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SPECIFIC TECHNOLOGICAL SOLUTIONS

Synthesis of Immunoactive Tris(2-hydroxyethyl)ammonium 1-R-Indol-3-ylsulfanyl(sulfonyl)acetates

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Abstract—Difficultly accessible 1-R-indol-3-ylsulfanyl(sulfonyl)acetic acids 1-R-IndYCH_2CO_2H (R = H, Me, Bn; Y = S, SO_2) **1a–Id** were prepared. Their reaction with tris(2-hydroxyethyl)amine yielded tris(2-hydroxyethyl) ammonium 1-R-indol-3-ylsulfanyl(sulfonyl)aceetates (protatranes) **2a–2d**. The immunoactive properties of **2a–2d** were studied. Protatranes **2a**, **2c**, and **2d** proved to be effective immunosuppressive agents (up to 99.5% inhibition of mice splenocyte proliferation in vitro).

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A wide range of physiologically active salts and ionic liquids were prepared previously by the reaction of biogenic amines (imidazoles, guanidines, alkanolamines, etc.) with biologically active arylchalcogenylacetic acids $RYCH_2CO_2H$ (R = Ar, indolyl, etc.; Y = O, S, SO, SO_2, Se) [1–3].

The tris(2-hydroxyethyl)amine (triethanolamine) derivatives, tris(2-hydroxyethyl)ammonium arylchalcogenylacetates $ArYCH_2CO_2^{-}\cdot HN^{+}(CH_2CH_2OH)_3$, whose cation has unique tricyclic "atrane" structure favoring penetration of the substance through cell membranes, are the most studied. We termed them protatranes. Protatranes combining the properties of their components exhibit synergistic effect and high and diverse physiological and pharmacological activity (antioxidant, antisclerotic, antiallergic, antitumor, antimetastatic, protective, growth-stimulating, enzyme-stimulating effects) [2].

Indole derivatives, in particular, indol-3-ylsulfanylacetic acids 1 and their salts, deserve particular attention as biologically active substances and intermediates for drug synthesis [4]. However, in contrast to aryloxyacetic acids (Y = O), acids 1 (Y = S) are difficultly accessible. Compounds **1** were prepared previously by prolonged (for up to 8 h) heating (to 85°C) in toxic alcohols (methanol, isopropanol, etc.) using expensive I_2 and KI, and also an equimolar amount of toxic hydrazine hydrate. The yield of **1** was 70–85%, and the purity was 92–93% [5].

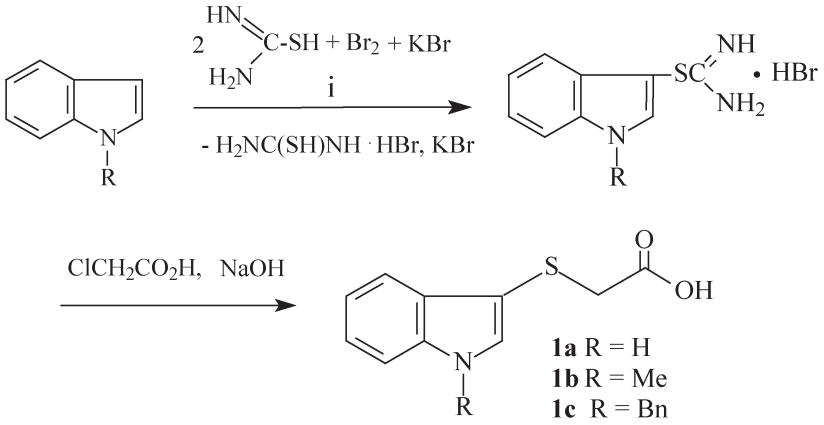
This study was aimed at developing an efficient procedure for preparing compounds **1** (Scheme 1) and their tris(2-hydroxyethyl)ammonium salts (protatranes) **2** (Scheme 2) and at evaluating their immunotropic activity (**2**).

Conditions: i, H_2O, 30°C, 3 h, yield up to 95%, purity up to 99.7%.

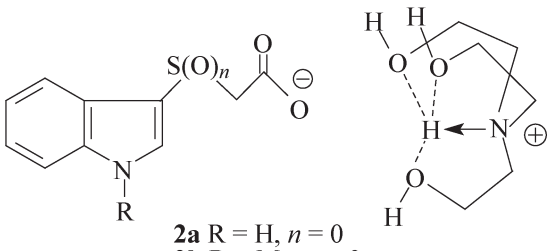
As shown in Scheme 1, we were able to exclude the use of alcohol in both steps of the synthesis. The reaction was performed in water at 30°C. Instead of I_2 and KI, we used 10–20 times cheaper Br_2 and KBr (NaBr) [6], which allows the product synthesis cost to be reduced.

Indole is brominated already at room temperature in 3-position. The 3-bromoindole formed reacts with thiourea to form indolylisothiouronium bromide. This reaction route ensures increased levels of the yield and purity of **1** due to exclusion of the formation of side oxidation products (indole disulfides etc.), occurring when using a strong oxidant, I_2 + KI. The use of hydrazine hydrate is not required. Oxidation of **1c** with hydrogen peroxide yielded 1-benzylindol-3-ylsulfonylacetic acid 1-Bn-Ind-$SO_2CH_2CO_2H$ (**1d**).

