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Antibacterial and Antifungal Activities of Nitroxoline Mannich Bases

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Abstract. The *in vitro* activity of nitroxoline and its Mannich bases against bacteria and fungi was investigated. Nitroxoline and its derivative with diisopropylamine as an amino component, exhibited the highest antimicrobial activity. The optimum hydro/lipophilic properties (log P), both for antibacterial and antifungal activity, are about log P values of 2. The least effective compound was this nitroxoline Mannich base which has diethanolamine as an amino component.

Introduction

Many derivatives of 8-hydroxyquinoline (oxine) have been tested for antibacterial [1–4, 11] and antiviral [9] activity. Oxine itself is known to be active against bacteria and fungi in very high dilution. A series of papers by *Albert et al.* [1, 2], dealing with oxine derivatives discuss the problems of antibacterial activity within a broad spectrum of compounds and influences of chemical constitutions on biological activities.

Both gram-positive and gram-negative bacterial strains are sensitive to 5-nitro-8-hydroxyquinoline (nitroxoline). This compound is particularly useful as an urogenital antiseptic because the resistance of microorganisms towards nitroxoline has not been observed [3]. Physicochemical properties of nitroxoline and its antimicrobial activity against several bacterial strains have been described by *Paljk et al.* [6, 8].

In the present work the results reflecting antibacterial and antifungal activities of structurally related nitroxoline derivatives are given. The compounds included in this study are Mannich bases of 5-nitro-8-quinolinole with various primary and secondary amines as amino components.

Materials and Methods

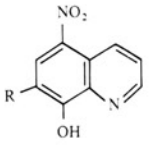
Materials. Nitroxoline 1 and nitroxoline Mannich bases 2–9 (table I) were prepared according to the procedure described in one of our earlier papers [7].

Test Microorganisms. All compounds given in table I were subjected to antibacterial and antifun-

gal screening against the following test microorganisms: *Sarcina lutea* (ATCC 9341), *Sarcina lutea* (FDA 1001), *Streptococcus faecalis* (ATCC 8043), *Staphylococcus albus, Staphylococcus aureus* (SG 511), *Staphylococcus aureus* (ATCC 5048), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 6538), *Micrococcus flavus* (ATCC 10240), *Micobacterium smegmatis, Brucella bronchiseptica* (ATCC 4617), *Klebsiella pneumoniae* (ATCC 10031), *Escherichia coli* (99-1), *Escherichia coli* (ATCC 10536), *Escherichia coli mutaflor, Candida albicans* and *Candida monosa.*

Assay. The diffusion technique generally used for antibiotic screening was adopted both for growth inhibition studies and determination of the minimum inhibitory concentration (MIC). Stock solutions (1 mg/ml) of the compounds were prepared in dimethylsulfoxide/phosphate buffer pH 7.4 (1:1). Working solutions of 0.5 and 0.25 mg/ml were prepared by dilution with phosphate buffer pH 7.4. The MICs were determined applying the oup-plate technique employing three bacterial strains against which the tested compounds showed the highest antibacterial activity, i.e. *M. flavus* (ATCC 10240), *K. pneumoniae* (ATCC 10031) and *S. aureus* (ATCC 5048).

Table I. Nitroxoline and nitroxoline Mannich bases



Compound No.	symbol	R	MW
1	5-NOK	H	190.20
2	5-NOK-Mo	$-CH_2N$ O	289.28
3	5-NOK-Pip	$-CH_2N$	287.31
4	5-NOK-Pyr	$-CH_2N$	273.30
5	5-NOK-DEyA	$-CH_2N(C_2H_5)_2$	275.31
6	5-NOK-DEA	$-CH_2N(CH_2CH_2OH)_2$	307.31
7	5-NOK-DPA	$-CH_2N[CH(CH_3)_2]_2$	303.37
8	5-NOK-MePzin	$-CH_2N$ NCH_3	302.34
9	5-NOK-EA·HCl	$-CH_2NHCH_2CH_2OH \cdot HCl$	336.25

Results and Discussion

Preliminary values for growth inhibition of different microorganisms treated with nitroxoline and its Mannich bases are given in table II.

