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SYNTHESIS AND ANTINOCICEPTIVE, ANTHELMINTIC, AND LARVICIDAL ACTIVITIES OF 4-(3,3-DIMETHYL-3,4-DIHYDROISOQUINOLIN-1-YLTHIO)ANILINE HYDROCHLORIDES

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Ritter cyclocondensation of 3,4-$(R)_2$-dimethylbenzylcarbinols with thiocyanatoanilines was used to obtain 4-[6,7-$(R)_2$-3,3-dimethyl-3,4-dihydroisoquinolin-1-ylthio]anilines (R = H, MeO). A benzo[*f*]isoquinoline derivative was obtained similarly. 4-Thiocyanatoaniline, 2-methyl-4-thiocyanatoaniline, 2-thiocyanato-4-aminoethylbenzene, and 4,4′-dithiocyanatodiphenylamine were used as the thiocyanatoanilines. The hydrochlorides of the obtained compounds were used for biological tests. All compounds exhibited an analgesic effect in the hot-plate test that exceeded that of metamizole sodium. Five of the seven compounds exhibited anthelmintic activity. The most active compound caused the death of worms 14.3 times faster than pyrantel. The larvicidal activity of the same compound was 1.63 times greater than that of diazinon for reducing the time of death of larvae.

Keywords: Ritter cyclocondensation; 3,4-$(R)_2$-dimethylbenzylcarbinols; thiocyanatoanilines; 4-[6,7-4-[6,7-$(R)_2$-3,3-dimethyl-3,4-dihydroisoquinolin-1-yl]anilines; hydrochlorides; antinociceptive activity; anthelmintic activity; larvicidal activity.

Derivatives of 3,3-dialkylisoquinoline that exhibited analgesic (antinociceptive) activity were previously investigated [1 – 9]. Compounds of this series also exhibited anthelmintic and larvicidal activity [10, 11]. Recently, 3-(3,3-dimethyl-3,4-dihydroisoquinolin-1-yl)anilines were prepared by us [12]. The structures of these compounds contained isoquinoline 1-arylthio groups, which are practically unknown in the literature. Tandem cyclization of isothiocyanates was one of few examples of their synthesis [13]. The arylthio moiety could be considered an analog of a benzyl moiety in which the methylene is replaced by an S atom. It is important to note that 1-benzylisoquinolines are the most numerous group of isoquinoline alkaloids and drugs and have a variety of activities [14].

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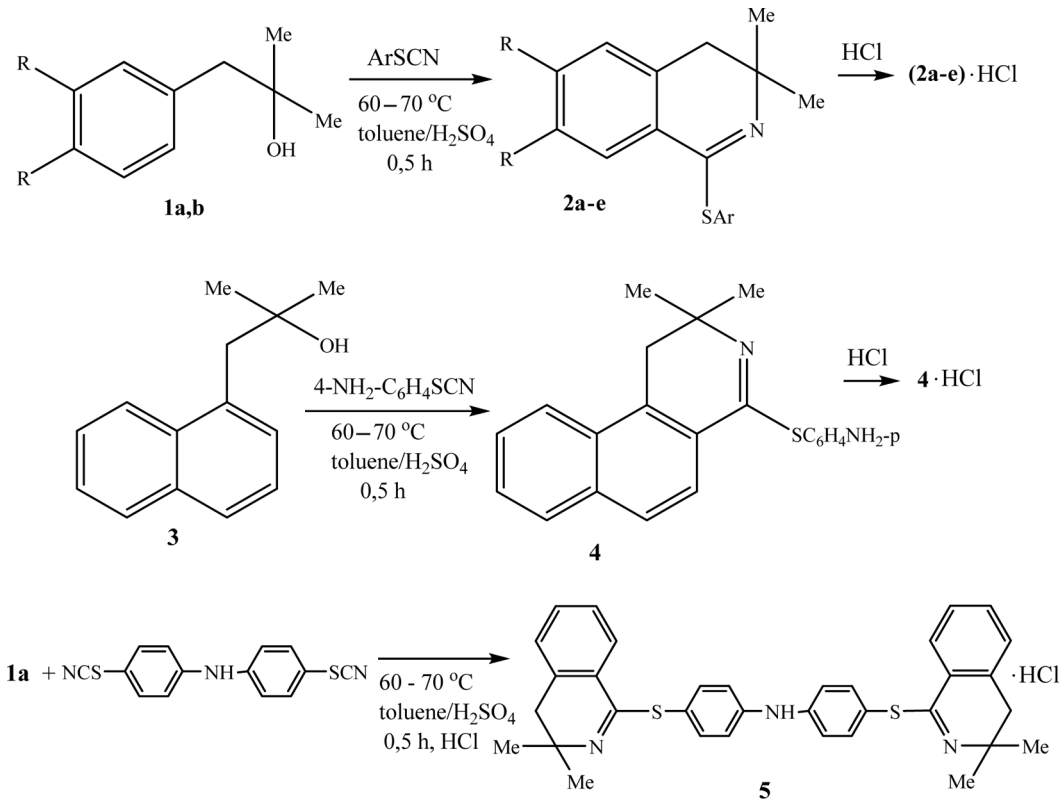
The aims of the present work were to synthesize and study the analgesic (antinociceptive), anthelmintic, and insecticidal (larvicidal) activity of several 4-(3,3-dimethyl-3,4-dihydroisoquinolin-1-ylthio)anilines and to establish their structure(activity relationship.

