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SEARCH FOR NEW DRUGS

SYNTHESIS AND ANTIVIRAL ACTIVITY OF SUBSTITUTED ETHYL-2-AMINOMETHYL-5-HYDROXY-1*H*-INDOLE-3-CARBOXYLIC ACIDS AND THEIR DERIVATIVES

A. V. Ivachtchenko,[1, 2, 3] P. M. Yamanushkin,[1] O. D. Mitkin,[1] V. M. Kisil,[2] O. M. Korzinov,[1, 4] V. Yu. Vedenskii,[2] I. A. Leneva,[5] E. A. Bulanova,[1] V. V. Bichko,[2] I. M. Okun',[2] A. A. Ivashchenko,[1, 4] and Ya. A. Ivanenkov[1]

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Novel substituted 5-hydroxy-2-aminomethyl-1*H*-indole-3-carboxylic acids and their derivatives were synthesized. The antiviral properties of these compounds were investigated in relation to bovine viral diarrhea virus (BVDV), hepatitis C virus (HCV), and influenza A/Aichi/2/69 (H3N2) virus. Of the compounds synthesized here, only the 5-hydroxy-2-(dimethylaminomethyl)-1-methyl-6-pyridin-3-yl- and 5-hydroxy-2-(dimethylaminomethyl)-1-methyl-6-fluoro-1*H*-indole-3-carboxylic acid ethyl ester hydrochlorides had significant activity against these viruses, these agents not only suppressing the replication of influenza A/Aichi/2/69 (H3N2) virus in cell cultures at micromolar concentrations, but also demonstrating high efficacy, greater than that of Arbidol, in a model of influenza pneumonia in mice infected with influenza A/Aichi/2/69 (H3N2) virus, when given at a dose of 25 mg/kg/day.

Keywords: 2-aminomethyl-5-hydroxy-1*H*-indole-3-carboxylic acids, antiviral activity, antiinfluenza activity, influenza A/Aichi/2/69 (H3N2), BVDV, HCV.

Substituted 2- and 4-aminomethyl-5-hydroxyl-1*H*-indole-3-carboxylic acids are known to have some degree of antiviral activity [1 – 3]. In particular, 1-benzyl-2-dimethylaminomethyl-3-carbethoxy-5-acetoxy-6-bromoindole hydrochloride demonstrated antiinfluenza activity in studies using a mouse model of influenza pneumonia [3]. We have previously described the synthesis and antiviral activity of a series of 2-aminomethyl-5-hydroxy-1*H*-indole-3-carboxylic acids and have shown them to have limited activity against influenza A/New Caledonia/20/99 (H1N1) virus, bovine viral diarrhea virus (BVDV), and HCV [4].

We describe here the synthesis and antiviral activities of novel derivatives of 2-aminomethyl-5-hydroxy-1*H*-indole-3-carboxylic acids (compounds **4 – 7**, **11**, **16 – 20**, **24**). Substituted ethyl esters of 2-aminomethyl-5-hydroxy-1*H*-indole-3-carboxylic acids **4 – 7** were prepared (scheme 1) from 1-substituted ethyl esters of 5-hydroxy-2-methyl-1*H*-indole-3-carboxylic acids **1a – e**, which, by analogy [5] with 1,2-dimethylindole (compound **1a**), were sequentially converted to 5-acetyloxy- (compounds **2a – e**) and 5-acetyloxy-6-bromoindoles (compounds **3a – e**). Interaction of these latter compounds with dimethylamine, pyrrolidine, and morpholine gave the corresponding 2-aminomethyl-6-bromoindoles (**4a – g**). 6-Bromoindoles **4b**,**c** were hydrogenated with hydrogen on 10% Pd/C to form indoles unsubstituted at position 6 (compounds **5a**, **b**); reaction of 6-bromoindole 4a with copper (I) cyanide in N-methylpyrrolidone (NMP) produced the corresponding 6-cyanoindole compound 6, while use of

[1] Chemical Diversity Research Institute, 114401 Khimki, Moscow District, Russia; e-mail: ypm@iihr.ru.
[2] ChemDiv Inc., 5506 Nancy Ridge Drive, San Diego, CA 92121 USA, Service: +1 877 ChemDiv; e-mail: av@chemdiv.com.
[3] ASAVI LLC, 1835 E. Hallandale Beach Blvd, #442, Hallandale Beach, 33009 USA; e-mail: av@asavillc.com.
[4] Moscow Physicotechnical Institute, 141700 Dolgoprudnyi, Moscow District, Russia; e-mail: a.ivashchenkio@mipt.ru.
[5] FGBU I. I. Mechnikov Science Research Institute of Vaccines and Sera, Russian Academy of Medical Sciences, Moscow, Russia; e-mail: wnyfd385@yandex.ru.

