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Substituted Amides of Pyrazine-2-carboxylic acids: Synthesis and Biological Activity.

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Abstract: Condensation of 6-chloro-, 5-*tert*-butyl- or 6-chloro-5-*tert*-butylpyrazine-2-carboxylic acid chloride with ring substituted anilines yielded a series of amides, which were tested for their *in vitro* antimycobacterial, antifungal and photosynthesis-inhibiting activities. The highest antituberculotic activity (72% inhibition) against *Mycobacterium tuberculosis* and the highest lipophilicity (log *P* = 6.85) were shown by the 3,5-bis-trifluoromethylphenyl amide of 5-*tert*-butyl-6-chloropyrazine-2-carboxylic acid (**2o**). The 3-methylphenyl amides of 6-chloro- and 5-*tert*-butyl-6-chloro-pyrazine-2-carboxylic acid (**2d** and **2f**) exhibited only a poor *in vitro* antifungal effect (MIC = 31.25-500 μmol·dm^{-3}) against all strains tested, although the latter was the most active antialgal compound (IC_{50} = 0.063 mmol·dm^{-3}). The most active inhibitor of oxygen evolution rate in spinach chloroplasts was the (3,5-bis-trifluoromethylphenyl)amide of 6-chloropyrazine-2-carboxylic acid (**2m**, IC_{50} = 0.026 mmol·dm^{-3}).

Keywords: Amides of pyrazinecarboxylic acid; antimycobacterial activity; antifungal evaluation; photosynthesis inhibition.

Introduction

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)[1,2]. 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]. Various compounds possessing –NHCO– grouping, *e.g.* substituted amides, acyl and thioacyl anilides, benzanilides, phenyl carbamates, etc., were found to inhibit photosynthetic electron transport [7—10]. Therefore, antifungal and photosynthesis-inhibiting evaluations of newly prepared pyrazine-2-carboxylic acid derivatives were additional areas of interest to us.

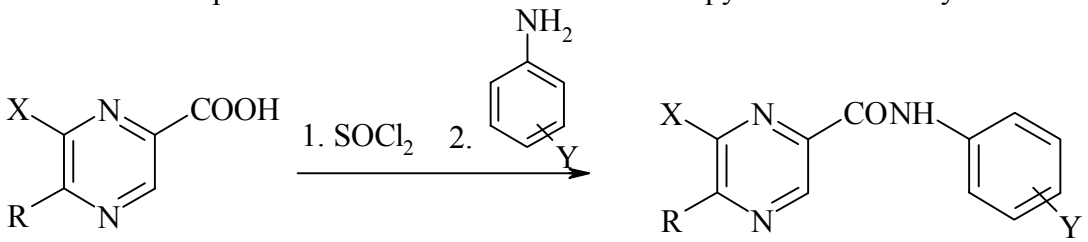
Amides of 2-alkylpyridine-4-carboxylic [11,12] and 2-alkylsulfanyl-4-pyridinecarboxylic [12,13] acids inhibited oxygen evolution rate in *Chlorella vulgaris* and their inhibitory activity depended on the lipophilicity of the compounds. Several esters of alkoxy substituted phenylcarbamic acids (APA) showed antialgal activity against *Chlorella vulgaris* [14-16]. The inhibitory efficiency of APA concerning chlorophyll production in *Chlorella vulgaris* depended on the lipophilicity of the alkoxy substituent and also on its position on the aromatic ring [14-16]. The antialgal activity of APA correlated with the antifungal activity of these compounds against *Candida albicans* [16]. We have recently reported the synthesis of a series of substituted amides prepared from some pyrazine-2-carboxylic acids and some aminophenols [17], halogenated or alkylated anilines [18].

The present study is concerned in the synthesis of another series of amides prepared from substituted pyrazine-2-carboxylic acids and alkylated (2-, 3-methyl-, 2,6-dimethyl-), alkoxylated (2-methoxy-) or halogenated (3-bromo-, 3,5-bis-trifluoromethyl-) anilines. The aim of this work is to search for the structure-activity relationships and to determine the importance of increased lipophilicity for antimycobacterial, antifungal and photosynthesis-inhibiting evaluation of newly prepared pyrazine-2-carboxylic acid amides.