Compounds **2a-e** were synthesized using Ritter cyclocondensations of carbinols **1a, b** with arylisocyanates containing an amine on the aromatic ring.

The reactions were performed in a heterophasic medium of toluene(H_2SO_4 at 60 – 70°C. The hydrochlorides (**2a-e**)**·HCl** were used for the pharmacological studies and were obtained by passing dry HCl through an EtOAc solution of the corresponding base. Benzo[*f*]isoquinoline **4·HCl** and bis-derivative **5·HCl** were prepared analogously.

EXPERIMENTAL CHEMICAL PART

IR spectra were taken on a Specord M-80 spectrometer. PMR spectra were recorded on a Bruker AMX 300 instrument (300 MHz) in $CDCl_3$ solution with HMDS internal standard (0.05 ppm vs. TMS).



The compounds were recrystallized from *i*-PrOH. Elemental analyses (C, H, N, S, Cl) agreed with those calculated. The purity of products was monitored using TLC with $CHCl_3$(Me_2CO (9:1) and detection by I_2 vapor.

The preparation of the bases **2a**-**e** was reported earlier [12]. The starting thiocyanates were prepared according to references given in a review [15].

4-[6,7-(R)$_2$-dimethyl-3,4-dihydroisoquinolin-1-ylthio]-aniline hydrochlorides [(2a-e)·HCl] and 4-(2,2-dimethyl-1,2-dihydrobenzo[*f*]isoquinoline-4-ylthio)aniline (4·HCl). A mixture of carbinol **1a** or **1b** or **3** (10 mmol) and the corresponding thiocyanate (10 mol) in toluene (100 mL) was treated dropwise with conc. H_2SO_4 (4 mL). For carbinol **1b**, glacial HOAc (2 mL) and H_2SO_4 (4 mL) were sequentially added. The mixture was stirred vigorously at 60 – 70°C for 30 min and poured into ice water (150 mL). The organic layer was separated. The aqueous phase was neutralized with NH_4OH solution. The resulting precipitate was filtered off, dried, and dissolved in EtOAc (100 mL). Dry HCl was passed through the solution to produce the hydrochloride, which was filtered off, dried, and recrystallized.

Bis[4-(3,3-dimethyl-3,4-dihydroisoquinolin-1-ylthio)-phenyl]amine hydrochloride (5·HCl) was prepared analogously to the preceding compounds from carbinol **1a** (3.0 mL, 20 mmol) and 4,4′-dithiocyanatodiphenylamine (2.83 g, 10 mmol).

EXPERIMENTAL BIOLOGICAL PART

Analgesic activity was assessed using outbred white mice of both sexes (18 – 22 g) in the hot-plate test of the paw thermal pain response [16]. The tested compounds were injected intraperitoneally at a dose of 50 mg/kg as suspensions in 2% starch solution 30 min before placing the animals onto a metallic plate heated to 54.5°C. The nociception parameter was the residence time of an animal on the hot plate before the onset of a defensive response (licking hind paws, jumping, withdrawing a hind paw). The effect was assessed 0.5, 1, 1.5, and 2 h after administration. Animals with an initial onset time of a defensive response ≤15 sec were used in the test. Each compound was tested in six animals.

Results were evaluated from the increase in the onset time of a defensive response as compared to the initial values. The control group of animals received 2% starch solution. The reference standard was metamizole sodium at a dose of 93 mg/kg, corresponding to ED_{50} [17, 18]. The dose of 50 mg/kg for the tested compounds and reference drug was chosen based on existing data for the activity and toxicity of structurally related isoquinolines [1 – 7]. Tests in these studies were conducted with an intraperitoneal dose of 50 mg/kg. It was advisable to use the same dose to compare the activity level with the effects of previously studied compounds. The chosen dose also agreed with a handbook [19].