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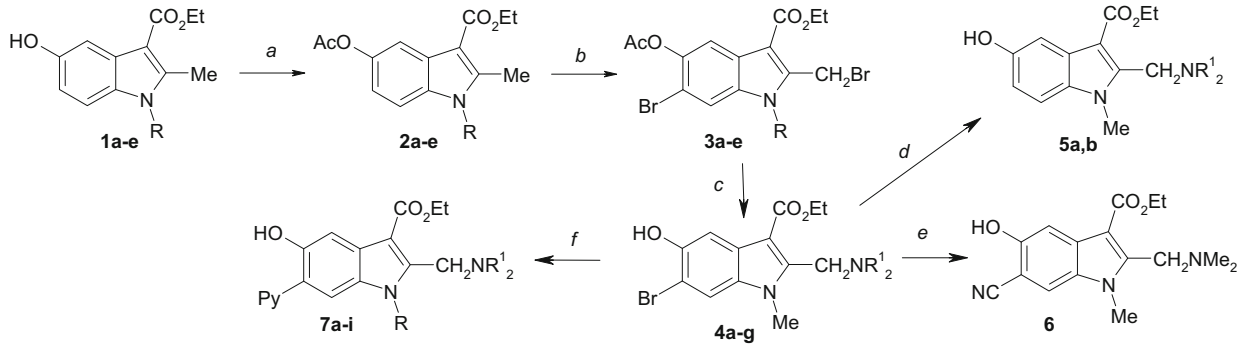
6-bromoindoles **4a – g** in the Suzuki reaction with pyridylboronic acids in the presence of dichloro-*bis*(triphenylphosphine)palladium (II) led to conversion to the corresponding 2-aminomethyl-5-hydroxy-6-pyridin-3(or 4)-yl-1*H*-indoles (compounds **7a – i**).

Scheme 1

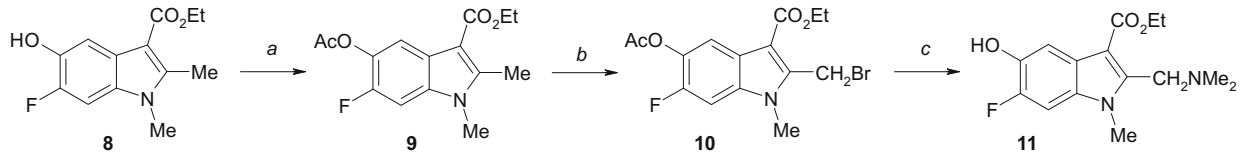


Reagents and conditions: (*a*) Ac_2O, pyridine; (*b*) Br_2, CCl_4, Δ; (*c*) $R^1{}_2NH$, PhH; (*d*) H_2, Pd/C, EtOH; (*e*) CuCN, N-methylpyrrolidone, 150 – 160°C, 24 h; (*f*) $PyB(OH)_2$, $Pd(PPh_3)_2Cl_2$, Na_2CO_3, EtOH, H_2O, 80 – 85°C.

