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SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF THE MAIN METABOLITE OF AFOBAZOLE AND ITS ANALOGS

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The methods and conditions for the synthesis of the main metabolite of the selective anxiolytic afobazole, 2-[2-(3-oxomorpholin-4-yl)ethylthio]-5-ethoxybenzimidazole, and its analogs are developed. It is shown that the main metabolite demonstrates an anxiolytic effect in a dose range similar to that of afobazole. It is found that the psychopharmacological profile of the other synthesized compounds (analogs) is characterized by a less pronounced anxiolytic component.

Key words: afobazole, synthesis of metabolites, metabolite analogs, pharmacological activity.

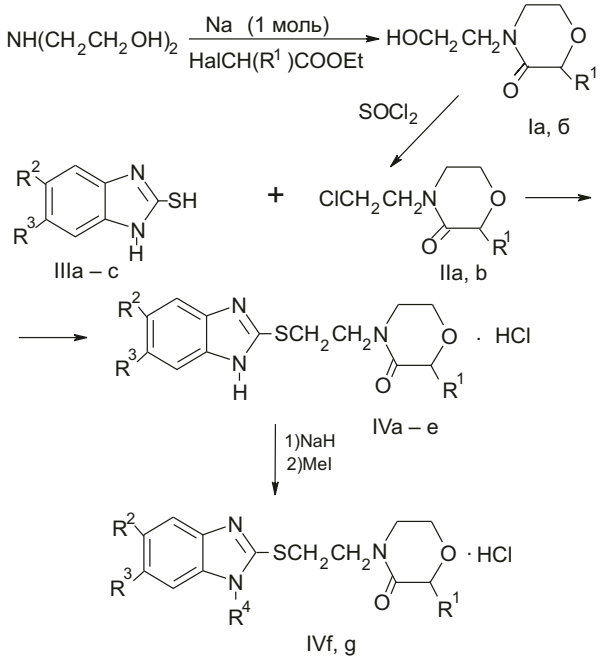
Many 2-mercaptobenzimidazole derivatives that exhibit various types of biological activity such as bacteriostatic, insecticidal, anthelminthic, antiulcer, anti-inflammatory, analgesic, etc. have been reported [1 – 4].

Studies of several S-derivatives of 2-mercaptobenzimidazole with a dialkylamino or N-containing heterocycle at the end of an alkyl chain discovered a series of compounds with anxiolytic properties. One of them, 2-(2-morpholinoethylthio)-5-ethoxybenzimidazole dihydrochloride, was incorporated into medical practice as a selective anxiolytic agent in 2005 under the name afobazole [5].

Studies of the pharmacokinetics and metabolism of afobazole using HPLC in combination with mass spectrometry found that one of the main biotransformation products was 2-[2-(3-oxomorpholin-4-yl)ethylthio]-5-ethoxybenzimidazole (**IVb**), which was named metabolite M-11 in a previous publication [6]. This metabolic pathway, oxidation of C-3 of the morpholine ring, agrees with other data in the literature [7].

The goal of the present work was to develop a synthetic method for this metabolite and several related compounds and to study their anxiolytic activity [8]. A route where N-derivatives of morpholin-3-one were used as intermediates turned out to be suitable for the synthesis of compounds of this type. Thus, alkylation of the monosodium salt of diethanolamine by the ethyl esters of chloroacetic or 2-bromopropionic acids and subsequent cyclization produced 4-(2-hydroxyethyl)morpholin-3-one (**Ia**) and 4-(2-hydroxyethyl)-2-methylmorpholin-3-one (**Ib**), respectively, under conditions analogous to those published [9]. Reaction of **Ia** or **Ib** with thionylchloride replaced the hydroxyl by chlorine and synthesized 4-(2-chloroethyl)morpholin-3-one (**IIa**) and 4-(2-chloroethyl)-2-methylmorpholin-3-one (**IIb**) (Scheme)

Scheme



I, **II**: a) R^1 = H; b) R^1 = Me;
III: a) $R^2 = R^3$ = H; b) R^2 = OEt, R^3 = H; c) $R^2 = R^3$ = Me;
IV: a) $R^1 = R^2 = R^3$ = H; b) $R^1 = R^3$ = H, R^2 = OEt; c) R^1 = H,