The largest inhibition zones were ob-

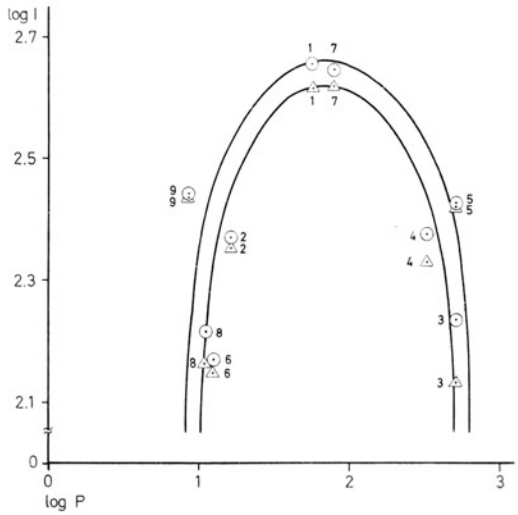


Fig. 1. Parabolic dependence of biological activity (I = inhibition zone) on partition coefficients (log P). O = *C. albicans;* △ = *C. monosa;* 1 = 5-NOK; 2 = 5-NOK-Mo; 3 = 5-NOK-Pip; 4 = 5-NOK-Pyr; 5 = 5-NOK-DEyA; 6 = 5-NOK-DEA; 7 = 5-NOK-DPA; 8 = 5-NOK-MePzin; 9 = 5-NOK-EA·HCl.

served with nitroxoline and its diisopropylamino Mannich base. 5-Nitro-8-hydroxyquinoline inhibited the most strongly the growth of tested fungi, *C. albicans* and *C. monosa*. Furthermore, plotting the values for calculated partition coefficients (log P) according to *Hansch and Leo* [5] against the observed antifungal activity, reflects typical parabolic dependence between these two parameters (fig. 1).

The same effect was observed with compound 7. *S. lutea* (FDA 1001), *S. aureus* (ATCC 5048), *M. flavus* (ATCC 10240) and *M. smegmatis* were the most sensitive bacterial strains towards nitroxoline and its diisopropylamino derivative. Relatively larger inhibition zones were observed in the presence of nitroxoline than in the presence of its Mannich base 7, when incubated with *S. aureus* (ATCC 5048), *S. lutea* (FDA 1001) and *M. flavus* (ATCC 10240), but reverse effect was observed by *M. smegmatis*. Comparative values for inhibition zones referring to fungi do not differ significantly from those obtained on bacteria.

B. bronchiseptica (ATCC 4617) was resistant to any of the tested compounds. Very poor inhibition of *S. faecalis* (ATCC 8043) was observed in the presence of nitroxoline 1 and its diisopropylamino Mannich base 7, whereas other derivatives did not cause any observable effect.

S. lutea (ATCC 9341), *S. albus*, *S. aureus* (SG 511), *S. aureus* (ATCC 6538), and *S. epidermidis* (ATCC 12228) were affected more or less with all nitroxoline derivatives, but relative sizes of inhibition zones prove, once again, that the strongest antibacterial agents are compounds 1 and 7. Nitroxoline Mannich base with diethylamine as amino component 6, exhibited only a poor growth inhibition of *S. aureus* (SG 511), *S. aureus* (ATCC 5048), *S. aureus* (ATCC 6538), *S. epidermidis* ATCC 12228) and *M. flavus* (ATCC 10240). Other tested microorganisms were only poorly inhibited by compound 6. Based upon these preliminary results the most interesting compounds were selected for further studies in order to determine relative sensitivities of some microorganisms (fig. 2). MIC determinations were carried out employing three bacterial strains, *M. flavus* (ATCC 10240), *K. pneumoniae* (ATCC 10031) and *S. aureus* (ATCC 5048) and using nitroxoline and its 5 derivatives. Corresponding MIC values are given in table III.

The concentration of only 3.1 μg/ml of nitroxoline and its diisopropylamino Mannich base (1 and 7) are sufficient to cause inhibition of *K. pneumoniae* (ATCC 10031) and *S. aureus* (ATCC 5048). *M. flavus* (ATCC 10240) seems to be slightly more resistant towards compounds 1 and 7, thus the MIC values are double in comparison with MIC for *K. pneumoniae* (ATCC 10031) and *S. aureus* (ATCC 5048).