Compound	R	Py	$NR^1{}_2$	Compound	R	Py	$NR^1{}_2$	Compound	R	Py	$NR^1{}_2$
4a	Me	–	NMe_2	**4g**	Ph	–	NMe_2	**7d**	Me	4-Py	NMe_2
4b	Me	–	Pyrrolidin	**5a**	–	–	Pyrrolidin	**7e**	Me	4-Py	Pyrrolidin
4c	Me	–	Morpholinyl	**5b**	–	–	Morpholinyl	**7f**	i-Pr	3-Py	NMe_2
4d	i-Pr	–	NMe_2	**7a**	Me	3-Py	NMe_2	**7g**	c-Pr	3-Py	NMe_2
4e	c-Pr	–	NMe_2	**7b**	Me	3-Py	Pyrrolidin	**7h**	Cy	3-Py	NMe_2
4f	Cy	–	NMe_2	**7c**	Me	3-Py	Morpholinyl	**7i**	Ph	3-Py	NMe_2

The ethyl ester of 2-dimethylaminomethyl-5-hydroxy-1-methyl-6-fluoro-1*H*-indole-3-carboxylic acid (compound **11**) was prepared (Scheme 2) using a method analogous to that for 6-bromo derivative **4a** from the corresponding 1,2-dimethyl-6-fluoro-1*H*-indole compound **7**, whose synthesis was described in [6].

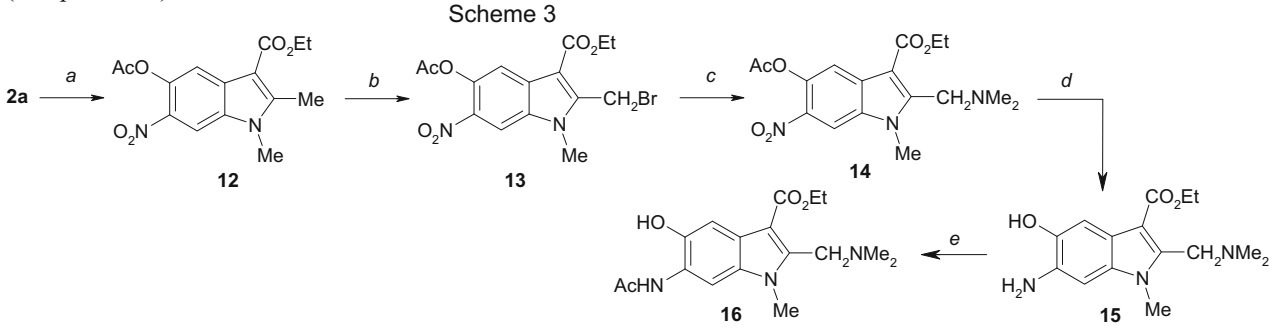
Scheme 2



Reagents and conditions: (*a*) Ac_2O, pyridine; (*b*) Br_2, CCl_4, Δ 4 h; (*c*) Me2NH, PhH.

The ethyl ester of 6-acetylamino-2-dimethylamiomethyl-5-hydroxy-1-methyl-1*H*-indole-3-carboxylic acid (compound **16**) was prepared using a five-stage synthesis (Scheme 3) from the ethyl ester of 5-acetoxy-1,2-dimethyl-1*H*-indole-3-carboxylic acid (compound **2a**).

Scheme 3

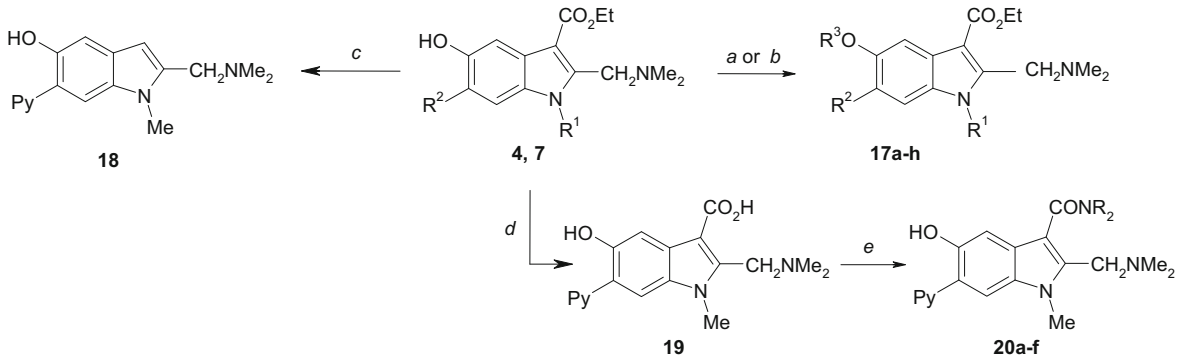


Reagents and conditions: (*a*) Ac_2O, HNO_3; (*b*) NBS (N-bromosuccinimide), CCl_4; (*c*) Me_2NH, PhH; (*d*) H_2, Pd/C, EtOH; (*e*) Ac_2O, EtOH.