Results and Discussion

The synthesis of amides is shown in Scheme 1. Condensation of the chlorides of 6-chloropyrazine-2-carboxylic (**1a**)[19], 5-*tert*-butyl-pyrazine-2-carboxylic (**1b**) [17] or 5-*tert*-butyl-6-chloropyrazine-2-carboxylic (**1c**) [17] acids with ring substituted anilines yielded a series of 18 substituted amides **2a-r** of the aforementioned substituted pyrazine-2-carboxylic acids.

Scheme 1. Preparation of substituted amides **2a-r** of pyrazine-2-carboxylic acids



1a-c **2a-r**

X = H, Cl
R = H, 1,1-*tert*-butyl

Y = 2-CH_3, 3-CH_3, 2,6-CH_3, 2-OCH_3, 3-Br, 3,5-CF_3

The melting points, yields, and elemental analyses of the compounds prepared **2a-r** are given in Table 3, and their spectral data in Tables 4 and 5. The structures were corroborated by 2D NMR spectroscopy using gHSQC and gHMBC experiments. The biological activities of the prepared amides **2a-r** with regards to *in vitro* antimycobacterial, antifungal and inhibition of oxygen evolution rate in spinach chloroplasts were investigated. The highest antituberculotic activity (72% inhibition) against *Mycobacterium tuberculosis* and also the highest lipophilicity (log *P* = 6,85) was shown by the 3,5-bis-trifluoromethylphenyl amide of 5-*tert*-butyl-6-chloropyrazine-2-carboxylic acid (**2o**). Some other amides (**2d**, **2f**, **2k**, **2l**) with higher than 20% inhibition were investigated. Three of them contain a *tert*-butyl moiety in position 5 of the pyrazine ring. The negative results of antimycobacterial screening allow us to make no conclusions regarding potential structure-activity relationships. Results of their antimycobacterial activity (MIC, % Inhibition) and calculated log *P* values of **2a-r** are shown in Table 1.

Table 1. Antimycobacterial activity (MIC, % inhibition), IC_{50} values for inhibition of oxygen evolution rate in spinach chloroplasts by compounds **2a-r** and calculated log *P* values of the compounds in comparison with standards rifampicine (RMP) and DCMU (see Experimental).

Compd.	MIC [μg ml^{-1}]	% Inhibition	IC_{50}[mmol dm^{-3}]	log *P*
2a	>6.25	0	1.072	2.72 ± 0.41
2b	>6.25	0	0.440	3.28 ± 0.40
2c	>6.25	0	0.244	4.41 ± 0.42
2d	>6.25	28	0.486	2.72 ± 0.41
2e	>6.25	11	0.148	3.28 ± 0.40
2f	>6.25	24	0.118	4.41 ± 0.42
2g	>6.25	6	-[a]	2.15 ± 0.42
2h	>6.25	18	0.286	2.72 ± 0.41
2i	>6.25	7	0.097	3.84 ± 0.43
2j	>6.25	19	0.313	3.46 ± 0.48