Scheme 1. Synthesis of 1-*R*-indol-3-ylsulfanylacetic acids.



Scheme 2. Tris(2-hydroxyethyl)ammonium 1-*R*-indol-3-ylsulfanyl(sulfonyl)acetates (protatranes) **2a–2d.**



The reaction of **1a–1d** with triethanolamine yielded tris(2-hydroxyethyl)ammonium 1-R-indol-3-ylsulfanyl-(sulfonyl)acetates, protatranes **2a–2d** (Scheme 2).

In contrast to the known procedure for preparing protatranes **2** (solvent ethanol, $T = 8°C$, 2–3 h, yield 80–90%, purity 93%) [5], the reaction is performed without a solvent; $T = 60°C$, 15–30 min, yield up to 99%, purity up to 99%.

The modern knowledge of the pathogenesis of diseases associated with disturbance of immune processes at mismatch of the regulatory effects and of the balance of the corresponding cytokines dictates the need for developing new immunoactive drugs for treating autoimmune, immunocomplex, immunodeficient, lymphoproliferative, tumor, and allergic diseases, and also complications in transplantations of organs, bone marrow, etc., associated with activation–deactivation of the immune system. Today there are no drugs that would be allowed for medical use and be capable of selectively altering the Th1/Th2 balance in the required direction [7].

In cooperation with the Institute of Basic and Clinical Immunology (Novosibirsk, Russia), we have studied the immunomodulating activity of protatranes **2a–2d**, following the known procedure [8].

Compounds **2a–2d** exhibit dose-dependent antiproliferative activity and inhibit spontaneous and mitogen-induced (concanavalin, Con A, Sigma) proliferation of spleen cells of experimental mice (see table).

Protatranes **2a**, **2c**, and **2d** are the most active (up to –99.5% inhibition of cell growth) and are therefore promising for further studies as immunosuppressive agents. The effect of **2a**, **2c**, and **2d** is comparable to that of the known immunosuppressive agent, cyclosporin A.

EXPERIMENTAL

The NMR spectra (solutions in D_2O or CD_3OD) were recorded with a Bruker DPX-400 spectrometer (^1H, 400.13 MHz; ^{13}C, 101.62 MHz) using HMDS as internal reference. The IR spectra were recorded with a Bruker IFS-25 spectrophotometer. The purity of the compounds was determined by potentiometric titration (EA-74 ion meter).

Synthesis of 1a–1c (general procedure). A solution of Br_2 (0.01 mol) and KBr (0.01 mol) in 25 mL of H_2O was added to a suspension of the corresponding indole (0.01 mol) and thiourea (0.02 mol) in 25 mL of H_2O. The mixture was stirred for 3 h at 30°C, after which a solution of 0.05 mol of NaOH in 15 mL of H_2O and a solution of 0.012 mol of monochloroacetic acid in 10 mL of H_2O were added. The mixture was kept for 30 min at pH 9–10, acidified with HCl (10%) to pH 1, and kept at 5°C for 24 h. The precipitate was filtered off and dried.

Effect of **2a–2d** on spontaneous and mitogen-stimulated proliferation of intact mice spleen cells in vitro (– or +, effect relative to control, %)[a]

Compound	Dosage, mg mL^{-1}	Proliferation	
		spontaneous	Con A
2a	3	+8.0	+15.1
	30	–30.0	–31.0
	300	–84.0	–48.0
2b	3	+71.0	–4.0
	30	+25.0	+5.5
	300	–49.0	–25.4
2c	3	–36.0	–19.0
	30	–74.5	–29.2
	300	–89.9	–99.5
2d	3	–1.5	–17.5
	30	–60.4	–29.9
	300	–75.7	–90.9

[a] The control (without adding **2a–2d**) is taken as 0%.

Synthesis of 1d. A mixture of 0.01 mol of 1c and 0.05 mol of 30% H_2O_2 in 30 mL of acetic acid was allowed to stand for 12 h. The solvent was distilled off, and the residue was recrystallized (H_2O/acetone, 45°C).

Synthesis of 2a–2d (general procedure). A mixture of 0.01 mol of appropriate acid 1a–1d and 0.01 mol of triethanolamine was stirred at 60°C for 15–30 min, cooled, washed with diethyl ether, and dried.

1a: yield 92%, colorless powder, mp 110°C, purity 99.6%. IR spectrum (KBr, ν, cm^{-1}): 1704 (C=O), 3498 (OH). ^{1}H NMR spectrum (CD_3OD), δ, ppm: 3.36 s (2H, SCH_2), 7.09–7.70 m (5H, Ind). ^{13}C NMR spectrum (CD_3OD), δ, ppm: 39.94 (SCH_2), 104.35–138.08 (Ind), 174.44 (C=O).

