Synthesis and Antimycobacterial, Antifungal, and Photosynthesis-Inhibiting Evaluation of Some Anilides of Substituted Pyrazine-2-carboxylic Acids

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Condensation of chlorides of substituted pyrazine-2-carboxylic acids with ring-substituted anilines yielded a series of anilides of 6-chloropyrazine-2-carboxylic, 5-(1,1-dimethylethyl)pyrazine-2-carboxylic or 6-chloro-5-(1,1-dimethylethyl)pyrazine-2-carboxylic acids. Products were tested for their antimycobacterial, antifungal, and photosynthesis-inhibiting activity. Some of them exhibited significative activity against *Mycobacterium tuberculosis* (inhibition > 50 % at MIC $=_{,}$ 12.5 μg cm^{-3}). The most active compound (88 %) was 6-chloro-5-(1,1-dimethylethyl)pyrazine-2-carboxylic acid 3-fluoroanilide.

Tuberculosis and the pathogen that causes it, *Mycobacterium tuberculosis*, have been known to medical science for well over a century. Even so, it is still a major global disease infecting one third of the worlds population and killing almost 3 million people each year [1, 2]. Recent years have seen increased incidence of tuberculosis in both developing and industrialized countries, the widespread emergence of drug-resistant strains and a deadly synergy with the human immunodeficiency virus (HIV). Pyrazinamide (PZA) is a nicotinamide analogue that has been used for almost 50 years as a first-line drug to treat tuberculosis [3]. PZA is bactericidal to semidormant mycobacteria and reduces total treatment time [4]. Although the exact biochemical basis of PZA activity *in vivo* is not known, under acidic conditions it is thought to be a prodrug of pyrazinoic acid, a compound with antimycobacterial activity [5]. The finding that PZA-resistant strains lose amidase (pyrazinamidase or nicotinamidase) activity and the hypothesis that amidase is required to convert PZA to pyrazinoic acid intracellularly led to the recent synthesis and study of various prodrugs of pyrazinoic acid [6].

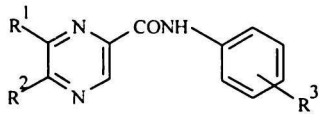
Research programs for the discovery of new antimycobacterial drugs are under way in many laboratories. Earlier studies [7, 8] have described synthesis and tuberculostatic activity of several anilides of unsubstituted pyrazine-2-carboxylic acid. We have recently reported the synthesis of a series of anilides prepared from some 2-alkylpyridine-4-carboxylic [9] or 5-alkylpyrazine-2-carboxylic [10] acids and some aminophenols.

The presented study is concerned in the synthesis of another series of compounds with halogenated (F, Cl) and/or alkylated (1,1-dimethylethyl, isopropyl) pyrazine and benzene rings. The aim of this work is to search for the structure—activity relationships and to determine the importance of increased hydrophobic properties for antimycobacterial activity. The prepared anilides possess the free amino hydrogen atom needed for binding to the receptor site, possibly by forming of hydrogen bond. Various compounds possessing this group, *e.g.* acyl and thioacyl anilides, benzanilides, phenyl carbamates, *etc.*, were found to inhibit photosynthetic electron transport [10—14]. Therefore antifungal and photosynthesis-inhibiting evaluations of newly prepared pyrazine-2-carboxylic acid derivatives were additional areas of our interest.

Condensation of chlorides of substituted pyrazine-2-carboxylic acids with ring-substituted anilines yielded a series of anilides of 6-chloropyrazine-2-carboxylic, 5-(1,1-dimethylethyl)pyrazine-2-carboxylic or 6-chloro-5-(1,1-dimethylethyl)pyrazine-2-carboxylic acids (see Tables 1 and 2). The hydrophobicity (log P values) of compounds was computed. Products were tested for their antimycobacterial, antifungal, and photosynthesis-inhibiting activity. The results of biological assays are summarized in Table 3.