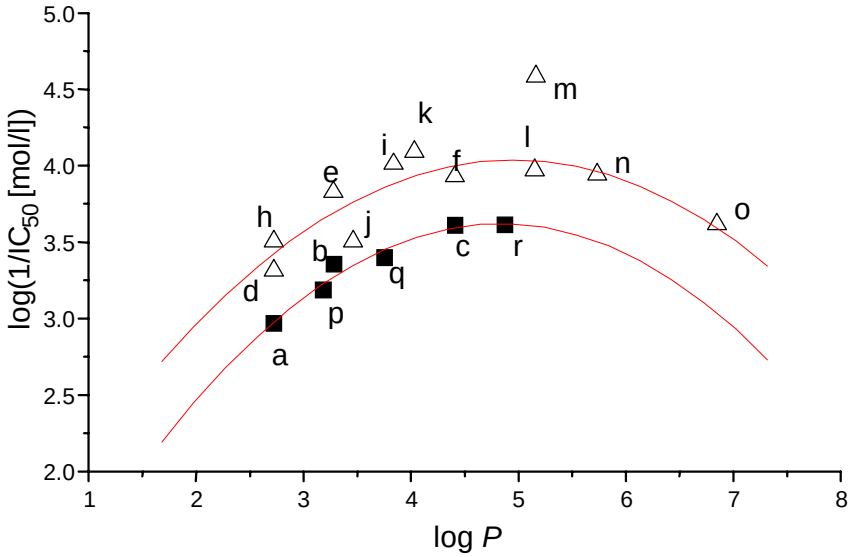
2k	>6.25	39	0.081	4.03 ± 0.48
2l	>6.25	20	0.107	5.15 ± 0.50
2m	>6.25	12	0.026	5.16 ± 0.54
2n	>6.25	10	0.114	5.73 ± 0.53
2o	>6.25	72	0.241	6.85 ± 0.55
2p	>6.25	0	0.649	3.18 ± 0.41
2q	>6.25	2	0.229	3.75 ± 0.40
2r	>6.25	13	0.242	4.87 ± 0.42
RMP	0.125	100	-	– 0.37 ± 0.35
DCMU[c]	-	-	0.0019	2.78 ± 0.38

[a] not measured

The evaluation of *in vitro* antifungal activity of the synthetized compounds showed that only compounds **2d** and **2f**, and partly compound **2l** having a considerable antifungal effect on all the fungal strains tested. The most susceptible was *Trichophyton mentagrophytes* strain (MIC = 62.5–1000 μmol·L^{-1}), especially towards compounds **2f**, **2h**, **2i** and **2l**. Another susceptible strain was *Absidia fumigatus* (MIC = 31.25–500 μmol·L^{-1}) towards compounds **2f** and **2j**.

The studied compounds inhibited photosynthetic electron transport in spinach chloroplasts, which was reflected in the inhibition of oxygen evolution rate. The photosynthesis inhibitory activity of the compounds has been expressed as IC_{50} values (see Table 1). The IC_{50} values varied in the range from 0.026 (**2m**) to 1.072 mmol·dm^{-3} (**2a**). In general, the photosynthesis-inhibiting activity of the studied compounds depended on their lipophilicity showing a quasi-parabolic trend. However, the studied compounds could be divided into two groups. The compounds with 2-CH_3 substituents on the phenyl ring (**2a**, **2b**, **2c**, **2p**, **2q** and **2r,** squares in Figure 1) had lower biological activity than the other investigated compounds with comparable log *P* values. Consequently, we assume that the methyl substituent in *ortho* position of the benzene ring is disadvantageous from the viewpoint of interactions with the photosynthetic apparatus. On the other hand, compound **2m** exhibited higher inhibitory activity than expected.

Figure 1. Quasi-parabolic dependence between photosynthesis inhibitory activity and log *P* of studied amides **2a-r**.



Additionally some inhibition of chlorophyll production in green algae *Chlorella vulgaris* was studied at the compounds **2f**, **2l**, **2m**, **2n**, **2o** and **2p**. Results of their antialgal activity are given in Table 2. The antialgal activity of these six studied compounds showed a quasi-parabolic dependence upon log *P* with maximum activity for compounds having log *P* in the range from 3.18 to 5.16 (see Figure 2). With the further increasing of the lipophilicity a dramatic decrease of antialgal activity was observed.

Figure 2. Quasi-parabolic dependence between antialgal activity and log *P* of studied amides **2f**, **2l**, **2m**, **2n**, **2o** and **2p**.

