Pharmaceutical Chemistry Journal, Vol. 28, No. 1, 1994

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NEW DERIVATIVES OF 1,4-DI-N-OXIDES OF QUINOXALINE

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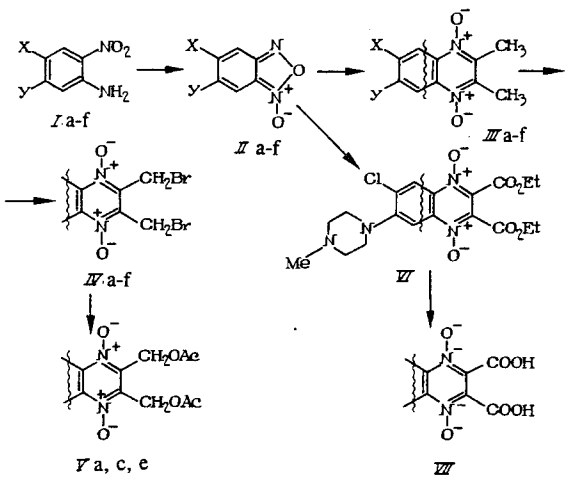
UDC 615.281:547.863.11].012.1.07

In the series of derivatives of 1,4-di-N-oxides of quinoxaline, compounds are known which exhibit high antibacterial activity: the drugs quinoxaline and dioxidine, and also analogs containing fluorine and chlorine atoms in the 6 position [1-3].

With the goal of searching for new drugs with a broad spectrum of antibacterial action, it was of interest to synthesize and study the biological activity of compounds containing chlorine or fluorine atoms in the 6 and 7 positions. Of special interest, in our opinion, was synthesis of compounds with chlorine or fluorine atoms in the 6 position and a substituted amino group in the 7 position, since an analogous moiety is found in the structure of antibacterial drugs of the quinolone carboxylic acid series [4].

We obtained compounds (V) (analogs of the drug quinoxidine) from benzofuroxanes (II). The starting compounds IIa-c were synthesized by oxidation of 4,5-substituted nitroanilines (Ia-c) by sodium hypochlorite in a water–alcohol base medium. When the synthesis of benzofuroxane IIg was attempted under these conditions, substitution of the fluorine by an ethoxy group occurred and instead of compound IIg, we isolated 5-ethoxy-6-chlorobenzofuroxane IIf. Accordingly, for synthesis of benzofuroxanes IId, e we used the method of pyrolysis of azides.

The benzofuroxanes IIa-f were reacted with methyl ethyl ketone in the presence of ammonia or amine; the di-N-oxides of the quinoxalines formed (IIIa-f) were brominated, and the bis-bromomethyl derivatives obtained (IVa, c-e) were reacted with triethylammonium acetate with formation of diacetates (Va, c-e). We could not substitute the bromine atoms by acetoxy groups in the di-N-oxide of bis-bromomethylquinoxaline (IVb) due to the low stability of this compound, isolated only in the form of the monobromohydrate.



I—V: a) X=Y=Cl; b) X=Cl, Y=N(CH$_2$CH$_2$)$_2$NMe; c) X=Cl, Y=N(CH$_2$CH$_2$)$_2$O; d) X=F, Y=Cl; e) X=Y=F; f) X=OEt, Y=Cl.

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0091-150X/94/2801-0017$12.50

TABLE 1. Physicochemical Properties of Synthesized Compounds

Compound	Yield, %	m.p., °C (solvent)	Empirical formula
IIb	71	115–6 (ethanol)	$C_{11}H_{13}ClN_4O_2$
IIc	82	128–30 (ethanol)	$C_{10}H_{10}ClN_3O_3$
IId	95	82–4 (ethanol)	$C_8H_5ClFN_2O_2$
IIe	52	56–8 (ethanol)	$C_8H_5F_2N_2O_2$
IIf	47	106–8 (ethanol)	$C_9H_7ClN_2O_3$
IIIa	91	228–9 (ethanol)	$C_{10}H_6Cl_2N_2O_2$
IIIb	87	193–5 (benzene)	$C_{15}H_{19}ClN_4O_2$
IIIc	62	191–3 (ethanol)	$C_{14}H_{16}ClN_3O_3$
IIId	60	209–11 (DMF)	$C_{10}H_5ClFN_2O_2$
IIIe	95	242–4 (ethanol)	$C_{10}H_6F_2N_2O_2$
IIIf	44	220–2 (acetone)	$C_{12}H_{13}ClN_2O_3$
IVa	80	212–4 (chloroform)[1]	$C_{10}H_6Br_2Cl_2N_2O_2$
IVb	21	200–2 (ethanol)	$C_{15}H_{18}Br_2ClN_4O_2$
IVc	24	130–2 (ethanol)	$C_{14}H_{15}Br_2ClN_3O_3$
IVd	75	183–5 (benzene)	$C_{10}H_6Br_2ClFN_2O_2$
IVe	76	179–81 (benzene)	$C_{10}H_6Br_2F_2N_2O_2$
IVf	70	194–5 (DMF)	$C_{12}H_{11}ClBr_2N_2O_3$
Va	77	201–3 (acetone)	$C_{14}H_{17}Cl_2N_2O_6$
Vb	26	194–5 (acetone)	$C_{16}H_{20}ClN_3O_7$
Vd	76	168–70 (ethylacetate)	$C_{14}H_{17}ClFN_2O_6$
Ve	70	152–4 (ethanol)	$C_{14}H_{17}F_2N_2O_6$
VI	43	129–31 (CCl_4 — PE = 1:1)	$C_{19}H_{23}ClN_4O_6$
VII	68	157–9	$C_{15}H_{15}ClN_4O_6$

