Synthesis and Antimicrobial Evaluation of Some Arylhydrazones of 4-[(2-Methylimidazo[1,2-a]pyridine-3-yl)azo]benzoic Acid Hydrazide

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A short series of arylhydrazones of 4-[(2-methylimidazo[1,2-a]pyridine-3-yl)azo]benzoic acid hydrazide was synthesized and tested for antimicrobial activity. All of the compounds show antimicrobial activity against *Escherichia coli*. A few members were also active against *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The interplanar angle (θ) between the aryl ring and the adjacent azomethine group of some representative compounds was measured by electronic absorption spectroscopy. No structure-activity-relationship between interplanar angle or lipophily and activity was found.

Synthese und antimikrobielle Wirksamkeit einiger Arylhydrazone von 4-[(2-methylimidazo[1,2-a]pyridin-3-yl)azo]benzoesäurehydrazid

Es wird über die Darstellung und die antimikrobielle Wirkung einiger Arylhydrazone von 4-[(2-methylimidazo[1,2-a]pyridin-3-yl)azo]benzoesäurehydrazid berichtet. Alle Substanzen hemmen das Wachstum von *Escherichia coli*. Einige Verbindungen sind auch wirksam gegen *Klebsiella pneumoniae*, *Staphylococcus aureus* und *Pseudomonas aeruginosa*. Durch Elektronenabsorptionsspektrometrie wurde der interplanare Winkel (θ) zwischen dem Arylrest und benachbarten Azomethingruppe einiger representativer Substanzen bestimmt. Zwischen Interplanar-Winkel bzw. Lipophilie und den Wirksamkeiten konnte keine Struktur-Wirkungs-Beziehungen festgestellt werden.

The hydrazone group plays an important role for the antimicrobial activity. For this purpose a number of compounds were prepared and tested against different bacterial strains[1-6].

The aim of the present investigation is to prepare arylhydrazides of the prototype **2**, with the imidazo[1,2-a]pyridine ring system which shows antimicrobial and anthelmintic activities[7] and to combine it with different aromatic aldehydes, in order to obtain more active compounds. In a number of occasions bioactivity of the drugs has been shown to be significantly related to the planarity of important functional groups[8-13] and thus the major thrust of this investigation was to prepare analogues of **4a** in which an aryl ring would be either coplanar or out of the plan in regard to the azomethine group and to explore whether a correlation between planarity and antimicrobial activity could be found. Modification of **4a** were accomplished using chloro, nitro, methoxy, methyl and in few cases with one example hydroxy, bromo, dimethoxy and methylenedioxy groups in the aldehyde part. As the lipid solubility can also play an important role in the activity of substances, the lipophily of **4a-o**, here R_M values, were calculated by reverse phase TLC according to[14-16].

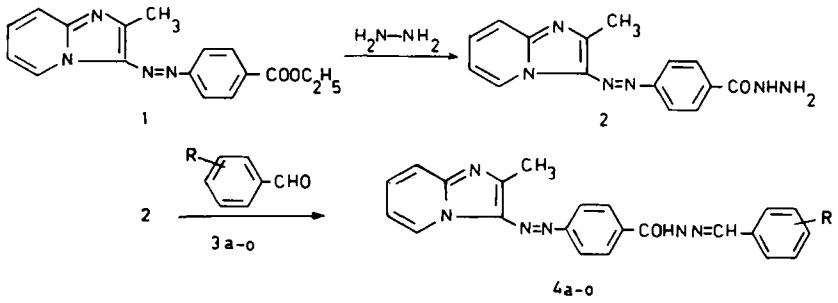
Results and Discussion

Synthesis and Spectroscopy

The hydrazide **2** was prepared by hydrazinolysis of the corresponding ester **1**[17]. The title compounds **4a-o** were obtained by reacting **2** with the corresponding aldehydes **3a-o** (scheme).