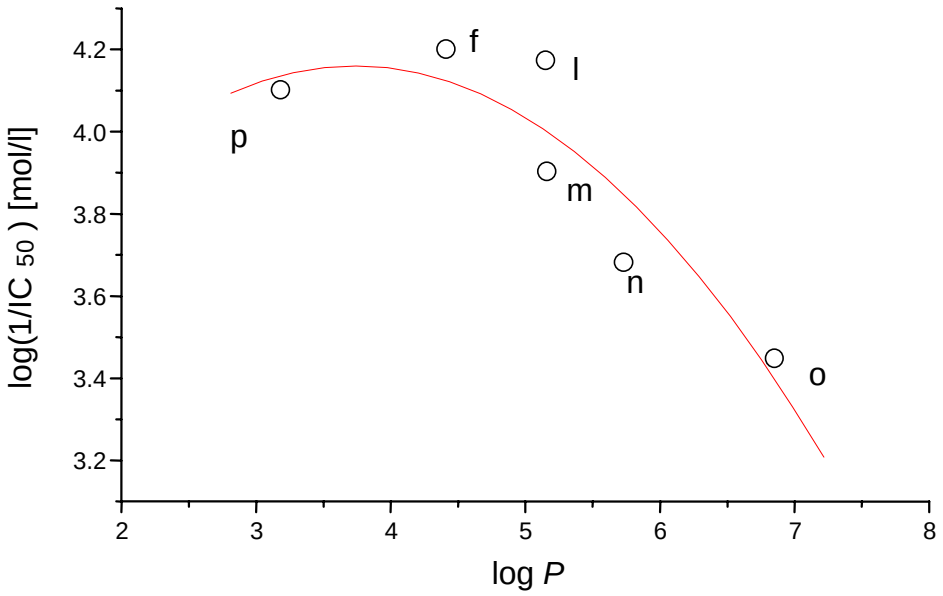


Table 2. IC_{50} values concerning inhibition of chlorophyll production in green algae *Chlorella vulgaris* by the tested anilides **2f**, **2l**, **2m**, **2n**, **2o** and **2p** and calculated log *P* values of the compounds in comparison with standard DCMU (see experimental).

Compd.	IC_{50}[mmol dm^{-3}]	log *P*
2f	0.063	4.41 ± 0.42
2l	0.067	5.15 ± 0.50
2m	0.125	5.16 ± 0.54
2n	0.208	5.73 ± 0.53
2o	0.356	6.85 ± 0.55
2p	0.079	3.18 ± 0.41
DCMU	0.0073	2.78 ± 0.38

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Experimental

General

Melting points were determined on a Kofler block, and are uncorrected. Elemental analyses were obtained using an EA 1110 CHNS-O CE apparatus (Fisons Instruments S.p.A., Milan). The IR spectra were recorded on a Nicolet Impact 400 spectrometer in KBr pellets. The ^{1}H and ^{13}C NMR spectra were measured for $CDCl_3$ solutions with a Varian Mercury - Vx BB 300 spectrometer operating at 300 MHz. Chemical shifts were recorded as δ values in parts per million (ppm), and were indirectly referenced to tetramethylsilane via the solvent signal (7.26 for ^{1}H and 77.0 for ^{13}C). Multiplicities are given together with the coupling constants (in Hz). Log *P* values were computed using a program ACD/Log P ver. 1.0 (Advanced Chemistry Development Inc., Toronto).

Synthesis of amides **2a-r**

A mixture of acid (*i.e.* 6-chloropyrazine-2-carboxylic [19], 5-*tert*-butylpyrazine-2-carboxylic [17] or 5-*tert*-butyl-6-chloropyrazine-2-carboxylic [17] acid, 0.05 mol) and thionyl chloride (5.5 mL, 75 mmol) in dry benzene (20 mL) was refluxed for about 1 h. Excess thionyl chloride was removed by repeated evaporation with dry benzene *in vacuo*. The crude acyl chloride dissolved in dry acetone (50 mL) was added dropwise to a stirred solution of the corresponding substituted aniline (50 mmol) in dry pyridine (50 mL) kept at room temperature. After the addition was complete, stirring was continued for another 30 min. The reaction mixture was then poured into cold water (200 mL) and the crude amide was collected and recrystallized from aqueous ethanol.

Table 3. Analytical data of the amides **2a-r**.