Alkoxy-2-dimethylaminomethyl-1*H*-indoles (compounds **17a – h**) were prepared (Scheme 4) by alkylation of the corresponding 5-hydroxy derivatives **4** and **7**, while 5-hydroxy-2-dimethylaminomethyl-1-methyl-6-pyridin-3-yl-1*H*-indole (com-

pound **18**) was prepared by acid hydrolysis and decarboxylation of the corresponding ester **7a**. In conditions of alkaline hydrolysis, compound **7a** yielded the acid (compound **19**), which was reacted with amines to form the corresponding amides (compounds **20a – f**).

Scheme 4

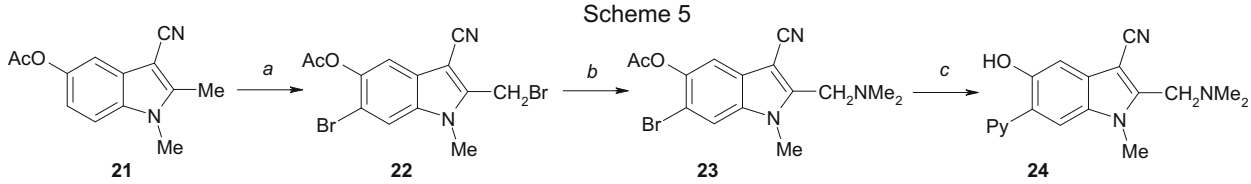


Reagents and conditions: (*a*) $Me_3N^+PhTsO^-$, Cs_2CO_3, DMF, 100°C; (*b*) *i*-PrBr, Cs_2CO_3, DMF; (*c*) HCl, Δ; (*d*) NaOH, Δ; (*e*) R^2NH, EDAC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide), HOBt (hydroxybenzotriazole), Et_3N, DMF.

Compound	R^1	R^2	R^3	Compound	R^1	R^2	R^3
17a	Me	3-Py	Me	**17e**	Ph	3-Py	Me
17b	*i*-Pr	3-Py	Me	**17f**	Me	6-F	Me
17c	*c*-Pr	3-Py	Me	**17g**	Me	3-Py	*i*-Pr
17d	Cy	3-Py	Me	**17h**	Me	NHAc	*i*-Pr

Hydroxy-2-dimethylaminomethyl-1-methyl-6-pyridin-3-yl-3-cyano-1*H*-indole (compound **24**) was prepared by bromination of 5-acetoxy-1,2-dimethyl-3-cyano-1*H*-indole (compound **21**), prepared as described in [7] by dimethylamination of the resulting dibromo derivative (compound **22**) and substitution of the bromine in the resulting 6-bromoindole (compound 23) with a pyridin-3-yl moiety using the Suzuki reaction with pyridyl-3-boronic acid (Scheme 5).

Scheme 5



Reagents and conditions: (*a*) Br_2, CCl_4, Δ; (*b*) Me_2NH, PhH; (*c*) $PyB(OH)_2$, $Pd(PPh_3)_2$, Na_2CO_3, EtOH, H_2O, 80-85°C.

EXPERIMENTAL CHEMICAL SECTION

The ^1H NMR spectra of the compounds synthesized here were taken in DMSO-d_6 solution on a Bruker DPX-400 (400 MHz, 27°C) spectrometer. GC-MS spectra were obtained using a Shimadzu 10Avp high-pressure liquid chromatograph with a Waters XBridge C18 3.5 μm (4.6 × 150 mm) column and an API 150 EX mass spectrometer (λ 220 and 254 nm). GC-MS/UV_{254} data showed that the purity of each synthesized compound was at least 98.0%. GC-MS and ^1H and ^{13}C NMR data were consistent with atomic formulas.

All reactions were run in solvents purified by standard methods.

Initial 6-bromo-1*H*-indoles **1a – e** were commercially available products from OOO Chemical Diversity Research Institute (Khimki, Moscow District).