By reaction of benzofuroxane IIb with the sodium salt of oxaloacetic acid we synthesized the 1,4-di-N-oxide of the diethyl ester of 6-chloro-7-(4′-methylpiperazinyl-1′)quinoxaline-2,3-dicarboxylic acid (VI), upon base hydrolysis of which we obtained the corresponding acid VII.

The structure of all the newly synthesized compounds were unambiguously confirmed by NMR and mass spectral data.

EXPERIMENTAL (CHEMICAL)

The mass spectra of the synthesized compounds were obtained on the MAT-112 spectrometer, ionizing potential 50 eV, ionization chamber temperature 140°C; the ^1H NMR spectra were obtained on the XL-200 spectrometer, internal standard TMS.

The physicochemical properties of the synthesized compounds are presented in Table 1. The elemental analysis results correspond to the calculated values.

5,6-Dichlorobenzofuroxane (IIa) was obtained by the familiar method in [5].

5-Chloro-6-(4′-methylpiperazinyl-1′)benzofuroxane (IIb). 5.69 g (21 mmoles) nitroaniline Ib was added to a solution of 14 g KOH in 175 ml ethanol, stirred at 35-40°C for 30 min, cooled down to 5°C. Then 322 ml 6% aqueous sodium hypochlorite solution was added at a rate such that the temperature of the reaction mass did not go above 22°C. It was held at this temperature for 1 h; the precipitate was filtered, washed with water, and recrystallized.

5-Chloro-6-morpholinobenzofuroxane (IIc) and 5-Ethoxy-6-chlorobenzofuroxane (IIf). Obtained analogously to compound IIb.

5-Fluoro-6-chlorobenzofuroxane (IId). A solution of 1.79 g (9 mmoles) nitroaniline Id in 15 ml acetic acid was added dropwise to a solution of 0.69 g (10 mmoles) $NaNO_2$ in 15 ml concentrated H_2SO_4 at a rate such that the temperature of the reaction mass did not go above 30°C; this was cooled down to 5°C and held at this temperature for 1 h. It was poured over ice and a solution of 0.58 g (9 mmoles) sodium azide in 5 ml water was added. The precipitate was filtered, dried under vacuum for 2 h, and dissolved in 10 ml acetic acid. The solution was boiled for 1 h, cooled, and decanted into water; the precipitate was filtered and recrystallized.

5,6-Difluorobenzofuroxane (IIe). Obtained analogously to compound IId.

1,4-Di-N-oxide of 2,3-Dimethyl-6,7-dichloroquinoxaline (IIIa). 3.33 g (16 mmoles) benzofuroxane IIa was added to a solution of 1.24 g (17 mmoles) methyl ethyl ketone in 5 ml DMF. This was heated up to 40°C and then 0.3 ml 25% aqueous ammonia solution was added. The reaction mass was heated up to 55-60°C, stirred at this temperature for 1 h, and cooled. The precipitate was filtered, washed with water, and recrystallized.

Di-N-oxides of quinoxalines (IIIc, d, f) were obtained analogously to compound IIIa.

1,4-Di-N-oxide of 2,3-Dimethyl-6-chloro-7-(4′-methylpiperazinyl-1′)quinoxaline (IIIb). 3.74 g (14 mmoles) benzofuroxane IIb was added to a solution of 1.06 g (15 mmoles) methyl ethyl ketone in 4 ml DMF. This was heated up to 40°C and 0.3 ml 25% aqueous ammonia solution was added. The reaction mass was heated up to 55-60°C, stirred at this temperature

TABLE 2. Antibacterial and Antifungal Activity of Synthesized Benzofuroxanes and Di-N-oxides of Quinoxalines *in Vitro*

