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DRUG SYNTHESIS METHODS AND MANUFACTURING TECHNOLOGY

DEVELOPMENT OF SYNTHESIS TECHNOLOGY FOR THE SELECTIVE ANXIOLYTIC DRUG AFOBAZOLE

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The optimum synthetic scheme, methods of obtaining intermediates, and stage-by-stage analytical control techniques have been developed for industrial manufacturing of the new anxiolytic drug afobazole. According to the scheme, 2-nitro-*p*-phenethidine reduction by sodium dithionite is followed by cyclization of the diamine with potassium ethylxanthate. Alkylation of 2-mercapto-5-ethoxybenzimidazole with 4-(2-chloroethyl)morpholine was carried out. Thus, the technology for obtaining afobazole parent substance of pharmacopoeial quality is worked out.

Key words: afobazole parent substance, phenacetin, nitration, sodium dithionite, reduction, cyclization, 2-mercapto-5-ethoxybenzimidazole, chloroethylmorpholine hydrochloride, 2-[2-(morpholino)ethylthio]-5-ethoxybenzimidazole dihydrochloride.

Afobazole (**I**), 2-[2-(morpholino)ethylthio]-5-ethoxybenzimidazole dihydrochloride, is a new original drug created at Zakusov Institute of Pharmacology, RAMS. It exhibits selective anxiolytic and neuroprotective activity [1]. The drug was approved for medical practice (registration number LS-00086, Nov. 3, 2005).

The goal of the present study was to develop the synthesis technology and production control for afobazole substance.

The key compounds in the synthesis of **I** are 2-mercapto-5-ethoxybenzimidazole and chloroethylmorpholine. Moreover, it is known that it is exceedingly difficult to introduce a hydroxyl or alkoxyl into a benzimidazole ring. Therefore, the benzimidazole ring is usually constructed from the corresponding substituted *o*-phenylenediamines for the synthesis of such compounds [2]. We selected available 4-ethoxyacetanilide (phenacetin, **II**) as the starting compound for preparing **I**. Thus, the synthesis of **I** consisted of five steps (Scheme).

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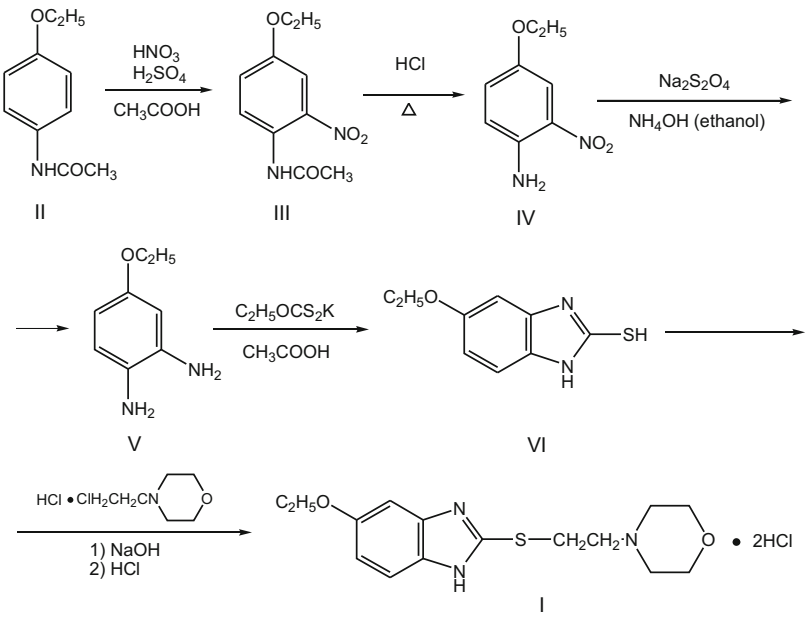
The synthesis of 3-nitrophenacetin (**III**) was reported several times [2 – 4]. We performed nitration of **II** in glacial acetic acid by a mixture of concentrated HNO_3 and H_2SO_4. The product **III** was readily hydrolyzed by refluxing in dilute HCl to 2-nitro-*p*-phenetidine (**IV**) [3].