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TABLE 1. Physicochemical Properties of the Synthesized Compounds

Compound	Yield, %	bp, °C/mm or mp, °C	Empirical formula	PMR spectra, ä, ppm
IIa	64	144 – 146/10	$C_6H_{10}ClNO_2$	3.54 (t, 2H, NC$\underline{H}_2$, morpholone), 3.69 and 3.73 (two m, 4H, $CH_2C\underline{H}_2Cl$), 3.89 (t, 2H, OC$\underline{H}_2CH_2$ morpholone), 4.17 (s, 2H, OC$\underline{H}_2$CO morpholone), ($CDCl_3$)
IIá	54	142 – 144/10	$C_7H_{12}ClNO_2$	1.42 (d, 3H, CH_3), 3.34 and 3.96 (ddd, 1H and ddd, 1H, C$\underline{H}_2$N morpholone), 3.6 – 3.83 (m, 6H, CH_2O morpholone, ClC$\underline{H}_2$C$\underline{H}_2$), 4.18 (q, 3H, C$\underline{H}CH_3$ morpholone) ($CDCl_3$)
IVa	80	182 – 184	$C_{13}H_{15}N_3O_2S$	3.74 (t, 2H, C$\underline{H}_2$N morpholone), 3.82 (t, 2H, C$\underline{H}_2$S), 4.00 (t, 2H, C$\underline{H}_2$N), 4.08 (t, 2H, OC$\underline{H}_2CH_2$ morpholone), 4.22 (s, 2H, OC$\underline{H}_2$CO morpholone), 7.72 – 7.76 (m, 3H, 4H-6H), 7.80 (dd, 1H, 7-H) (D_2O)
IVb	80	178 – 182	$C_{15}H_{20}ClN_3O_3S$	1.64 (t, 3H, $OCH_2C\underline{H}_3$), 3.72 (t, 2H, C$\underline{H}_2$N morpholone), 3.82 (t, 2H, C$\underline{H}_2$S), 3.98 (t, 2H, C$\underline{H}_2$N), 4.05 (t, 2H, OC$\underline{H}_2CH_2$ morpholone), 4.25 (s, 2H, OC$\underline{H}_2$CO morpholone), 4.35 (q, 2H, C$\underline{H}_2CH_3$), 7.30 (d, 1H, 6-H), 7.35 (br.s, 1H, 4-H), 7.74 (d, 1H, 7-H) (D_2O)
IVc	86	236 – 238	$C_{15}H_{20}ClN_3O_2S$	2.50 (s, 6H, $2CH_3$), 3.68 (t, 2H, C$\underline{H}_2$N morpholone), 3.76 (t, 2H, C$\underline{H}_2$S), 3.95 (t, 2H, C$\underline{H}_2$N), 4.04 (t, 2H, OC$\underline{H}_2CH_2$ morpholone), 4.22 (s, 2H, OC$\underline{H}_2$CO morpholone), 7.49 (s, 2H, ArH) (D_2O)
IVd	85	86 – 88	$C_{16}H_{22}ClN_3O_3S$	1.5 (d, 3H, CHC$\underline{H}_3$ morpholone), 1.61 (t, 3H, $OCH_2C\underline{H}_3$), 3.60 and 4.20 (ddd, 1H and ddd, 1H, C$\underline{H}_2$N morpholone), 3.80 – 4.08 (m, 6H, $CH_2C\underline{H}_2O$ morpholone, SC$\underline{H}_2$C$\underline{H}_2$N), 4.3 (q, 2H, OC$\underline{H}_2CH_3$), 4.39 (q, 1H, C$\underline{H}CH_3$ morpholone), 7.25 (dd, 1H, 6-H), 7.26 (d, 1H, 4-H), 7.62 (d, 1H, 7-H) (D_2O)
IVe	87	194 – 196	$C_{16}H_{22}ClN_3O_2S$	1.46 (d, 3H, CHC$\underline{H}_3$ morpholone), 2.5 (s, 6H, $2CH_3$), 3.59 and 4.18 (ddd, 1H and ddd, 1H, CH_2N morpholone), 3.75 – 4.00 (m, 6H, $CH_2C\underline{H}_2O$ morpholone, SC$\underline{H}_2$C$\underline{H}_2$N), 4.30 (q, 1H, C$\underline{H}CH_3$ morpholone), 7.5 (s, 2H, ArH) (D_2O)
IVe	83	132 – 134	$C_{16}H_{22}ClN_3O_2S$	2.59 and 2.61 (s, 3H and s, 3H, $2CH_3$), 3.70 (t, 2H, C$\underline{H}_2$N morpholone), 3.82 (t, 2H, C$\underline{H}_2$S), 3.97 (t, 2H, C$\underline{H}_2$N), 4.00 (s, 3H, NCH_3), 4.10 (t, 2H, OC$\underline{H}_2CH_2$ morpholone), 4.25 (d, 2H, OC$\underline{H}_2$CO morpholone), 7.65 (s, 6H, ArH) (D_2O)
IVg	86	124 – 126	$C_{17}H_{24}ClN_3O_2S$	1.51 (d, 3H, CHC$\underline{H}_3$ morpholone), 2.54 and 2.55 (s, 3H, and s, 3H, $2CH_3$), 2.60 and 4.20 (ddd, 1H and ddd, 1H, C$\underline{H}_2$N morpholone), 3.75 – 4.05 (m, 6H, $CH_2C\underline{H}_2O$ morpholone, SC$\underline{H}$2C$\underline{H}_2$N), 4.00 (s, 3H, NCH_3), 4.30 (q, 1H, C$\underline{H}CH_3$ morpholone), 7.62 (s, 2H, ArH) (D_2O)