Compd.	X	R	Y	Formula M. w.	% Calculated / % Found C	H	N	F	Cl	Br	M.p./°C Yield/%
2a	Cl	H	2-CH_3	$C_{12}H_{10}ClN_3O$	58.19	4.07	16.97	-	14.31	-	97-99
				247.7	58.02	4.14	16.86	-	14.19	-	75
2b	H	$(CH_3)_3C$	2-CH_3	$C_{16}H_{19}N_3O$	71.35	7.11	15.60	-	-	-	80-81
				269,3	71.48	7.08	15.67	-	-	-	84
2c	Cl	$(CH_3)_3C$	2-CH_3	$C_{16}H_{18}ClN_3O$	63.26	5.97	13.83	-	11.67	-	114-15
				303.8	63.15	5.82	13.96	-	11.86	-	78
2d	Cl	H	3-CH_3	$C_{12}H_{10}ClN_3O$	58.19	4.07	16.97	-	14.31	-	83-84
				247.7	58.08	4.11	16.80	-	14.48	-	79
2e	H	$(CH_3)_3C$	3-CH_3	$C_{16}H_{19}N_3O$	71.35	7.11	15.60	-	-	-	94-95
				269,3	71.41	7.22	15.77	-	-	-	85
2f	Cl	$(CH_3)_3C$	3-CH_3	$C_{16}H_{18}ClN_3O$	63.26	5.97	13.83	-	11.67	-	98-99
				303.8	63.40	6.08	14.01	-	11.74	-	84
2g	Cl	H	2-OCH_3	$C_{12}H_{10}ClN_3O_2$	54.66	3.82	15.94	-	13.45	-	71-72
				263.7	54.57	3.93	16.01	-	13.35	-	85
2h	H	$(CH_3)_3C$	2-OCH_3	$C_{16}H_{19}N_3O_2$	67.35	6.71	14.73	-	-	-	77-78
				285.3	67.16	6.68	14.62	-	-	-	88
2i	Cl	$(CH_3)_3C$	2-OCH_3	$C_{16}H_{18}ClN_3O_2$	60.09	5.67	13.14	-	11.09	-	118-19
				319.8	60.16	5.59	13.23	-	11.07	-	82
2j	Cl	H	3-Br	$C_{11}H_7BrClN_3O$	42.27	2.26	13.44	-	11.34	25.56	99-100
				312.5	42.37	2.25	13.41	-	11.48	25.60	83
2k	H	$(CH_3)_3C$	3-Br	$C_{15}H_{16}BrN_3O$	53.91	4.83	12.57	-	-	23.91	113-14
				334.2	54.03	4.97	12.61	-	-	23.77	75
2l	Cl	$(CH_3)_3C$	3-Br	$C_{15}H_{15}BrClN_3O$	48.87	4.10	11.40	-	9.62	21.67	104-105
				368.7	48.79	4.22	11.28	-	9.77	21.78	62
2m	Cl	H	3,5-CF_3	$C_{13}H_6ClF_6N_3O$	42.24	1.64	11.37	30.84	9.59	-	132-133
				369.7	42.21	1.66	11.33	30.77	9.46	-	88
2n	H	$(CH_3)_3C$	3,5-CF_3	$C_{17}H_{15}F_6N_3O$	52.18	3.86	10.74	29.13	-	-	135-137
				391.3	52.02	3.84	10.72	29.17	-	-	89
2o	Cl	$(CH_3)_3C$	3,5-CF_3	$C_{17}H_{14}ClF_6N_3O$	47.96	3.31	9.87	26.77	8.33	-	98-99
				425.8	48.01	3.41	9.63	26.56	8.51	-	88
2p	Cl	H	2,6-CH_3	$C_{13}H_{12}ClN_3O$	59.66	4.62	16.06	-	13.55	-	121-122
				361.7	59.70	4.70	16.09	-	13.67	-	75
2q	H	$(CH_3)_3C$	2,6-CH_3	$C_{17}H_{21}N_3O$	72.06	7.47	14.83	-	-	-	84-85
				283.4	72.09	7.45	14.84	-	-	-	78
2r	Cl	$(CH_3)_3C$	2,6-CH_3	$C_{17}H_{20}ClN_3O$	64.25	6.34	13.22	-	11.16	-	145-146
				317.8	64.19	6.40	13.18	-	11.17	-	68

Table 4. IR and ^{1}H-NMR spectral data of the amides **2a-r**.