All new anilides of pyrazine-2-carboxylic acid syn-

Table 1. Characteristics of Compounds *I*—*XII*



Compound	R^1	R^2	R^3	Formula M_r	w_i(calc.)/% w_i(found)/%					M.p./°C Yield/%
					C	H	N	Cl	F	
I	Cl	H	3-F	$C_{11}H_7ClFN_3O$	52.50	2.80	16.70	14.09	7.55	143—144
				251.6	52.38	2.83	16.61	14.19	7.48	87
II	Cl	H	2,4-F	$C_{11}H_6ClF_2N_3O$	49.00	2.24	15.58	13.15	14.09	123—124
				269.6	48.92	2.28	15.66	13.25	14.18	81
III	Cl	H	4-Cl	$C_{11}H_7Cl_2N_3O$	49.28	2.63	15.67	26.45	–	145—146
				268.1	49.35	2.70	15.76	26.37	–	73
IV	Cl	H	4-$CH(CH_3)_2$	$C_{14}H_{14}ClN_3O$	60.98	5.12	15.24	12.86	–	65—66
				275.7	61.07	5.16	15.30	12.78	–	59
V	H	$(CH_3)_3C$	3-F	$C_{15}H_{16}FN_3O$	65.92	5.90	15.37	–	6.95	127—129
				273.3	65.78	5.71	15.45	–	6.82	84
VI	H	$(CH_3)_3C$	2,4-F	$C_{15}H_{15}F_2N_3O$	61.85	5.19	14.43	–	13.04	114—115
				291.3	61.61	5.03	14.38	–	13.15	74
VII	H	$(CH_3)_3C$	4-Cl	$C_{15}H_{16}ClN_3O$	62.18	5.57	14.50	12.24	–	187—189
				289.8	62.27	5.53	14.41	12.35	–	83
VIII	H	$(CH_3)_3C$	4-$CH(CH_3)_2$	$C_{18}H_{23}N_3O$	72.70	7.80	14.13	–	–	131—132
				297.4	72.52	7.68	14.22	–	–	78
IX	Cl	$(CH_3)_3C$	3-F	$C_{15}H_{15}ClFN_3O$	58.54	4.91	13.65	11.52	6.17	142—144
				307.8	58.42	4.98	13.61	11.67	5.99	82
X	Cl	$(CH_3)_3C$	2,4-F	$C_{15}H_{14}ClF_2N_3O$	55.31	4.33	12.90	10.88	11.66	126—127
				325.7	55.18	4.40	13.04	10.68	11.49	73
XI	Cl	$(CH_3)_3C$	4-Cl	$C_{15}H_{15}Cl_2N_3O$	55.57	4.66	12.96	21.87	–	175—177
				324.2	55.38	4.82	13.09	21.77	–	65
XII	Cl	$(CH_3)_3C$	4-$CH(CH_3)_2$	$C_{18}H_{22}ClN_3O$	65.15	6.68	12.66	10.68	–	174—175
				331.8	65.09	6.70	12.66	11.77	–	68

Table 2. IR and ^{1}H NMR Parameters of Compounds *I*—*XII*

Compound	IR	^{1}H NMR, δ										
	$\tilde{\nu}(\nu(C{=}O))$	Pyrazine			Benzene					$CH(CH_3)_2$	$(CH_3)_3$	NH
	cm^{-1}	H-3	H-5	H-6	H-2	H-3	H-4	H-5	H-6			
I	1660	9.39	8.82	–	7.71, m	–	6.90, m	7.28—7.41, m, 2H		–	–	9.42
II	1660	9.38	8.82	–	–	6.95, m	–	6.95, m	8.43, m	–	–	9.56
III	1660	9.41	8.84	–	7.74, m	7.39, m	–	7.39, m	7.74, m	–	–	9.35
IV	1660	9.42	8.82	–	7.68, m	7.27, m	–	7.27, m	7.68, m	2.93, 1H; 1.26, d, 6H	–	9.37
V	1660	9.39	–	8.62	7.72, dt J_1 = 10.71 Hz J_2 = 2.20 Hz	–	6.86, m	7.21—7.41, m, 2H		–	1.45, s 9H	9.70
VI	1670	9.37	–	8.65	–	6.94, m	–	6.94, m	8.51, m	–	1.45, s 9H	9.85
VII	1670	9.41	–	8.64	7.73, m	7.37, m	–	7.37, m	7.73, m	–	1.45, s 9H	9.70
VIII	1640	9.42	–	8.64	7.69, m	7.27, m	–	7.27, m	7.69, m	2.93, 1H; 1.26, d, 6H	1.44, s 9H	9.62
IX	1670	9.27	–	–	7.72, dt J_1 = 10.71 Hz J_2 = 2.20 Hz	–	6.88, m	7.28—7.41, m, 2H		–	1.55, s 9H	9.40
X	1690	9.25	–	–	–	6.94, m	–	6.94, m	8.42, m	–	1.55, s 9H	9.53
XI	1660	9.28	–	–	7.73, m	7.38, m	–	7.38, m	7.73, m	–	1.56, s 9H	9.39
XII	1660	9.36	–	–	7.50, m	7.19, m	–	7.19, m	7.50, m	2.88, 1H; 1.26, d, 6H	1.42, s 9H	9.67