Compound	MIC, μg/ml							
	St. aureus ATCC 6538-p	Bac. subtilis ATCC 6633	E. coli ATCC 25922	Pr. vulgaris ATCC 6896	Ps. aeruginosa ATCC 27853	M. canis 3/83	C. albicans 885–653	Tr. gypseum 5/85
IIb	3,9	3,9	15,6	>250	>250	125	31,2	125
IIc	1,0	7,8	>250	>250	>250	3,9	15,6	15,6
IId	3,9	15,6	62,5	>250	>250	7,8	31,2	15,6
IIe	31,2	62,5	62,5	>250	>250	7,8	62,5	7,8
IIf	2,0	3,9	>250	>250	>250	15,6	15,6	7,8
IIIa	250	>250	>250	>250	>250	>250	>250	>250
IIIb	>250	>250	>250	>250	>250	>250	>250	>250
IIIc	>250	>250	>250	>250	>250	>250	>250	>250
IIId	125	125	>250	>250	>250	>250	>250	>250
IIIe	250	250	>250	>250	>250	>250	>250	>250
IIIf	250	>250	>250	>250	>250	>250	>250	>250
IVa	125	>250	>250	>250	>250	125	62,5	125
IVc	31,2	31,2	>250	>250	>250	15,6	31,2	7,8
IVd	7,8	3,9	250	250	>250	7,8	15,6	7,8
IVe	125	250	250	250	>250	7,8	31,2	15,6
IVf	62,5	31,2	>250	>250	>250	125	125	31,2
Va	125	>250	>250	>250	>250	>250	>250	>250
Vd	7,8	3,9	>250	>250	>250	>250	>250	>250
VI	31,2	31,2	>250	>250	>250	>250	>250	>250
VII	>250	>250	>250	>250	>250	>250	>250	>250

for 1 h, and cooled down to room temperature. 20 ml water was added and it was filtered. The filtrate was extracted with chloroform and the extract was dried over Na_2SO_4. After evaporation under vacuum, the residue was recrystallized.

1,4-Di-N-oxide of 2,3-Dimethyl-6,7-difluoroquinoxaline (IIIe). 0.47 g (6.4 mmoles) butylamine was added to a solution of 1 g (5.8 mmoles) benzofluroxane IIe in 2.6 g (32 mmoles) methyl ethyl ketone. This was stirred for 1 h at room temperature and 10 ml petroleum ether was added. The precipitate was filtered and recrystallized.

1,4-Di-N-oxide of 2,3-bis-Bromomethyl-6,7-dichloroquinoxaline (IVa). A mixture of 6.27 g (24 mmoles) di-N-oxide IIIa, 10 ml DMF, and 10 ml CH_2Cl_2 was heated up to 53°C. 11.65 g (73 mmoles) bromine was added dropwise. It was stirred at this temperature for 3 h and then cooled down to room temperature. The reaction mass was diluted with 60 ml water, the residue was filtered, washed with water, and recrystallized.

Di-N-oxides of quinoxalines (IVb-f) were obtained analogously to compound IVa. The di-N-oxide of quinoxaline IVb was isolated as the bromohydrate by dilution of the reaction mass with methylene chloride. The precipitate was filtered and recrystallized.

1,4-Di-N-oxide of 6,7-Dichloro-2,3-bis(acetoxymethyl)quinoxaline (Va). 2.89 g (29 mmoles) triethylamine was added dropwise to a solution of 3.43 g (57 mmoles) glacial acetic acid in 40 ml acetone at 20-25°C. This was held for 15 min and then 4 g (9.6 mmoles) di-N-oxide IVa was added. The mixture was boiled for 1 h and then cooled down to room temperature. The precipitate was filtered, washed with water, and recrystallized.

Di-N-oxides of quinoxalines (Vc-e) were obtained analogously to compound Va.

1,4-Di-N-oxide of the Diethyl Ester of 6-Chloro-7-(4′-methylpiperazinyl-1′)quinoxaline-2,3-dicarboxylic Acid (VI). A mixture of 1.41 g (5.25 mmoles) benzofuroxane IIb, 0.26 g (5.47 mmoles) of the sodium salt of the diethyl ester of oxaloacetic acid, and 0.07 g (1.3 mmoles) ammonium chloride in 10 ml isopropanol was stirred at room temperature for 4 h and then evaporated under vacuum. 10 ml water was added and it was extracted with chloroform. The extract was washed with water twice and dried over Na_2SO_4. The solution was evaporated down to a volume of 15 ml and diluted with 30 ml hexane. The precipitate was filtered and recrystallized.

1,4-Di-N-oxide of 6-Chloro-7-(4′-methylpiperazinyl-1′)quinoxaline-2,3-dicarboxylic Acid (VII). A mixture of 0.63 g (1.43 mmoles) ester VI and 12.6 ml 2.5 N NaOH was stirred at room temperature for 40 min and filtered. The solution was acidified by 2.5 N HCl to pH 1. The precipitate was filtered and washed with petroleum ether.