Compd.	IR (cm^{-1})	^{1}H-NMR (δ, ppm; J in Hz)
2a	1692 (C=O) 3377 (NH)	2.40s (CH_3), 7.10-7.17m (H5′), 7.22-7.33m (H3′- H4′), 8.11-8.15m (H6′), 8.81s (H5), 9.40s (H3), 9.42bs (NH)
2b	1685 (C=O) 3358 (NH)	1.45s [$C(CH_3)_3$], 2.40s (CH_3), 7.10td (J=7.70, H5′), 7.20-7.33m (H3′- H4′), 8.26d J=7.70, H6′), 8.65dd (J=1.37, H5), 9.41dd (J=1.37, H3), 9.71bs (NH)
2c	1695 (C=O) 3360 (NH)	1.56s [$C(CH_3)_3$], 2.40s (CH_3), 7.12td (J=7.41, 1.37, H5′), 7.21-7.32m (H3′-H4′), 8.18-8.13m (H6′), 9.28s (H3), 9.42s (NH)
2d	1692 (C=O) 3369 (NH)	2.39s (CH3), 6.98-7.03m (H6′), 7.28t (J=7.96, H5′), 7.52-7.61m (H2′-H4′), 8.80s (H5), 9.35bs (NH), 9.39bs (H3)
2e	1684 (C=O) 3356 (NH)	1.45s[$C(CH_3)_3$], 2.38s(CH3), 6.98d (J=7.69, H6′), 7.27t (J=7.69, H5′), 7.50-7.56m (H4′), 7.61-7.65m (H2′), 8.62d (J=1.51, H6), 9.40d (J=1.51,H3), 9.61s (NH)
2f	1694 (C=O) 3374 (NH)	1.55s [$C(CH_3)_3$], 2.39s (CH_3), 6.97-7.02m (H6′), 7.28t (J=7.69, H5′), 7.51-7.57m (H4′), 7.59-7.63m (H2′), 9.27s (H3), 9.32bs (NH)
2g	1690 (C=O) 3377 (NH)	3.97s (OCH_3), 6.94dd (J=7.96, 1.64, H3′), 7.03td (J=7.69, 1.51, H5′), 7.13td (J=7.69, 1.51, H4′), 8.52dd (J=7.96, 1.64, H6′), 8.78s (H5), 9.38s (H3), 10.04s (NH)
2h	1691 (C=O) 3356 (NH)	1.45s [$C(CH_3)_3$], 3.96s (OCH_3), 6.94dd (J=7.96, 1.64, H3′), 7.02td (J=7.69, 1.53, H5′), 7.11td (J=7.69, 1.53, H4′), 8.59dd (J=7.96, 1.64, H6′), 8.68d (J=1.37, H6), 9.39d (J=1.37, H3), 10.27bs (NH)
2i	1695 (C=O) 3369 (NH)	1.55s [$C(CH_3)_3$], 3.97s (OCH_3), 6.94dd (J=7.97, 1.51, H3′), 7.02td (J=7.97, 1.51, H5′), 7.12td (J=7.97, 1.51, H4′), 8.53dd (J=7.97, 1.51, H6′), 9.26s (H3), 10.01bs (NH)
2j	1701 (C=O) 3369 (NH)	7.35-7.22m (H5′, H6′), 7.67ddd (J=7.96, 1.92, 1.37, H4′), 8.01t (J=1.92, H2′), 8.82s (H5), 9.38bs (H3), 9.38bs (NH)
2k	1692 (C=O) 3352	1.45s [$C(CH_3)_3$], 7.21-7.32m (H5′,H6′), 7.66dt (J=7.65, 1.92, H4′), 8.03t (J=1.92, H2′), 8.62d (J=1.65, H6), 9.38d (J=1.65, H3), 9.66bs (NH)
2l	1697 (C=O) 3360 (NH)	1.55s [$C(CH_3)_3$], 7.22-7.34m (H5′, H6′), 7.66dt (J=7.69, 1.92, H4′), 8.02t (J=1.92, H2′), 9.26s (H3), 9.36bs (NH)
2m	1681 (C=O) 3368 (NH)	7.70bs (H4′), 8.87bs (H5, H2′, H6′) 9.41s (H3), 9.66bs (NH)
2n	1699 (C=O) 3346 (NH)	1.46s[$C(CH_3)_3$], 7.66bs (H4′), 8.28bs (H2′, H6′), 8.64d (J=1.51, H6), 9.41d (J=1.51, H3), 9.94bs (NH)
2o	1686 (C=O) 3370 (NH)	1.56s[$C(CH_3)_3$], 7.68bs (H4′), 8.29bs (H2′, H6′), 9.29s (H3), 9.63bs (NH)
2p	1691 (C=O) 3356 (NH)	2.28s (CH_3), 7.10-7.21m (H3′, H4′, H5′), 8.83s (H5), 8.94bs (NH), 9.39s (H3)
2q	1667 (C=O) 3370 (NH)	1.46s [$C(CH_3)_3$], 2.29s (CH3), 7.09-7.19m (H3′, H4′, H5′), 8.65d (J=1.37, H6), 9.16bs (NH), 9.40d (J=1.37, H3)
2r	1710 (C=O) 3291 (NH)	1.57s [$C(CH_3)_3$], 2.28s (CH_3), 7.07-7.20m (H3′, H4′, H5′), 8.91bs (NH), 9.27s (H3)