$R^2 = R^3 = Me$; d) $R^1 = Me$, $R^2 = OEt$, $R^3 = H$; e) $R^1 = R^2 = R^3 = Me$; f) $R^1 = H$, $R^2 = R^3 = R^4 = Me$; g) $R^1 = R^2 = R^3 = R^4 = Me$.

Condensation of the resulting chloro derivatives (**IIa, b**) with substituted 2-mercaptobenzimidazoles (**IIIa** – **c**) under conditions analogous to those for afobazole production, i.e., refluxing in aqueous alcohol in the presence of NaOH, produced a mixture of a large number of inseparable compounds. Compounds **IVa-e** were synthesized directly as the hydrochlorides by conducting the reaction under milder conditions, refluxing the chloroethylmorpholin-3-ones (**IIa, b**) with 2-mercaptobenzimidazole (**IIIa**) and its analogs (**IIIb, c**) in anhydrous EtOH without base. The N-alkyl derivatives (**IVf, g**) were prepared by alkylation of the corresponding compounds **IVc, e** with MeI in DMF in the presence of NaH. Compounds **IVc** and **IVe** were chosen as an example of the methylation because they had two identical substituents (methyls) in the 5- and 6-positions. This made the benzimidazole system symmetric. Therefore, the N atoms in it were equivalent. The alkylation produced pure **IVf** and **IVg**. If **IVb** or **IVd** with a substituent (ethoxy) only in the

TABLE 2. Effect of **IVb** on Behavior of Balb/c Mice in the Elevated Plus-Maze Test

Drug	*n*	Time, s			Number of entries	
		in center	in open arms	in closed arms	in open arms	in closed arms
Control	15	27.4 ± 1.9	11.7 ± 2.5	258.3 ± 3.3	9.1 ± 0.6	8.0 ± 0.6
0.1 mg/kg	10	38.1 ± 5.5	49.7 ± 9.1*	212.2 ± 6.2*	9.4 ± 0.9	7.8 ± 0.4
0.5 mg/kg	10	36.4 ± 5.7	65.1 ± 8.4*	199.5 ± 5.9*	7.0 ± 0.5	7.2 ± 0.6
1.0 mg/kg	10	42.8 ± 4.7	49.2 ± 5.7*	208.0 ± 5.0*	9.2 ± 0.7	7.9 ± 0.5

* $p < 0.01$ compared with the control.