The constitutions of **2** and **4a-o** were confirmed by UV, IR, ^{1}H-NMR, and MS data and by elemental analysis. Data of **4a-o** are shown in Table 1.

In the UV spectra of **4a-o** four main bands at 204; 234; 294-304, and 406-408 nm attributed to the benzenoid,



For **a** - **o** see Table 1

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Table 1. Data of **4a-o**

Compound	R	Mp.[°C]	Yield [%]	Molecular formula (mol.wt.)	Elemental Analysis (calcd./found) C	H	N	MS* m/z (rel.int.)
4a	H	262-263	59	$C_{22}H_{18}N_6O$	69.1	4.74	22.0	383 (M+1)(6), 382 ($M^{+\cdot}$)(23),
				(382.4)	68.9	4.83	22.0	263 (100)
4b	2-Cl	239-241	69	$C_{22}H_{17}ClN_6O$	59.5	4.54	18.9	418 (M+2)(4), 417 (M+1)(83),
				1.5 H_2O(443.9)	59.6	4.25	18.8	416 ($M^{+\cdot}$)(10), 263 (100)
4c	3-Cl	241-243	54	$C_{22}H_{17}ClN_6O$	63.4	4.11	20.2	418 (M+2)(12), 417 (M+1)(9),
				(416.9)	63.4	3.90	20.3	416 ($M^{+\cdot}$)(35), 263 (100)
4d	4-Cl	269-271	75	$C_{22}H_{17}ClN_6O$	63.4	4.11	20.2	418 (M+2)(5), 417 (M+1)(4),
				(416.9)	63.1	3.97	20.2	416 ($M^{+\cdot}$)(15), 263 (100)
4e	2-OCH_3	216-217	60	$C_{23}H_{20}N_6O_2$	62.9	5.28	19.1	413 (M+1)(6), 412 ($M^{+\cdot}$)(22),
				1.5 H_2O(439.5)	63.1	5.04	18.9	263 (100)
4f	3-OCH_3	236-238	63	$C_{23}H_{20}N_6O_2$	61.6	5.39	18.7	413 (M+1)(10),412 ($M^{+\cdot}$)(35),
				2 H_2O(448.5)	61.6	5.19	18.9	263 (100)
4g	2-CH_3	216-218	39	$C_{23}H_{20}N_6O$	69.7	5.09	21.2	397 (M+1)(8), 396 ($M^{+\cdot}$)(28),
				(396.4)	69.8	5.39	21.1	263 (100)
4h	3-CH_3	248-250	35	$C_{23}H_{20}N_6O$	69.6	5.09	21.2	397 (M+1)(9), 396 ($M^{+\cdot}$)(32)
				(396.4)	70.0	5.21	21.4	263 (100)
4i	4-CH_3	251-253	55	$C_{23}H_{20}N_6O$	66.6	5.35	20.3	397 (M+1)(7), 396 ($M^{+\cdot}$)(24),
				1 H_2O(405.5)	66.5	5.21	20.1	263 (100)
4j	3,4-O-CH_2-O	238-240	49	$C_{23}H_{18}N_6O_3$	64.8	4.25	19.7	427 (M+1)(10), 426 ($M^{+\cdot}$)(36),
				(426.4)	64.6	4.22	19.5	263 (100)
4k	3,4-(OCH_3)$_2$	254-255	60	$C_{24}H_{22}N_6O_3$	65.1	5.01	19.0	443 (M+1)(8), 442 ($M^{+\cdot}$)(29),
				(442.5)	65.0	4.95	19.0	263 (100)
4l	2-OH,4-Br	168-170	61	$C_{22}H_{17}BrN_6O_2$	53.3	3.87	17.0	479 (M+2+1)(4), 478 (M+2)(18),
				1 H_2O(495.3)	53.5	3.77	16.7	477 (M+1)(5), 476 ($M^{+\cdot}$)(19),
								263 (100)
4m	2-NO_2	259-261	76	$C_{22}H_{17}N_7O_3$	58.1	4.44	21.6	428 (M+1)(5), 427 ($M^{+\cdot}$)(19),
				1.5 H_2O(454.5)	58.3	4.40	21.3	78 (100)
4n	3-NO_2	267-269	80	$C_{22}H_{17}N_7O_3$	58.1	4.44	21.6	428 (M+1)(4), 427 ($M^{+\cdot}$)(16),
				1.5 H_2O(454.5)	58.2	4.26	21.7	78 (100)
4o	4-NO_2	286-287	82	$C_{22}H_{17}N_7O_3$	57.0	4.57	21.2	428 (M+1)(6), 427 ($M^{+\cdot}$)(23),
				2 H_2O(463.5)	56.9	4.67	21.0	263 (100)