Table 3. Antimycobacterial (% Inhibition at MIC = 12.5 μg cm^{-1}), Antifungal Activity (MIC against *Trichophyton mentagrophytes*), Photosynthesis-Inhibiting Activity (IC_{50}), and Lipophilicity (Calculated log P) of Compounds *I*—*XII* in Comparison with Standards

Compound	Inhibition	MIC (after 72 h/120 h)	IC_{50}	log P
	%	μmol dm^{-3}	mmol dm^{-3}	
I	28	250/250	0.565	2.74 ± 0.42
II	1	500/500	0.539	2.67 ± 0.42
III	65	>500/>500	0.486	3.25 ± 0.41
IV	73	125/250	0.118	3.60 ± 0.44
V	71	>500/>500	0.313	3.31 ± 0.41
VI	44	>500/>500	0.371	3.24 ± 0.41
VII	0	>250/>250	1.502	3.81 ± 0.41
VIII	71	>500/>500	0.110	4.16 ± 0.43
IX	88	>500/>500	0.129	4.43 ± 0.43
X	21	>500/>500	0.106	4.36 ± 0.42
XI	24	>500/>500	0.043	4.94 ± 0.42
XII	4	250/>1000	0.052	5.28 ± 0.45
Rifampicine	100[a]	–	–	0.49 ± 0.74
Ketoconazole	–	0.98/1.95[a]	–	4.01 ± 0.66
Atrazine	–	–	0.001[a]	1.03 ± 0.62

a) See Experimental.

thesized were evaluated in agreement with an international program of the TAACF (Tuberculosis Antimicrobial Acquisition & Coordinating Facility of the National Institute of Allergy and Infectious Diseases of the Southern Research Institute, Birmingham, Alabama, USA) for an *in vitro* antituberculosis activity screening. Five of the tested compounds were quite active *in vitro* against *M. tuberculosis* (> 50 % inhibition at MIC = 12.5 μg cm^{-3}). 6-Chloro-5-(1,1-dimethylethyl)pyrazine-2-carboxylic acid 3-fluoroanilide (*IX*, 88 % inhibition) was the most active compound representing the prospective leading structure for the next series of anilides. The activity dropped or disappeared either at the compounds with lower lipophilicity or at the compounds with higher lipophilicity parameters, *i.e.* at the derivatives with two halogen atoms or two alkyl groups. However, the exact conclusions can be drawn after the enlargement of studied series.

None of the compounds studied was effective against majority of fungal pathogens tested (MIC > 0.5—2.0 μmol dm^{-3}). Several compounds exhibit only weak activity against *Trichophyton mentagrophytes* (MIC = 0.125—1.0 μmol dm^{-3}). There was found no correlation between antimycobacterial and antifungal activity in the series of prepared compounds.

A pronounced increase in inhibitory effects upon the oxygen evolution rate in spinach chloroplasts system was found with the more lipophilic compounds (*XI*, *XII*). The introduction of chlorine to the pyrazine moiety (*IV*, *IX*—*XII*) leads to an increase in photosynthesis-inhibiting activity. However, the current series in comparison with previous series of similar anilides (with one quite active compound) [10] is not promising. Probably the loss of phenolic moiety from benzene ring is the main reason for the decreasing of photosynthesis-inhibiting effect.