Arbidol was prepared as described in [5]. The ^1H NMR spectrum (DMSO-d_6), δ, ppm, was: 9.38 (s, 1H), 8.97 (s, 1H), 8.03 (s, 1H), 7.35 (m, 5H), 4.84 (s, 2H), 4.74 (s, 2H), 4.19 (q, J 7.2 Hz, 2H), 3.70 (s, 3H), 2.74 (s, 5H), 1.25 (t, J 7.2 Hz, 3H).

GC-MS (ESI): The content of the main substance was 99.62%, $[M + H]^+$ 477.479. $C_{22}H_{25}BrN_2O_3S \cdot HCl$.

Hydrochlorides of substituted ethyl esters of 6-bromo-2-aminomethyl-5-hydroxy-1*H*-indole-3-carboxylic acids (4a – g), general preparation method. A solution of 2.5 mmol of one of compounds **3a – e** in 30 ml of benzene was supplemented with 15 ml of a 1 M solution of the corresponding amine in benzene. The reaction was mixed for 1 h, filtered, and evaporated in vacuo; the residue was dissolved in 30 ml of ethanol, supplemented with 420 mg (7.5 mmol) of KOH in 20 ml of etha-

nol and mixed for 12 h. The solution was neutralized with 0.435 ml of hydrochloric acid and evaporated in vacuo; the residue was dissolved in 100 ml of dichloromethane, washed with 10% potash solution, dried over Na_2SO_4, and evaporated in vacuo; the residue was chromatographed on silica gel eluted with chloroform:triethylamine (40:1). This yielded compounds **4a – g**, which were converted to the hydrochlorides by addition of the equivalent quantity of 3 M HCl solution in dioxane to solutions of bases **4a – g** in 5 ml of acetone; the resulting precipitates were collected by filtration, washed with ether, and dried in vacuo. Yields were 42 – 71%.

6-Bromo-2-((dimethylamino)methyl)-5-hydroxy-1-methyl-1*H*-indole-3-carboxylic acid ethyl ester hydrochloride (4a). ^{1}H NMR (DMSO-d_6), δ, ppm: 9.74 (broad s, 1H), 7.71 (s, 1H), 7.62 (s, 1H), 4.27 (q, J 7.0 Hz, 2H), 3.95 (s, 2H), 3.75 (s, 3H), 2.19 (s, 6H), 1.36 (t, J 7.0 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 355, 357. $C_{15}H_{19}BrN_2O_3$.

6-Bromo-5-hydroxy-1-methyl-2-(pyrrolidin-1-ylmethyl)-1*H*-indole-3-carboxylic acid ethyl ester hydrochloride (4b). ^{1}H NMR (DMSO-d_6), δ, ppm: 9.73 (s, 1H), 7.70 (s, 1H), 7.62 (s, 1H), 4.25 (q, J 7.0 Hz, 2H), 4.15 (s, 2H), 3.76 (s, 3H), 3.32 (s, 4H), 1.66 (s, 4H), 1.36 (t, J 7.0 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 381, 383. $C_{17}H_{21}BrN_2O_3$.

6-Bromo-5-hydroxy-1-methyl-2-(morpholinomethyl)-1*H*-indole-3-carboxylic acid ethyl ester hydrochloride (4c). ^{1}H NMR (DMSO-d_6), δ, ppm: 9.75 (s, 1H), 7.73 (s, 1H), 7.62 (s, 1H), 4.27 (q, J 7.0 Hz, 2H), 4.04 (s, 2H), 3.77 (s, 3H), 3.52 (m, 4H), 2.43 (m, 4H), 1.37 (t, J 7.0 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 397, 399. $C_{17}H_{21}BrN_2O_4$.

6-Bromo-2-((dimethylamino)methyl)-5-hydroxy-1-isopropyl-1*H*-indole-3-carboxylic acid ethyl ester hydrochloride (4d). GC-MS (ESI) $[M + H]^+$ 383, 385. $C_{17}H_{23}BrN_2O_3$.