* Only (M+2), (M+1), $M^{+\cdot}$, and the base peaks are given.

heterocyclic ring, -HC-N= and azo groups appeared. The IR spectrum of **2** shows the hydrazide carbonyl band at 1640 cm^{-1}. In the IR spectra of **4a-o**, besides characteristic stretching bands, a supplementary -HC=N- stretching band at 1670 cm^{-1} was observed. In the ^{1}H-NMR spectrum of **2** a singlet at 2.75 ppm assigned to C-2-CH_3 of the heterocyclic ring just downfield to the DMSO-d_6 impurity signal was found. A singlet, exchangeable with D_2O, for the NH_2 group appeared at 4.57 ppm. Aromatic protons were observed as a multiplet between 7.26-8.00 ppm. A doublet and a singlet exchangeable with D_2O at 9.75 and 9.89 ppm attributed to H-5 of the imidazo[1,2-a]pyridine ring and to CONH were also present. The ^{1}H-NMR spectra of **4a-o** besides the common group signals with **2** show a singlet for the azomethine-H at ~8.95 ppm, not exchangeable with D_2O, and a singlet at ~12.2 ppm for the hydrazone group, exchangeable with D_2O. NMR spectral data of some representative derivatives are given in the experimental part. In the MS all compounds gave molecular ion peaks. For **4a-o** a common fragmentation pattern with m/z = 263, which with the exception of **4m**, **4n** forms the base peak, corresponding to the loss of Ar-CH=NNH' fragment and m/z = 119 of low intensity corresponding to the cleavage of the 2-methylimidazo[1,2-a]pyridine ring was always observed.

The hydrazones are capable of existing in *syn* and *anti* configuration[8]. Examination of the ^{1}H-NMR spectra of **4a-o** in DMSO-d_6 revealed that only one isomer was present in solution. The *Dreiding* models show that in the *anti* configuration the aryl ring was coplanar with the azomethine bonding but in the *syn* configuration, non bonded interaction between the secondary amide proton and ortho protons of the aryl ring inhibited coplanarity of the phenyl ring with the azomethine group[13]. It is likely, therefore, that *anti-syn* isomerization was impeded and the hydrazones **4a-o** had the *anti* configurations. The planarity of representative compounds were calculated by electron absorption spectrometry. The interplanar angel θ between the aryl ring and the adjacent azomethine (-HC=N-) linkage was obtained from the equation according to *Braude* and *Sondheimer*[18].

Results are presented in Table 2. For this purpose the third absorption band corresponding to -HC=N- grouping was used.

Table 2. Selected UV data of some representative compounds

Compound	λmax (nm) for the 3 th absorption band	ε	Interplanar angle θ
4b	302	30 000	12
4c	298	31 106	-
4d	304	31 258	-
4g	298	30 439	25
4h	298	33 200	19
4i	296	37 355	-
4m	304	30 476	25
4n	294	27 350	31
4o	298	37 179	-

Compounds with ortho methyl or nitro, chloro substituents caused a lack of coplanarity of the aryl ring with the adjacent azomethine linkage of approximately 25° for methyl and nitro groups and 12° for the chlorine atom. Methyl and nitro substituents in the meta position also inhibited the coplanarity. The meta chloro substituent showed a coplanarity with the adjacent unsaturated group.