Anthelmintic activity was tested using pyrantel and levamisole as reference drugs. These drugs were chosen ac-

TABLE 1. Properties of Synthesized Compounds

Compound	R	Ar	Empirical formula	mp, °C	Yield, %
2a · HCl	H	4-NH_2-C_6H_4	$C_{17}H_{18}N_2S \cdot HCl$	94 – 96	78
2b · HCl	H	4-NH_2-3-Me-C_6H_3	$C_{18}H_{20}N_2S \cdot HCl$	220 – 222	75
2c · HCl	H	1-CO_2Et-4-NH_2-C_6H_4	$C_{20}H_{22}N_2O_2S \cdot HCl$	145 – 147	68
2d · HCl	MeO	4-NH_2-C_6H_4	$C_{19}H_{22}N_2O_2S \cdot HCl$	178 – 180	71
2e · HCl	MeO	1-CO_2Et-4-NH_2-C_6H_4	$C_{22}H_{26}N_2O_4S \cdot HCl$	173 – 175	67
4 · HCl	–	–	$C_{21}H_{20}N_2S \cdot HCl$	180 – 182	62
5 · HCl	–	–	$C_{34}H_{33}N_3S_2 \cdot HCl$	160 – 161	69

cording to a handbook [20] and their broad use in practice [21]. Larvicidal activity was assessed using the reference drugs pirimiphos, diazinon, and imidacloprid, which are the insecticides currently used most often [22, 23]. All these reference drugs were also used by us earlier [12, 24, 25]. Therefore, their use for evaluating and comparing the results with those from previous studies was also justified. Tablets of pyrantel (Polfa, Poland), levamisole (decaris; Gedeon Richter, Hungary), imidacloprid (tanrec; ZAO Avgust, Russia), diazinon (Fox & Co., Russia), and pirimiphos (OOO Syngenta, Russia) were used in the tests.

Anthelmintic activity was studied using rainworms and the Nikolaev method [26, 27]. A solution (5 mL, 0.5%) of the tested compound was placed into a Petri dish. Five rainworms selected for their length (5 – 8 cm) and diameter (3 – 5 mm) were immersed in it. The onset time of death of each worm was recorded as the cessation of locomotor activity in response to mechanical irritation. The worms could live for ~1 d in purified H_2O (control).

Larvicidal activity was studied similarly to anthelmintic activity. An aqueous solution (5 mL, 0.1%) of the tested compounds was placed into a Petri dish. Five *Chironomidae* (midge) larvae were immersed in it. The onset time of death of each larva was recorded as the cessation of locomotor activity in response to mechanical irritation.

Test results were statistically processed using the Student coefficient. Differences with $p < 0.05$ were considered statistically significant [28]. The number of tests was 6 for determining anthelmintic activity and 10 for studying insecticidal activity. Lack of an anthelmintic or larvicidal effect for 200 min was considered unpromising for further observation.

Digital data were statistically processed using STATISTICA for Windows 6.0 software.

RESULTS AND DISCUSSION

The obtained hydrochlorides [**(2a-e)·HCl**] were colorless crystalline compounds that were difficultly soluble in H_2O. Table 1 presents their characteristics and yields.

The structures of the target products were proven using PMR spectral data (Table 2). Spectra were recorded in DMSO-d_6. A singlet for the three NH_3^+ protons formed upon protonation of the aromatic amine N atom was common to spectra of all hydrochlorides [29]. Protonation shifted the NH resonance by ~3 ppm to stronger field as compared to the base (NH_2) [12]. This resonance was observed at

TABLE 2. PMR Spectra of Synthesized Compounds, δ, ppm

Compound	6H, 2Me, c	2H, 4(2)-CH_2, c	Aromatic protons	2MeO	Me and CO_2Et on aromatic ring	NH_3^+
2a · HCl	1.30	3.30	7.20 – 7.45 (m, 8H)	–	–	8.10
2b · HCl	1.18	3.28	7.38 – 8.40 (m, 7H)	–	2.16 (s, 3H, CH_3)	9.37
2c · HCl	1.32	3.47	7.30 – 8.38 (m, 7H)	–	1.35 (t, 3H, CH_3CH_2O, J 7.3 Hz), 4.34 (q, 2H, CH_3CH_2O, J 7.3 Hz)	8.73
2d · HCl	1.27	3.31	7.10 – 8.2 (m, 4H), 7.01 (s, 5–H), 8.07 (s, 8–H)	3.81 s 3.83 s	–	9.10
2e · HCl	1.22	3.27	7.03 – 8.08 (m, 4H), 6.82 (s, 5–H), 8.11 (s, 8–H)	3.78 s 3.80 s	1.32 (t, 3H, CH_3CH_2O, J 7.4 Hz), 4.33 (q, 2H, CH_3CH_2O, J 7.4 Hz)	8.87
4 · HCl	1.20	3.10	7.24 – 8.24 (m, 10H)	–	–	9.32
5 · HCl	1.23	2.95	7.15 – 7.78 (m, 12H)	–	–	8.02