Table 5. ^{13}C NMR spectral data of the amides **2a-r**.

Compd.	^{13}C NMR (75 MHz, $CDCl_3$) δ, ppm, J in Hz
2a	159.3, 147.5, 147.4, 144.2, 142.1, 134.9, 130.6, 128.6, 127.0, 125.5, 121.9, 17.6
2b	167.7, 160.9, 142.9, 141.7, 139.1, 135.5, 130.4, 127.9, 126.9, 124.8, 121.4, 37.0, 29.7, 17.6
2c	164.5, 159.7, 145.7, 141.3, 140.2, 135.1, 130.5, 128.5, 126.9, 125.3, 121.7, 39.0, 28.2, 17.6
2d	159.2, 147.4, 147.4, 144.0, 142.2, 139.2, 136.7, 129.0, 126.0, 120.5, 117.1, 21.5
2e	167.6, 161.0, 142.9, 141.5, 139.1, 138.9, 137.3, 128.9, 125.4, 120.3, 116.8, 37.0, 29.7, 21.5
2f	164.4, 159.7, 145.7, 141.2, 140.2, 139.1, 137.0, 128.9, 125.7, 120.5, 117.0, 39.0, 28.3, 21.5
2g	159.2, 148.7, 147.5, 147.3, 144.5, 142.1, 126.6, 124.8, 121.1, 120.0, 110.1, 55.9
2h	167.4, 161.0, 148.6, 142.9, 141.9, 139.2, 127.2, 124.2, 121.1, 119.7, 110.1, 55.8, 37.0, 29.7
2i	164.2, 159.7, 148.7, 145.8, 141.6, 140.1, 126.9, 124.6, 121.0, 119.9, 110.1, 55.9, 38.9, 28.3
2j	159.4, 147.8, 147.5, 143.5, 142.2, 138.1, 130.5, 128.1, 122.9, 122.8, 118.4, 29.7
2k	168.1, 161.1, 143.0, 141.0, 139.0, 138.7, 130.4, 127.5, 122.8, 122.6, 118.1, 37.1, 29.7
2l	164.9, 159.9, 145.8, 140.7, 140.3, 138.3, 130.4, 127.9, 122.8, 118.4, 39.0, 28.2
2m	159.9, 148.4, 147.7, 142.9, 142.4, 138.3, 132.7 (q, J=33.5 Hz), 123.0 (q, J=272.8 Hz), 119.7, 118.5 (q, J=3.7 Hz)
2n	168.7, 161.6, 143.2, 140.3, 139.1, 138.9, 132.6 (q, J=33.7 Hz), 123.1 (q, J=272.9 Hz), 119.4 (d, J=2.9 Hz), 117.8 (q, J=3.8 Hz), 37.2, 29.7
2o	165.6, 160.4, 146.0, 140.4, 140.0, 138.5, 132.6 (q, J=33.2 Hz), 123.0 (q, J=272.9 Hz), 119.6 (q, J=3.2 Hz), 118.1 (q, J=4.0 Hz), 39.2, 28.2
2p	159.8, 147.5, 143.9, 142.3, 135.3, 132.7, 128.3,, 127.7, 30.9, 18.5
2q	167.6, 161.4, 143.0, 141.4, 139.1, 135.3, 133.3, 128.2, 127.4, 37.0, 29.7, 18.5
2r	164.5, 160.2, 145.9, 141.0, 140.3, 135.4, 132.9, 128.3, 127.6, 39.0, 28.3, 18.5