1b: yield 93%, colorless powder, mp 106°C, purity 99.7%. IR spectrum (KBr, ν, cm^{-1}): 1700 (C=O), 3475 (OH). ^{1}H NMR spectrum (CD_3OD), δ, ppm: 3.39 s (2H, SCH_2), 3.72 s (3H, N–Me), 7.15–7.75 m (5H, Ind). Found, %: C 59.78, H 4.99, N 6.37. Calculated for $C_{11}H_{11}NO_2S$, %: C 59.71, H 5.01, N 6.33.

1c: yield 95%, colorless powder, mp 108°C, purity 99.7%. IR spectrum (KBr, ν, cm^{-1}): 1701 (C=O), 3435 (OH). ^{1}H NMR spectrum (CD_3OD), δ, ppm: 3.36 s (2H, SCH_2), 5.23 s (2H, NCH_2), 7.06–7.72 m (10H, Bnz, Ind). ^{13}C NMR spectrum (CD_3OD), δ, ppm: 38.55 (SCH_2), 102.86–137.29 (Bnz, Ind), 172.83 (C=O).

1d: yield 92%, colorless powder, mp 165°C, purity 98.6%. IR spectrum (KBr, ν, cm^{-1}): 1380 (SO_2), 1700 (C=O), 3290 (OH). ^{1}H NMR spectrum (CD_3OD), δ, ppm: 4.26 s (2H, SO_2CH_2), 5.48 s (2H, CH_2–C_6H_5), 7.20–8.03 m (10H, Bnz, Ind). ^{13}C NMR spectrum (CD_3OD), δ, ppm: 49.47 (CH_2–C_6H_5), 60.44 (SO_2CH_2), 110.33–134.43 (Bnz, Ind), 163.80 (C=O).

2a: yield 97%, colorless powder, mp 93°C, purity 99.0%. IR spectrum (KBr, ν, cm^{-1}): 1591 (C=O), 2700 (N + H), 3308 (OH). ^{1}H NMR spectrum (D_2O), δ, ppm: 3.19 t (6H, NCH_2), 3.37 s (2H, SCH_2), 3.77 t (6H, OCH_2), 7.11–7.70 m (5H, Ind). ^{13}C NMR spectrum (D_2O), δ, ppm: 50.91 (NCH_2), 57.11 (SCH_2), 57.37 (OCH_2), 105.88–138.96 (Ind), 177.27 (C=O). Found, %: C 54.01, H 6.77, N 7.80, S 9.09. Calculated for $C_{16}H_{24}N_2O_5S$ (%): C 53.91, H 6.78, N 7.86, S 8.99.

2b: yield 99%, colorless powder with mp 90°C, purity 98.0%. IR spectrum (KBr, ν, cm^{-1}): 1600 (C=O), 2720 (N + H), 3310 (OH). ^{1}H NMR spectrum (D_2O), δ, ppm: 3.20 t (6H, NCH_2), 3.30 s (2H, SCH_2), 3.70 s (3H, N–Me), 3.75 t (6H, OCH_2), 7.12–7.75 m (5H, Ind), 180.07 (C=O).

2c: yield 96%, colorless powder with mp 94°C, purity 98.6%. IR spectrum (KBr, ν, cm^{-1}): 1607 (C=O), 2750 (N + H), 3300 (OH). ^{1}H NMR spectrum (D_2O), δ, ppm: 3.32 t (6H, NCH_2), 3.36 s (2H, SCH_2), 3.83 t

(6H, OCH_2), 5.44 s (2H, CH_2–C_6H_5), 7.23–7.98 m (10H, Bnz, Ind). ^{13}C NMR spectrum (D_2O), δ, ppm: 38.00 (SCH_2), 49.43 (CH_2–C_6H_5), 54.73 (NCH_2), 54.80 (OCH_2), 110.15–135.52 (Bnz, Ind), 179.17 (C=O).

2d: yield 96%, colorless powder with mp 136°C, purity 98.1%. IR spectrum (KBr, ν, cm^{-1}): 1380 (SO_2), 1598 (C=O), 2800 (N + H), 3320 (OH). ^1H NMR spectrum (D_2O), δ, ppm: 3.39 t (6H, NCH_2), 3.88 t (6H, OCH_2), 4.19 s (2H, SO_2CH_2), 5.35 s (2H, CH_2–C_6H_5), 7.12–7.88 m (10H, Bnz, Ind). ^{13}C NMR spectrum (D_2O), δ, ppm: 37.10 (SCH_2), 48.07 (CH_2–C_6H_5), 55.03 (NCH_2), 55.10 (OCH_2), 61.54 (SO_2CH_2), 110.13–134.00 (Bnz, Ind), 176.88 (C=O).

CONCLUSIONS

1-R-Indol-3-ylsulfanyl(sulfonyl)acetic acids and their tris(2-hydroxyethyl)ammonium salts (protatranes), difficultly accessible previously, were synthesized by a new efficient method. These compounds are of interest as pharmacologically active substances and precursors of immunomodulating and immunosuppressive drugs.

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