Microbiology

The biological activity was tested against seven representative gram-positive and gram-negative bacterial strains. **2** has no antimicrobial activity. **4a-o** showed notable antimicrobial activity at the concentration of 100 µg/ml against *Escherichia coli*. With the exception of **4a** the derivatives are at least active against two microorganisms. *Micrococcus luteus*, *Bacillus subtilis* and *Proteus vulgaris* were not sensitive against the tested compounds. **4i** was the only substance which was active against four bacteria namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. **4a-o** inhibited particularly the growth of gram-negative strains. For all substances MIC values were 100 µg/ml. Results are presented in Table 3.

Finally no significant structure-activity-relationship between the angle of non-planarity of lipophily and antimicrobial activity was observed. Only the substances which are more hydrophilic proved to be active against only one or two microorganisms. For this class of substances some other factors such as electronegativity can play a role.

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Experimental Part

Mp.: Büchi apparatus according to Dr. Tottoli (uncorrected). - UV-Spectra: Carl Zeiss PMQ II spectrophotometer (methanol). - IR spectra: Perkin-Elmer 1420 spectrophotometer (KBr). - ^{1}H-NMR spectra: Bruker 250 MW (250 MHz). -MS: Varian Mat CH 7A instruments.

4-[(2-Methylimidazo[1,2-a]pyridine-3-yl)azo]benzoic acid hydrazide (**2**)

0.02 mol (6.61 g) **1**[17] were refluxed in ethanol, with 25 ml hydrazine hydrate for 5 h. After staying overnight, separated crystals were filtered off and crystallized from ethanol (yield 75%), m.p. 245°C. $C_{15}H_{14}N_6O \cdot 1/2\ H_2O$ (303.3) calcd. C 59.4 H 4.62 N 27.7 found C 59.6 H 4.80 N 27.6. - IR: 3275; 1640; 1515 cm^{-1}. - ^{1}H-NMR δ (ppm) (DMSO-d_6): 2.75 (s, 3H, CH_3), 4.57 (s, 2H, NH_2), 7.26-8.0 (m, 7H, Ar-H), 9.75 (d, 1H, C-5 imidazo-py.-H), 9.89 (s, 1H, CONH). - MS: m/z (rel.int.): 295 (M+H; 25), 192 (28), 146 (100), 133 (59).

General procedure for the synthesis of **4a-o**

A mixture of the appropriate aldehyde **3a-o** (2 mmol) and **2** (2.2 mmol) in 200 ml ethanol after addition of 0.1 ml conc. H_2SO_4 was heated under reflux for 2 h. After cooling the resulting precipitate was collected, washed with ethanol and crystallized from the same solvent.

NMR Spectroscopy

For **a-n** see Table 1

4a (DMSO-d_6): 2.76 (s, 3H, CH_3), 7.3-8.12 (m, 12H, Ar-H), 8.51 (s, 1H, CH=N), 9.82 (d, 1H, C-5 imidazo-py.-H), 11.96 (s, 1H, NH*).

4i ($CDCl_3$): 2.37 (s, 3H, CH_3), 2.83 (s, 3H, CH_3), 7.12-7.94 (m, 11H, Ar-H), 8.43 (s, 1H, CH=N), 9.73 (d, 1H, C-5 imidazo-py.-H), 10.0 (s, 1H, NH*).

4j (DMSO-d_6): 2.75 (s, 3H, CH_3), 6.11 (s, 2H, -CH_2-), 7.0-8.11 (m, 10H, Ar-H), 8.41 (s, 1H, CH=N), 9.80 (d, 1H, C-5 imidazo-py.-H), 11.88 (s, 1H, NH*).

4n (DMSO-d_6): 2.78 (s, 3H, CH_3), 7.37-8.18 (m, 11H, Ar-H), 8.59 (s, 1H, CH=N), 9.82 (d, 1H, C-5 imidazo-py.-H), 12.22 (s, 1H, NH*).