Various reagents are used to reduce nitro groups in aromatic compounds. Thus, a nitro group was reduced by H_2 in the presence of platinum oxide in order to prepare 2,3-diamino-1,4-dimethoxybenzene [5]. The reduction of 6-nitro-2,5-xylidine by powdered iron in HCl gave the diamine [6], which was also obtained in 60% yield using sodium dithionite in aqueous medium as the reductant [7]. This same method of reduction was used for reductive cyclization in the preparation of imidazo[1,2-*a*]quinoxaline derivatives [8]. Hydrogen sulfide, sodium or ammonium sulfide, and zinc in alkaline medium are often used as reductants. The synthesis of 1-methylbenzimidazolin-2-thione from *N*-methylnitroaniline was reported [9]. The nitro group of the former was reduced by powdered Zn in alkaline medium. Then, the resulting diamine was treated without isolation with CS_2. The target 3,4-diaminophenetole (**V**) was obtained in 83% yield.

Scheme



In industry, nitro groups are usually reduced by H_2 over Raney nickel. Thus, **IV** was reduced in EtOH [10]. After the catalyst was removed, the solvent was evaporated in a stream of CO_2. The solid was crystallized from hydrocarbons to afford **V** in 75% yield (mp 79 – 81°C). The diamine base **V** was a grayish-lilac crystalline powder that was unstable and easily oxidized in air.

We tested various methods for reducing the nitro group in the step for producing **V**. These included H_2 over Raney Ni or in the presence of Pd on C, hydrazine hydrate over Raney Ni, sodium sulfide, and sodium dithionite. As a result, we developed a convenient method for reducing intermediate **IV** by sodium dithionite in alkaline aqueous-alcohol medium that was also applied to the synthesis of **I**. When the reduction was finished, the alcohol solution of **V** was separated and used for the cyclization step (step 4).

Cyclization using CS_2 in alkaline aqueous-alcohol medium was used to prepare 2-mercaptobenzimidazole derivatives [9, 11]. Preparation of 5-substituted-2-mercaptobenzimidazoles via reaction of the corresponding *o*-phenylenediamines with diethyldithiocarbmate in H_2O was reported [12]. Studies in which potassium ethylxanthate was used as the condensing agent for the synthesis of 2-mercaptobenzimidazole and its 5-ethoxy derivative were described [13, 14].

We used the method described in a patent [14] that involved heating diamine **V** in aqueous-alcohol with potassium ethyl- or butylxanthate to synthesize 5-ethoxy-2-mercaptobenzimidazole (**VI**).

Alkylation of 2-mercaptobenzimidazole derivatives is known to occur almost exclusively at the S atom under rather mild conditions. Thus, 2-methylthiobenzimidazole was prepared in 85% yield by shaking 2-mercaptobenzimidazole with methyliodide in aqueous base (1 N) at room temperature [15]. 1-Methyl-2-methylthiobenzimidazole was synthesized in 73% yield under analogous conditions [9]. Alkylation of **VI** by ethylbromide was carried out in anhydrous EtOH at 70°C for 4 h [14]. Removal of solvent afforded 2-ethylthio-5-ethoxybenzimidazole hydrobromide in >90% yield.

We synthesized **I** by alkylation of **VI** with 4-(2-chloroethyl)morpholine hydrochloride in the presence of NaOH in aqueous EtOH.

Crude **I** was purified by recrystallization from anhydrous EtOH at a 1:10 ratio and from propanol-2 at a 1:80 ratio with added activated carbon (5%). The yields were 76.5 and 79%, respectively. The products were dried to constant mass at 90 – 95°C for 6 – 8 h.

The content of residual solvents in the products was determined by GC. Up to 2% solvent was detected in the sample that was recrystallized from propanol-2. These results were confirmed by PMR spectroscopy. The spectrum of this sample taken in D_2O (δ, ppm) exhibited at strong field a resonance for CH_3 protons of propanol-2. The methine proton was observed at 4.0 ppm. These resonances disappeared after prolonged drying in vacuo.