EXPERIMENTAL (BIOLOGICAL)

The antibacterial and antifungal activity of the synthesized compounds were studied *in vitro* by twofold dilution series on a liquid nutrient medium: Hottinger's broth (for the bacteria) and Saburo's medium (for the fungi). As the test bacteria we used *S. aureus* ATCC 6538-p, *Bac. subtilis* ATCC 6633, *E. coli* ATCC 25922, *Ps. aeruginosa* ATCC 27853; in experiments with fungi, test strains of *M. canis* 3/83, *Tr. gypseum* 5/85, and *C. albicans* ATCC 885-653. The microbial load in experiments with bacteria was $1 \cdot 10^5$ CFU/ml; with the fungi, $1 \cdot 10^6$ CFU/ml. The bacteria were incubated at 37°C for 18 h; the fungi, at 25°C for 24 h in experiments with *C. albicans* and for 5 days with the dermatophyte fungi *M. canis* and *Tr. gypseum*. The compounds were tested at a concentration of 250 μg/ml and lower.

In the *in vivo* experiments, we studied the chemotherapeutic efficacy of eight compounds: IIIa, b, d, e, IVa, Va, VI, VII. The experiments were done on models of septicemia in mice induced by *Ps. aeruginosa* 165, *S. typhi* 4446, *St. aureus* 178. The infectious dose for intraperitoneal injection was $1 \cdot 10^6$ CFU for *Ps. aeruginosa*, $1 \cdot 10^7$ CFU for *S. typhi*, and $1 \cdot 10^8$ CFU for *St. aureus*. The compounds were injected in doses of 200-400 mg/kg; the compounds IIIa, b, d, e, IVa, Va were used *per os*, while the compounds VI and VII were used subcutaneously. The results of the experiments were assessed from the survival rate of the animals on the 10th day of the experiment compared with the control group of mice.

As we see from Table 2, compounds IIc, d, f, IVc exhibited pronounced activity *in vitro* (MIC 2-7.8 μg/ml) with respect to the Gram-positive bacteria (*St. aureus, Bac. subtilis*) and pathogenic fungi, while compounds IIe and IVe were active only against the fungi. Compounds Vd and IIb were active with respect to the Gram-positive bacteria, while compounds IVf and IVc were moderately active with respect to *St. aureus, Bac. subtilis* and the fungi. None of the compounds inhibited the growth of Gram-negative bacteria, except for IIb and IIe, which at a concentration of 15.6-62.5 μg/ml displayed moderate activity with respect of *E. coli*.

The components studied *in vivo* displayed very weak therapeutic action or were inactive for the experimental septicemia in mice induced by *Ps. aeruginosa, S. typhi*, and *St. aureus*.

Thus, in the *in vitro* experiments, we established that the benzofuroxanes IIb-f, containing halogen atoms in the 5 and 6 positions and substituted amino groups, exhibit pronounced activity with respect to Gram-positive bacteria and pathogenic fungi.

For derivatives of di-N-oxides of quinoxalines, *in vitro* we observe some dependence of the biological action of these compounds on the type of substituent. Thus, none of the investigated di-N-oxides of quinoxalines with methyl substituents in the 2 and 3 positions display antibacterial and antifungal activity. Introduction of bromomethyl substituents into the 2 and 3 positions of the di-N-oxides of quinoxalines leads to the appearance of pronounced or moderate activity with respect to pathogenic fungi. Only the bis-bromomethyl derivatives with an asymmetric structure, having different substituents in the 6 and 7 positions, exhibit activity with respect to Gram-positive bacteria.

Of the two investigated bis-acetoxymethyl derivatives of di-N-oxides of quinoxalines, only the compound with an asymmetric structure (Vd) displayed pronounced activity with respect to Gram-positive bacteria.

None of the studied compounds were chemotherapeutically effective in the models of septicemia in mice induced by *Ps. aeruginosa, S. typhi*, and *St. aureus*.

REFERENCES

1. A. S. Elina, L. G. Tsyrul'nikova, and O. Yu. Magidson, USSR Inventor's Certificate 207,917; Izobreteniya, No. 3, 19 (1968).
2. K. A. Belozerova, A. S. Elina, O. Yu. Magidson, et al., USSR Inventor's Certificate 428,626; Otkrytiya, No. 18, 151 (1975).
3. I. S. Musatova, A. S. Elina, E. N. Padeiskaya, et al., Khim.-farm. Zh., No. 8, 934-938 (1982).
4. R. G. Glushkov, I. B. Levshin, N. B. Marchenko, and E. N. Pedeiskaya, Khim.-farm. Zh., No. 9, 1048-1064 (1984).
5. F. B. Mallory, S. L. Manatt, and C. S. Wood, J. Am. Chem. Soc., **87**, No. 23, 5433-5438 (1965).