Antimycobacterial Assay

Antimycobacterial evaluation was carried out in Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Southern Research Institute, Birmingham, Alabama, USA, which is a part of National Institutes of Health (NIH). Primary screening of all compounds were conducted at 6.5 or 12.5 μg·ml^{-1} against *Mycobacterium tuberculosis* H_{37}Rv in BACTEC 12B medium using the BACTEC 460 radiometric system [20]. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of 99% relative to controls. For the results see Table 1.

In vitro antifungal susceptibility testing

Broth microdilution test [21,22] was used for the assessment of *in vitro* antifungal activity of ketoconazole (standard) and the synthetized compounds against *Candida albicans* ATCC 44859, *Candida tropicalis* 156, *Candida krusei* E28, *Candida glabrata* 20/I, *Trichosporon beigelii* 1188, *Trichophyton mentagrophytes* 445, *Aspergillus fumigatus* 231, and *Absidia corymbifera* 272. The

procedure was performed with twofold compound dilutions in RPMI 1640 buffered to pH 7.0 with 0.165 mol morpholinopropanesulfonic acid. The final concentrations of the compounds ranged from 1000 to 0.975 μmol/L. Drug free controls were included. The MICs were determined after 24 and 48 h of static incubation at 35°C. In the case of *Trichophyton mentagrophytes* the MICs were determined after 72 and 120 h of incubation.

Study of inhibition 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 method described in Ref. [23]. The compounds were dissolved in dimethyl sulfoxide (DMSO) because of their low water solubility. The DMSO volume fractions used (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. IC_{50} value for the standard, a selective herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea, DCMU (DIURON) was measured about 1.9 μmol dm^{-3}. For the results see Table 1.

Study of inhibition of chlorophyll production in green algae Chlorella vulgaris

The algae *Chlorella vulgaris* were cultivated statically at room temperature according to Sidóová *et al.* [24] (photoperiod 16 h light/8 h dark; illumination 4000 lx; pH = 7.2). The effect of compounds **2f, 2l, 2m, 2n, 2o** and **2p** on algal chlorophyll (Chl) content was determined after 4-day cultivation in the presence of the tested compounds, expressing the response as percentage of the corresponding values obtained for control. The Chl content in the algal suspension was determined spectrophotometrically (Specord UV VIS, Zeiss Jena, Germany) after extraction into *N*,*N*-dimethylformamide according to Inskeep and Bloom [25]. The Chl content in the suspensions at the beginning of cultivation was 0.5 mg dm^{-3}. Because of their low water solubility, the tested compounds were dissolved in DMSO. DMSO concentration in the algal suspensions did not exceed 0.25 v/v % and the control samples contained the same DMSO amount as the suspensions treated with the tested compounds. IC_{50} value for the standard, a selective herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea, DCMU (DIURON) was measured about 7.3 μmol dm^{-3}. For the results see Table 2.

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Sample availability: Samples of the compounds mentioned in this paper are available from MDPI.