6-Bromo-2-((dimethylamino)methyl)-5-hydroxy-1-cyclopropyl-1*H*-indole-3-carboxylic acid ethyl ester hydrochloride (4e). ^{1}H NMR ($CDCl_3$), δ, ppm: 7.66 (s, 1H), 7.61 (s, 1H), 6.33 (broad s, 1H), 4.38 (q, J 7.2 Hz, 2H), 4.15 (s, 2H), 3.41 (m, 1H), 2.38 (s, 6H), 1.43 (t, J 7.2 Hz, 3H), 1.22 (m, 2H), 1.08 (m, 2H). GC-MS (ESI) $[M + H]^+$ 381, 383. $C_{17}H_{21}BrN_2O_3$.

6-bromo-2-((dimethylamino)methyl)-5-hydroxy-1-cyclohexyl-1*H*-indole-3-carboxylic acid ethyl ester hydrochloride (4f). GC-MS (ESI) $[M + H]^+$ 423, 425. $C_{20}H_{27}BrN_2O_3$.

6-Bromo-2-((dimethylamino)methyl)-5-hydroxy-1-phenyl-1*H*-indole-3-carboxylic acid ethyl ester hydrochloride (4g). GC-MS (ESI) $[M + H]^+$ 417, 419. $C_{20}H_{21}BrN_2O_3$.

2-Substituted ethyl esters of 5-hydroxy-1-methyl-1*H*-indole-3-carboxylic acids (5a,b), general method. Pd/C (10%, 40 mg) was added to a solution of 1 mmol of compounds **4b** or c in 15 ml of ethanol and the reaction was mixed under hydrogen for 12 h. The mixture was filtered through Celite and evaporated in vacuo. This produced compounds **5a, b** with quantitative yield.

5-Hydroxy-1-methyl-2-(pyrrolidin-1-ylmethyl)-1*H*-indole-3-carboxylic acid ethyl ester (5a). ^{1}H NMR (DMSO-d_6), δ, ppm: 8.95 (s, 1H), 7.40 (d, J 1.6 Hz, 1H), 7.29 (d, J 8.8 Hz, 1H), 6.71 (dd, J_1 8.8 Hz, J_2 1.6 Hz, 1H), 4.26 (q, J 7.0 Hz, 2H), 4.16 (s, 2H), 3.76 (s, 3H), 3.32 (broad m, 4H), 1.66 (broad m, 4H), 1.35 (t, J 7.0 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 303. $C_{17}H_{22}N_2O_3$.

5-Hydroxy-1-methyl-2-(morpholin-4-ylmethyl)-1*H*-indole-3-carboxylic acid ethyl ester (5b). ^{1}H NMR (DMSO-d_6), δ, ppm: 8.97 (s, 1H), 7.40 (d, J 1.6 Hz, 1H), 7.32 (d, J 8.8 Hz, 1H), 6.72 (dd, J_1 8.8 Hz, J_2 1.6 Hz, 1H), 4.27 (dq, J_1 7.2 Hz, J_2 1.2 Hz, 2H), 4.06 (s, 2H), 3.77 (s, 3H), 3.53 (broad m, 4H), 2.44 (broad m, 4H), 1.36 (dt, J_1 7.2 Hz, J_2 1.2 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 319. $C_{17}H_{22}N_2O_4$.

5-Hydroxy-1-methyl-2-((methylamino)methyl)-6-cyano-1*H*-indole-3-carboxylic acid ethyl ester hydrochloride (6). A solution of 1.42 g (4 mmol) of compound **4a** in 20 ml of NMP was supplemented with 1.075 g (12 mmol) of CuCN and the mixture was held under argon at 150 – 160°C for 24 h. After cooling, the mixture was poured into 200 ml of 10% ammonia, the precipitate was collected by filtration, dried in vacuo, and chromatographed on silica gel with chloroform:triethylamine (40:1) as eluant. The yield was 325 mg (27%). The hydrochloride was prepared by addition of an equivalent quantity of 3 M HCl solution in dioxane to a solution of the base in 5 ml of acetone; the resulting precipitate was collected by filtration, washed with ether, and dried in vacuo. ^{1}H NMR (DMSO-d_6), δ, ppm: 10.73 (s, 1H), 10.06 (broad s, 1H), 8.12 (s, 1H), 7.70 (s, 1H), 4.84 (d, J 4.0 Hz, 2H), 4.36 (q, J 7.0 Hz, 2H), 3.93 (s, 3H), 2.84 (d, J 4.0 Hz, 6H), 1.42 (t, J 7.0 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 302. $C_{16}H_{19}N_3O_3$.