* Exchangeable with D_2O

Microbiology

In vitro antimicrobial activity was tested against three gram-positive (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 9341) and four gram-negative (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* UC 57, *Pseudomonas aeruginosa* ATCC 10145, *Proteus vulgaris* NHB 60201) bacteria. Sterile nutrient agar medium (2%, pH 7.2) was melted and allowed to cool to 48°C. Each 100 ml was inoculated with 1 ml of 24 h broth culture and 15 ml was

Table 3. Microbiological activity of **4a-o** against different bacteria

	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**	**4k**	**4l**	**4m**	**4n**	**4o**
Staphylococcus aureus	-	+	+	+	-	+	-	+	+	+	-	-	+	+	-
Micrococcus luteus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Pseudomonas aeruginosa	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Proteus vulgaris	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

transferred to sterile Petri dishes of 10 cm diameter. After the solidifaction with sterile metal cylinders four holes of 9 mm diameter were cut in agar. Each hole was filled with 0.2 ml solutions containing 100; 10; 1 µg/ml of the tested compound. A control for the solvent was included for each organism. After evaporation of the solvent Petri dishes were incubated at 37°C for 24 h. The average inhibition zone diameters were measured for each compound.

References

1 Ng.Ph. Buu-Hoi, Ng.D. Xuong, Ng.H. Ham, F. Binon, and R. Royer, J. Chem. Soc. *1953*, 1358.

2 C.R. Craig, Arch. Int. Pharmacodyn. Thér. *165*, 328 (1967); C.A. *66*, 103795e (1967).

3 V.S. Jolly and V. Singal, J. Indian Chem. Soc. *58*, 724 (1981); C.A. *95*, 115406 r (1981).

4 V.S. Jolly and P. Ittyerah, Agra Univ. J. Res. Sci. *28*, 7 (1979); C.A. *95*, 150057 n (1981).

5 N.S. Habib and M.A. Khalil, J. Pharm. Sci. *73*, 982 (1984).

6 S. Rollas, S. Büyüktimkin, N. Büyüktimkin, M. Ülgen, and E. Yemni, Pharmazie *43*, 511 (1988).

7 R.J. Bochis, L.E. Olen, F.S. Waksmunski, H. Mrozik, P. Eskola, P. Kulsa, G. Wilks, J.E. Taylor, J.R. Egerton, D.A. Ostling, and G. Olson, J. Med. Chem. *24*, 1518 (1981).

8 J.R. Dimmock, J.M. Brenner, and O.A. Phillips, Pharmazie *42*, 376 (1987).

9 D.W. Adamson, P.A. Barrett, J.W. Billinghurst, A.W. Green, and T.S.G. Jones, Nature (London) *168*, 204 (1951).

10 W.H. Laarhoven, R.J.F. Nivard, and E. Havinga, Experientia *17*, 214 (1961).

11 A.J.H. Smith and F. Went, Proc. Acad. Sci. Amsterdam *38*, 852 (1935); C.A. *30*, 26008 (1936).

12 R. Willette, Amer. J. Pharm. Educ. *34*, 662 (1970).

13 J.R. Dimmock, G.B. Baker, and W.G. Taylor, Canad. J. Pharm. Sci. *7*, 100 (1972).

14 J.K. Seidel and K.J. Schaper, Chemische Struktur und biologische Aktivität von Wirkstoffen, p. 257, Verlag Chemie, Weinheim, New York, 1979.

15 S. Büyüktimkin, J. Fac. Pharm. Istanbul, *21*, 26 (1985); C.A. *107*, 51388t (1987).

16 S. Büyüktimkin, J. Fac. Pharm. Istanbul, *21*, 37 (1985); C.A. *107*, 51389u (1987).

17 Z. Cesur and N. Ergenç, J. Fac. Pharm. Istanbul, in press (1989).

18 E.A. Braude and F. Sondheimer, J. Chem. Soc. *1955*, 3754.

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