EXPERIMENTAL

Melting points were determined on a Kofler apparatus. Purity of products was checked by TLC on Silufol UV 254 plates (Kavalier, Votice) using toluene—acetone (φ_r = 1:1) or light petroleum—ethyl acetate (φ_r = 1:1). Samples for elemental analysis were vacuum-dried at about 100 Pa over phosphorus pentoxide at room temperature. Elemental analyses were obtained using an EA 1110 CE instrument (Fisons Instruments S.p.A., Milan). The IR spectra were recorded on a Nicolet Impact 400 spectrometer in KBr pellets. ^{1}H NMR spectra were measured for solutions in $(CD_3)_2SO$ with a BS 497 (Tesla, Brno) 100 MHz apparatus. log P Values were computed using a program ACD/Log P ver. 1.0 (Advanced Chemistry Development Inc., Toronto).

Anilides *I*—*XII*

A mixture of acid (*i.e.* 6-chloropyrazine-2-carboxylic [15], 5-(1,1-dimethylethyl)pyrazine-2-carboxylic [10] or 6-chloro-5-(1,1-dimethylethyl)pyrazine-2-carboxylic [10] acids, 0.05 mol) and thionyl chloride (5.5 cm^3, 75 mmol) in 20 cm^3 of dry benzene was refluxed for about 1 h. Excess of thionyl chloride was removed by repeated evaporation with dry benzene *in vacuo*. The crude acyl chloride dissolved in 50 cm^3 of dry acetone was added dropwise to a stirred solution of the corresponding substituted aniline (50 mmol) in 50 cm^3 of dry pyridine keeping at the room temperature. After the addition was complete, stirring continued for

another 30 min. The reaction mixture was then poured into 200 cm^3 of cold water and the crude anilide was collected and recrystallized from aqueous ethanol. The yields and analytical data are given in Table 1, the IR and ^1H NMR parameters are given in Table 2.

Antimycobacterial Evaluation

In vitro evaluation of antimycobacterial activity (primary screening) was conducted at 12.5 mg cm^{-3} against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA) [16]. Activity was expressed as % inhibition, where standard rifampicine exerted a minimal inhibitory concentration (MIC) 0.25 mg cm^{-3} against *M. tuberculosis*. For the results see Table 3.

Antifungal Evaluation

The prepared compounds were tested for their antifungal activity by the microdilution broth method. The procedure was performed with twofold compound dilutions in RPMI 1640 medium buffered to pH 7.0 with morpholinopropanesulfonic acid (0.165 mol) (Sigma). The final concentrations of the evaluated compounds ranged from 1.000 to 0.975 μmol dm^{-3}. Drug-free controls were included. The MIC's were determined after 24 h and 48 h of static incubation at 35 °C. In case of *Trichophyton mentagrophytes* the MIC's were recorded after 72 h and 120 h incubation. The MIC of the compounds *I—XII* was measured in *Candida albicans* ATCC 44859, *C. tropicalis* 156, *C. krusei* E28, *C. glabrata* 20/I, *Trichosporon beigelii* 1188, *Trichophyton mentagrophytes* 445, *Aspergillus fumigatus* 231, and *Absidia corymbifera* 272 [17]. Standard ketoconazole exerted the MIC = 0.98—1.95 μmol dm^{-3} against studied fungal strains. The results are summarized in Table 3.

Measurement of Oxygen Evolution Rate in Spinach Chloroplasts

The oxygen evolution rate in spinach chloroplasts was investigated spectrophotometrically (Specord UV VIS, Zeiss, Jena) in the presence of an electron acceptor 2,6-dichlorophenol—indophenol, by the method described in Ref. [18]. The compounds were dissolved in dimethyl sulfoxide (DMSO) because of their low water solubility. The used DMSO volume fractions (up to 5 vol. %) did not affect the oxygen evolution. The inhibitory efficiency of the studied compounds has been expressed by IC_{50} values, *i.e.* by molar concentration of the compounds causing 50 % decrease in the oxygen evolution relative to the untreated control. Comparable IC_{50} value for a selective herbicide atrazine [19] is about 1.0 μmol dm^{-3}. The results are summarized in Table 3.

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