Hydrochlorides of substituted ethyl esters of 2-aminomethyl-5-hydroxy-1*H*-indole-3-carboxylic acids (7a – i), general method. Pyridylboronic acid (197 mg, 1.6 mmol), Na_2CO_3 (212 mg, 2 mmol) and water (5 ml) were added to a solution of 1 mmol of one of compounds **4a – g** in 10 ml of ethanol. The reaction vessel was ventilated with argon and $Ph(PPh_3)_2Cl_2$ (70 mg, 0.1 mmol) and $NaBH_4$ (1 mg) were added. The reaction was mixed under argon at 80 – 85°C for 12 h. After cooling, solvent was evaporated in vacuo and the residue was dissolved in 50 ml of dichloromethane, washed with 10% potash solution, dried over Na_2SO_4, and evaporated in vacuo; the residue was chromatographed on silica gel with hexane:ethyl acetate:triethylamine (7:3:1) as eluant. This produced compounds **7a – i**, which were converted to hydrochlorides by addition of the equivalent quantity of 3 M hydrochloride in dioxane to the solution of base **7a – i** in 5 ml of acetone; the resulting precipitate was collected by filtration, washed with ether, and dried in vacuo. Yields were 22 – 56%.

2-((Dimethylamino)methyl)-5-hydroxy-1-methyl-6-(pyridin-3-yl)-1*H*-indole-3-carboxylic acid ethyl ester dihydrochloride (7a). ^{1}H NMR (DMSO-d_6), δ, ppm: 10.25 (broad s, 1H), 10.17 (s, 1H), 9.14 (s, 1H), 8.81 (d, J 5.6 Hz, 1H), 8.77 (d, J 8.0 Hz, 1H), 8.04 (dd, J_1 8.0 Hz, J_2 5.6 Hz, 1H), 7.89 (s, 1H), 7.76 (s, 1H), 4.87 (s, 2H), 4.38 (q, J 7.2 Hz, 2H), 4.00 (s, 3H), 2.85 (s, 6H), 1.44 (t, J 7.2 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 354. $C_{20}H_{23}N_3O_3$.

5-Hydroxy-1-methyl-6-(pyridin-3-yl)-2-(pyrrolidin-1-ylmethyl)-1*H*-indole-3-carboxylic acid ethyl ester dihydrochloride (7b). ^{1}H NMR (DMSO-d_6), δ, ppm: 10.77 (broad s, 1H), 10.14 (s, 1H), 9.14 (s, 1H), 8.81 (d, J 6.0 Hz, 1H), 8.78 (d, J 8.0 Hz, 1H), 8.04 (dd, J 1 8.0 Hz, J 2 6.0 Hz, 1H), 7.88 (s, 1H), 7.75 (s, 1H), 4.95 (s, 2H), 4.37 (q, J 7.0 Hz, 2H), 4.02 (s, 3H), 3.49 (broad m, 2H), 3.30 (broad m, 2H), 2.07 (broad m, 2H), 1.95 (broad m, 2H), 1.43 (t, J 7.0 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 380. $C_{22}H_{25}N_3O_3$.

5-Hydroxy-1-methyl-2-(morpholinomethyl)-6-(pyridin-3-yl)-1*H*-indole-3-carboxylic acid ethyl ester (7c). ^{1}H NMR (DMSO-d_6), δ, ppm: 9.38 (s, 1H), 8.81 (s, 1H), 8.49 (m, 1H), 8.02 (m, 1H), 7.63 (s, 1H), 7.50 (s, 1H), 7.43 (m, 1H), 4.29 (dk, J_1 7.2 Hz, J_2 1.6 Hz, 2H), 4.08 (s, 2H), 3.83 (d, J 1.6 Hz, 3H), 3.54 (broad m, 4H), 2.46 (broad m, 4H), 1.39 (dt, J_1 7.2 Hz, J_2 1.6 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 396. $C_{22}H_{25}N_3O_4$.