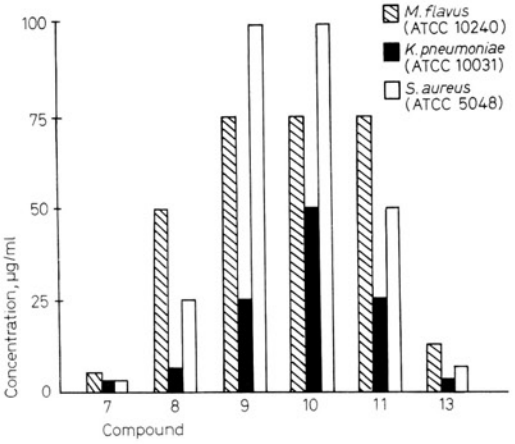


Fig. 2. Relative sensitivity of some microorganisms treated with quinoline Mannich bases.

Table II. Antibacterial and antifungal activities of quinoline Mannich bases (diameters of inhibition zones, mm × 10)

Compound	*S. lutea* (ATCC 9341)		*S. lutea* (FDA 1001)		*S. faecalis* (ATCC 8043)		*S. albus*		*S. aureus* (SG 511)		*S. aureus* (ATCC 5048)		*S. epidermidis* (ATCC 12228)		*S. aureus* (ATCC 6538)	
1	329	340	381	440	261	281	363	383	351	368	381	403	386	403	353	371
2	218	236	235	272	–	–	192	212	285	293	213	228	261	271	183	207
3	163	196	203	238	–	–	±[1]	160	249	264	238	256	212	225	175	205
4	162	188	175	206	–	–	±	175	249	264	228	245	222	236	–	–
5	176	195	210	246	–	–	162	189	255	271	259	273	255	271	±	195
6	–	–	±	175	–	–	–	–	160	180	155	205	178	160	–	156
7	306	323	377	393	248	262	354	370	320	340	369	385	376	398	330	340
8	135	137	133	171	±	±	123	133	220	230	185	208	167	152	131	133
9	165	187	177	218	–	–	193	219	238	256	255	283	168	206	155	177
Concentration[2]	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2

[1] ± = minimally observable inhibition, and – = no inhibition.
[2] T_2 = 0,5 mg/ml, and T_1 = 0,25 mg/ml.

Table III. MIC values for nitroxoline and its Mannich bases tested against various bacteria

Compound	MIC values, μg/ml		
	M. flavus (ATCC10240)	*K. pneumoniae* (ATCC10031)	*S. aureus* (ATCC5048)
1	6.25	3.1	3.1
2	50.00	6.25	25.0
3	75.0	25.0	100.0
4	75.0	50.0	100.0
5	75.0	25.0	50.0
6	125.0	75.0	250.0
7	12.5	3.1	6.3
8	125.0	75.0	100.0
9	125.0	50.0	250.0

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M. flavus (ATCC 10240)		*M. smegmatis*		*B. bronchiseptica* (ATCC 4617)		*K. pneumoniae* (ATCC 10031)		*E. coli* (99-1)		*E. coli* (ATCC 10536)		*E. coli mutaflor*		*C. albicans*		*C. monosa*	
331	371	355	415	–	–	321	335	300	345	355	390	290	303	429	455	403	420
195	250	223	235	–	–	251	253	235	255	300	313	225	245	207	236	208	225
205	220	193	205	–	–	208	223	202	243	185	252	205	230	148	172	121	135
180	252	197	215	–	–	203	220	±	245	210	280	180	205	174	243	136	214
188	251	310	350	–	–	235	249	193	270	258	340	233	243	220	273	223	263
164	195	–	–	–	–	–	–	–	223	–	235	–	–	–	±	–	148
320	364	378	420	–	–	307	319	255	315	327	375	271	288	420	445	403	419
168	239	–	–	–	–	115	115	190	236	170	265	±	±	152	165	140	146
180	205	–	–	–	–	222	225	203	230	288	310	220	188	253	278	233	276
T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2

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