Samples of **I** that were recrystallized from anhydrous EtOH did not contain residual solvent after drying. However, they contained moisture in an amount less than 3%. Therefore, we used anhydrous EtOH for further separation and purification of **I**.

Compound **I** was a white or almost white crystalline powder that was very soluble in H_2O, soluble in $CHCl_3$ and alcohols, and practically insoluble in acetone, ether, and toluene. The structure of **I** was confirmed by IR and PMR spectra. The IR spectrum of **I** (KBr, cm^{-1}) exhibited a broad band with a maximum at 3420 that was characteristic of an NH group; 1615, 1505, 1465, C=N and C=C of the

benzimidazole ring; 1635, NH bending vibrations of ammonium ion; 1450 – 1300, bending vibrations of C–H protons; and a series of bands at 1180 and 1050, Ar–O–Alk vibrations.

The PMR spectrum in DMSO-d_6 (δ, ppm) showed resonances for all fragments of **I**: 1.37 (t, 3H, CH_3); 3.37 (m, 4H, CH_2NCH_2); 3.58 (t, 2H, CH_2S); 3.91 (m, 4H, CH_2OCH_2); 3.93 (t, 2H, CH_2N); 4.08 (q, 2H, CH_2CH_3); 7.04 (dd, 1H, H-6); 7.15 (d, 1H, H-4); 7.59 (d, 1H, H-7).

EXPERIMENTAL PART

PMR spectra were recorded in DMSO-d_6 with TMS internal standard on a Bruker AC-250 instrument using standard Bruker programs. IR spectra were taken in KBr pellets on a Perkin—Elmer 580 spectrophotometer (Sweden).

Residual solvent was determined by GC on a Chrom-5 instrument (Czechslovakia) with a flame-ionization detector. Separation was carried out in a glass column (2.4 m, ID 3 mm) packed with 15% carbowax 1500 sorbent on Chromaton N-AW-DMCS (0.200 – 0.250 mm) with N_2 carrier gas (40 mL/min), H_2 flow rate 40 mL/min; and air flow rate 400 mL/min. The vaporizer temperature was 160°C; detector, 130°C; programmed thermostat 70°C for 4 min, increase to 120°C at 20°C/min; and 120°C for 6 min.

TLC was performed on Kieselgel 60 F_{254} plates (Merck) using solvent system hexane:EtOAc:EtOH:NH_4OH (conc.) (5:3:1:1) with detection in UV light and I_2 vapor.

3-Nitrophenacetin (III). A solution of **II** (242 g, 1.35 mol) in glacial acetic acid (550 mL) cooled to 8 – 10°C was stirred for 1 h, treated with a nitrating mixture consisting of conc. HNO_3 (108 mL, 151.2 g, 1.54 mol) and conc. H_2SO_4 (85 mL, 143.3 g, 1.46 mol) at a rate such that the temperature of the mixture did not rise above 12°C, stirred for 0.5 h, poured into cold (10 – 15°C) water (2.5 L), and stirred for 0.5 h. The precipitate was filtered off, washed with H_2O until neutral, and dried at 55 – 65°C to constant mass to afford **III** (280 – 290 g, 92 – 95%), bright yellow shiny crystals, mp 99 – 104°C (lit. [2] mp 101 – 103°C; [3] mp 103 – 104°C).

2-Nitro-*p*-phenetidine (IV). A mixture of **III** (290 g), conc. HCl (300 mL), and H_2O (300 mL) was stirred and refluxed for 20 – 30 min. The end of the reaction was determined by TLC and the disappearance of the spot of the starting material. The mixture was poured with stirring into H_2O (2.62 L) and stirred for 1 h. The precipitate was filtered off, washed with H_2O (4 × 200 mL), and dried at 60 – 70°C to constant mass to afford a brick-red crystalline powder, mp 111 – 113°C (lit. [2, 3] mp 111 – 112°C). Yield 201 g (85.3%). Product **IV** was used in the following step without further purification.

