2** and **3** (Table S2), and the pyridazinoindoles **4**−**7** (Table S3), including yields, melting points, ^{1}H NMR data, and elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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