TABLE 3. Antinociceptive, Anthelmintic, and Larvicidal Activity of Synthesized Compounds

Compound	Latent period of defensive response,* time, s	Anthelmintic activity, worm lifespan, min	Larvicidal activity, larva lifespan, min
2a · **HCl**	20.83 ± 0.97	>200	>200
2b · **HCl**	20.50 ± 0.69	18.0 ± 1.22	10.4 ± 1.43
2c · **HCl**	23.67 ± 0.68	40.60 ± 4.23	>200
2d · **HCl**	19.83 ± 0.72	35.60 ± 3.03	38.8 ± 3.47
2e · **HCl**	22.25 ± 0.95	15.0 ± 0.63	19.60 ± 0.81
4 · **HCl**	21.30 ± 0.44	>200	>200
5 · **HCl**	20.75 ± 0.49	38.60 ± 10.62	>200
Control: 2% starch solution	10.50 ± 0.35	–	–
Reference drugs			
Metamizole sodium	16.60 ± 3.40**	–	–
Pyrantel		215.0 ± 0.37	–
Levamisole		20.20 ± 2.08	–
Pirimiphos		–	24.50 ± 1.69
Diazinon		–	17.0 ± 1.87
Imidacloprid		–	43.50 ± 3.39

* $p < 0.05$ vs. control and standard.

5.0 – 5.81 ppm in spectra of the bases; at 8.02 – 9.37 ppm, in spectra of the hydrochlorides. The singlet for the CH_2 protons (2.95 – 3.47 ppm) also shifted to stronger field. The spectra also contained resonances for protons of the corresponding substituents, i.e., methyl, methoxy, and carbomethoxy groups, the integrated intensities of which agreed with the given number of protons.

IR spectra of the bases contained absorption bands for C=N in the range 1625 – 1630 cm^{-1} and for the primary amine in the ranges 3475 – 3460 and 3380 – 3370 cm^{-1} (ν_{as} and ν_s). Spectra of esters **2c** and **2e** contained a band for carbonyl stretching vibrations near 1725 cm^{-1}.

All seven compounds were tested for analgesic (antinociceptive) activity (Table 3). The studies showed that all compounds were more active than metamizole sodium. They increased the latent period for a defensive response by 19.83 – 23.67 sec while the reference drug metamizole sodium increased it by 16.60 sec. Hydrochloride **2c** with a carboethoxy group was the most active and had a value of 23.67 sec, which was 1.42 times greater than the effect of metamizole sodium.

TABLE 3 shows that five of seven compounds exhibited anthelmintic activity. The lifespan of the worms was 15.00 – 40.60 min, which represented much greater activity than pyrantel (215.0 min). Hydrochloride **2e** containing an ester group on the aniline and 6- and 7-methoxy groups on the isoquinoline ring was the most active and caused the death of worms in 15 min, which was statistically significantly more active than levamisole (20.20 min) and 14.3 times greater than pyrantel.

Four of the seven hydrochlorides tested for larvicidal activity were active with larva lifespans of 10.4 – 38.8 min. Hydrochloride **2b·HCl** was the most active with respect to duration of the effect (10.4 min) and 1.63 times greater than the most active reference drug diazinon (17.0 min). It is interesting that the anthelmintic and larvicidal activities were most potent for compounds with substituents on the aniline (**2b·HCl** and **2e·HCl**).

A comparison of the analgesic activity of the tested compounds with structurally analogous 1-benzylisoquinolines [30] showed that the S-containing compounds had activities approximately the same as 1-benzyl derivatives. However, a distinct enhancement of the anthelmintic and larvicidal activities was observed if the CH_2 was replaced by S [31].

Possible biological targets of the studied compounds were discussed before [9 – 11, 25, 31].

It could be concluded based on the results that further searching for 4-(3,3-dimethyl-3,4-dihydroisoquinolin-1-ylthio)aniline hydrochlorides with antinociceptive, anthelmintic, and larvicidal activity is warranted if substituents on the aniline aromatic ring are considered.

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