3,4-Diaminophenetole (V). A solution of **IV** (108 g) in EtOH (1 L) and conc. NH_4OH (500 mL) was stirred at 20 – 30°C and treated in portions with sodium dithionite ($Na_2S_2O_4$, 380 g). After the addition was complete, the mixture was stirred vigorously, treated with a thin stream of H_2O (1350 mL) keeping the temperature below 35°C, and stirred for 0.5 h (TLC monitoring). The layers were separated when the reaction was finished. The upper organic layer was separated. The aqueous layer was extracted with EtOH (100 mL). The combined alcohol extract was treated with activated carbon (20 g) and stirred for 1 h. The solution was filtered to remove the carbon and afford an alcohol solution (1900 mL, 1760 g) containing **V** (88.35 g) calculated per dry compound (98.3% of theoretical). The solution was used in the next step.

2-Mercapto-5-ethoxybenzimidazole (VI). An alcohol solution (1.9 L) containing **V** (0.58 mol) was treated with potassium ethylxanthate (110 g, 0.68 mol), and refluxed with stirring for 3.5 – 4 h until the starting diamine disappeared (TLC monitoring). When the reaction was finished, the mixture was cooled to 60°C, treated with H_2O (650 mL) and activated carbon (20 g), and refluxed for 10 min. The hot solution was filtered through a heated filter. The filtrate was cooled to 30°C, stirred, treated with glacial acetic acid (50 mL), and stirred at 5 – 10°C for 10 – 12 h. The resulting precipitate of **VI** was filtered off, washed with H_2O until the rinsings were colorless (5 × 100 mL), and dried to constant mass at 80 – 90°C. The product was a light-beige amorphous powder (90 g, 80%). Crude **VI** was crystallized from propanol-2 at a 1:20 ratio with 10% activated carbon. The solution was refluxed for 10 min, filtered, cooled with stirring to 0 – 5°C, and stored for 6 – 8 h. The precipitate was separated by filtration, washed with cold alcohol (50 mL), and dried at 80 – 90°C (5 – 6 h) to afford a white powder with a cream tint, mp 242 – 246°C (lit. [14] mp 244 – 246°C), mass loss on drying <1.5%, purity >97%.

2-[2-(Morpholino)ethylthio]-5-ethoxybenzimidazole dihydrochloride (I). A solution of NaOH (48.8 g, 1.2 mol) in EtOH (1.2 L) and H_2O (0.2 L) was stirred, treated with **VI** (101 g, 0.52 mol), stirred for 5 min, treated with 4-(2-chloroethyl)morpholine hydrochloride (119 g, 0.64 mol) in H_2O (100 mL), and stirred and refluxed for 2.5 – 3 h. When the reaction was finished (content of **VI** < 0.5% by TLC), the EtOH was distilled off. The solid was treated with H_2O (0.9 L). Base **I** was extracted by $CHCl_3$ (0.8 L, 1 × 0.3 L and 2 × 0.25 L). The combined extracts were washed with H_2O (2 × 0.1 L), treated with anhydrous Na_2SO_4 and activated carbon (20 g), and stirred for 4 – 5 h. The desiccant and carbon were filtered off. The solvent was evaporated. The resulting oil was dissolved in anhydrous acetone (H_2O content <0.3%), cooled to 15 – 20°C, treated with HCl (20 – 25%) in anhydrous EtOH until the pH was 1 – 2, and stirred at 5 – 10°C for 4 – 5 h. The precipitate of **I** was filtered off, washed with anhydrous acetone until the mother liquor was colorless, and dried at 85 – 95°C to constant mass to afford crude **I** (156 g). Crude **I** was crystallized from 10 volumes of anhydrous EtOH with added activated carbon (5 – 7%). The precipitate of **I** was filtered off, washed with anhydrous acetone, and dried at 95 – 100°C to constant

mass. The resulting product was an almost white crystalline powder, mp 192 – 195°C, 120 g (76.9%). The purity of the product was >99%; mass loss on drying, <3%; impurity content, <0.5% (TLC). Yield of **I** 60.7% calculated per **IV**; 31.6% calculated per **II**.

Thus, we developed synthesis technology for **I** consisting of five steps with high yields of intermediates and the target product. The method was simple to scale up, which is important for industrial manufacturing of drugs.

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