TABLE 3. Effect of **IVb** on Behavior of Mongrel Mice in the Elevated Plus-Maze Test

Drug	*n*	Time, s			Number of entries	
		in center	in open arms	in closed arms	in open arms	in closed arms
Control	23	20.1 ± 2.2	10.1 ± 3.3	269.5 ± 4.4	4.7 ± 0.6	3.9 ± 0.4
0.1 mg/kg	6	27.0 ± 5.5	20.8 ± 4.0*	252.2 ± 5.2*	6.5 ± 0.9	6.2 ± 0.5*
0.5 mg/kg	10	46.3 ± 8.1*	29.5 ± 10.9*	214.2 ± 11.9*	7.2 ± 0.9*	5.9 ± 0.6*
1.0 mg/kg	10	46.6 ± 9.4*	77.2 ± 29.2*	176.2 ± 28.4*	3.7 ± 0.7	3.2 ± 0.7

* $p < 0.05$ compared with the control.

TABLE 4. Effect of **IVc-g** on Behavior of Mongrel Rats in the Elevated Plus-Maze Test

Group	*n*	Latent time, s	Time, s			Number of entries		
			in center	in open arms	in closed arms	in center	in open arms	in closed arms
Control	32	6.3 ± 1.0	20.9 ± 3.0	19.1 ± 4.2	260.0 ± 6.4	2.1 ± 0.4	1.4 ± 0.3	3.0 ± 0.4
IVc	16	11.2 ± 3.2	33.0 ± 5.7	46.4 ± 18.6	220.8 ± 17.5*	2.9 ± 0.6	1.7 ± 0.5	3.4 ± 0.7
IVd	16	4.9 ± 1.1	19.6 ± 4.8	11.9 ± 4.2	268.5 ± 6.4	1.6 ± 0.4	0.7 ± 0.2	2.6 ± 0.5
IVe	16	6.9 ± 1.6	26.3 ± 4.0	73.6 ± 20.3*	200.1 ± 20.0*	3.3 ± 0.6	3.1 ± 0.9	4.4 ± 0.7
IVf	16	5.7 ± 1.0	30.6 ± 6.8	64.6 ± 23.8	204.8 ± 23.1*	3.1 ± 0.9	1.9 ± 0.5	3.3 ± 0.9
IVg	16	7.6 ± 1.6	38.3 ± 4.4*	27.8 ± 8.4	233.9 ± 11.5*	3.7 ± 0.7*	1.6 ± 0.4	4.3 ± 0.7

* $p < 0.05$ compared with the control.

5-position were used, the alkylation formed in each instance a mixture of two difficultly separated positional isomers because of the asymmetry of the benzimidazole system.

The structures of all prepared compounds were confirmed by elemental analysis and PMR spectra. Resonances in spectra were assigned using double homonuclear resonance.

EXPERIMENTAL CHEMICAL PART

The course of reactions and purity of products were monitored using TLC on Silufol UV-254 plates with detection in UV light and by I_2 vapor. Table 1 presents the physicochemical and spectral characteristics of the synthesized compounds. Elemental analyses of the compounds agreed with those calculated from the empirical formulas.

PMR spectra were recorded at 25°C on a Bruker AC 250 spectrometer with TMS internal standard.

4-(2-Hydroxyethyl)morpholin-3-ones (Ia, b). Metallic Na (1.15 g, 0.05 mol) and freshly distilled diethanolamine (5.25 g, 4.77 mL, 0.05 mol) were dissolved in anhydrous dioxane (20 mL) with heating. The mixture was cooled to room temperature, treated slowly with the ethyl ester of chloroacetic or 2-bromopropionic acid, gradually brought to reflux, refluxed for 1 h, cooled, and filtered to remove NaCl. The precipitate was washed several times with dioxane. The dioxane was distilled off. The remaining oil (**Ia, b**) was used without further purification in the next step.

4-(2-Chloroethyl)morpholin-3-ones (IIa, b). Thionylchloride (23.8 g, 15 mL, 0.2 mol) was dissolved in $CHCl_3$ (20 mL), treated slowly at <30°C with a solution of the oil resulting from the previous step in $CHCl_3$ (15 mL), stirred for 1 h, poured into ice water, washed with cold $NaHCO_3$ solution and H_2O, and dried over Na_2SO_4. The solvent was distilled off. The residue was vacuum distilled.

2-[2-(3-Oxomorpholin-4-yl)ethylthio]benzimidazole hydrochlorides (IVa – e). A mixture of the appropriate 2-mercaptobenzimidazole **III** (0.0025 mol) and 4-(2-chloroethyl)morpholin-3-one (**IVa, b**) (0.003 mol) in anhydrous EtOH (10 mL) was refluxed for 1 h. The solvent was distilled off. The solid was recrystallized from MeOH:Et_2O to afford **IVa** – **e**.

An aqueous solution of **IVb** was treated with $NaHCO_3$ and extracted with EtOAc. The extract was dried over Na_2SO_4. The solvent was distilled off to afford **IVb** base as an oil. PMR spectrum ($CDCl_3$, ä, ppm, *J*/Hz): 1.40 (t, 3H, $C\underline{H}_3CH_2O$), 3.30 (t, 2H, CH_2S), 3.49 (t, 2H, morpholone CH_2N), 3.70 (t, 2H, morpholone $CH_2C\underline{H}_2O$), 4.04 (q, 2H, $O\underline{H}_2CH_3$), 4.21 (s, 2H, morpholone $OC\underline{H}_2CO$), 6.84 (dd, 1H, $J_{6,7} = 2.5$, $J_{4,6} = 8.5$, 6-H), 7.01 (d, 1H, $J_{4,6} = 2.5$, 4-H), 7.43 (d, 1H, $J_{6,7} = 8.5$, 7-H).

1,5,6-Trimethyl-2-[2-(3-oxomorpholin-4-yl)ethylthio] benzimidazole hydrochlorides (IVf, g). Sodium hydride (0.12 g, 0.03 mol, 60%) was added to anhydrous DMF (7 mL), stirred for 30 min, treated with a solution of **IVc** or **IVe** (0.001 mol) in anhydrous DMF (5 mL), stirred for 1 h, treated with MeI (0.1 mL, 0.23 g, 0.002 mol), stirred for 0.5

h, poured into water, and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and filtered. The solvent was distilled off. The solid was dissolved in anhydrous Et_2O and treated with HCl in Et_2O. The resulting precipitate was filtered off and recrystallized from MeOH:Et_2O to afford **IVf** or **IVg**.

EXPERIMENTAL BIOLOGICAL PART

We used male Balb/c and mongrel mice (20 – 22 g) for the study of afobazole metabolite **IVb**. Anxiolytic activity was estimated in an elevated plus-maze (EPM) test [10]. The method consisted essentially of an analysis, on one hand, of the fear response of the animals in an unfamiliar space and height and, on the other, of exploratory activity in a new situation.

The residence time in open and closed maze arms, the time in the maze central square, and the number of entries into the open and closed arms were counted quantitatively. The total observation time of each animal was 5 min.

The anxiolytic activities of **IVc** – **g** were also estimated in EPM tests [11]. We used male mongrel rats (200 – 250 g) in the experiment. The total observation time of each animal was 5 min.

The results were processed statistically using unifactorial dispersion analysis and nonparametric analysis for the independent variables (Mann—Whitney U-test).

RESULTS AND DISCUSSION

Compound **IVb** was injected i.p. 30 min before the experiment at doses of 0.1, 0.5, and 1.0 mg/kg. It stimulated the behavior of mice by increasing the residence time in the open arms and decreasing it in the closed arms (Tables 2 and 3). Furthermore, the number of entries into the open and closed maze arms increased for the mongrel mice (Table 3).

Compounds **IVc, e**,**f, g** were injected i.p. in mongrel rats 30 min before the experiment at a dose of 1.0 mg/kg. They caused a statistically significant reduction of the residence time in the closed maze arms with a simultaneous increase of the residence time in the open arms (the latter was statistically significant only for **IVe**) (Table 4). Furthermore, **IVg** increased reliably both the residence time of animals in the EPM center and the number of passes through it. This may indicate that the exploratory behavior of the animals increased. Compound **IVd** at the studied dose did not affect the behavior of the rats as compared with the control.

Thus, the results of the present investigation demonstrate that **IVb** exhibits anxiolytic activity. The dose range and type of activity of the compound are similar to the pharmacological effect of afobazole [12]. The anxiolytic activities of **IVc, e**,**f, g** were less pronounced than that of **IVb**.

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