2-((Dimethylamino)methyl)-5-hydroxy-1-methyl-6-(pyridin-4-yl)-1*H*-indole-3-carboxylic acid ethyl ester dihydrochloride (7d). ^{1}H NMR (DMSO-d_6), δ, ppm: 10.42 (s, 1H), 10.22 (broad s, 1H), 8.90 (d, J 6.2 Hz, 2H), 8.39 (d, J 6.2 Hz, 2H), 7.99 (s, 1H), 7.79 (s, 1H), 4.88 (s, 2H), 4.38 (q, J 7.2 Hz, 2H), 4.01 (s, 3H), 2.86 (s, 6H), 1.44 (t, J 7.2 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 354. $C_{20}H_{23}N_3O_3$.

5-Hydroxy-1-methyl-6-(pyridin-4-yl)-2-(pyrrolidin-1-ylmethyl)-1*H*-indole-3-carboxylic acid ethyl ester dihydrochloride (7e). ^{1}H NMR (DMSO-d_6), δ, ppm: 10.69 (broad s, 1H), 10.40 (s, 1H), 8.90 (d, J 6.0 Hz, 2H), 8.40 (d, J 6.0 Hz, 2H), 7.99 (s, 1H), 7.78 (s, 1H), 4.96 (s, 2H), 4.38 (q, J 7.2 Hz, 2H), 4.04 (s, 3H), 3.49 (broad m, 2H), 3.32 (broad m, 2H), 2.07 (broad m, 2H), 1.95 (broad m, 2H), 1.43 (t, J 7.2 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 380. $C_{22}H_{25}N_3O_3$.

2-((Dimethylamino)methyl)-5-hydroxy-1-isopropyl-6-(pyridin-3-yl)-1*H*-indole-3-carboxylic acid ethyl ester dihydrochloride (7f). ^{1}H NMR (DMSO-d_6), δ, ppm: 10.16 (s, 1H), 9.76 (broad s, 1H), 9.11 (d, J 1.2 Hz, 1H), 8.83 (d, J 5.6 Hz, 1H), 8.75 (d, J 8.0 Hz, 1H), 8.05 (dd, J_1 8.0 Hz, J_2 5.6 Hz, 1H), 7.92 (s, 1H), 7.81 (s, 1H), 5.12 (m, 1H), 4.90 (d, J 2.4 Hz, 2H), 4.38 (q, J 7.0 Hz, 2H), 2.85 (s, 6H), 1.66 (d, J 6.8 Hz, 6H), 1.44 (t, J 7.0 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 382. $C_{22}H_{27}N_3O_3$.

2-((Dimethylamino)methyl)-5-hydroxy-6-(pyridin-3-yl)-1-cyclopropyl-1*H*-indole-3-carboxylic acid ethyl ester dihydrochloride (7g). ^{1}H NMR (DMSO-d_6), δ, ppm: 10.15 (s, 1H), 10.10 (broad s, 1H), 9.12 (d, J 1.6 Hz, 1H), 8.82 (d, J 5.6 Hz, 1H), 8.73 (d, J 8.0 Hz, 1H), 8.04 (dd, J_1 8.0 Hz, J_2 5.6 Hz, 1H), 7.79 (s, 1H), 7.76 (s, 1H), 4.89 (d, J 4.0 Hz, 2H), 4.37 (q, J 7.2 Hz, 2H), 3.70 (m, 1H), 2.88 (d, J 4.0 Hz, 6H), 1.42 (t, J 7.2 Hz, 3H), 1.34 (m, 2H), 1.12 (m, 2H). GC-MS (ESI) $[M + H]^+$ 380. $C_{22}H_{25}N_3O_3$.

2-((Dimethylamino)methyl)-5-hydroxy-6-(pyridin-3-yl)-1-cyclohexyl-1*H*-indole-3-carboxylic acid ethyl ester dihydrochloride (7h). ^{1}H NMR (DMSO-d_6), δ, ppm: 10.11 (s, 1H), 9.62 (broad s, 1H), 9.09 (m, 1H), 8.82 (m, 1H), 8.70 (broad m, 1H), 8.03 (broad m, 1H), 7.90 (s, 1H), 7.79 (s, 1H), 4.92 (d, J 2.6 Hz, 2H), 4.64 (broad m, 1H), 4.39 (q, J 7.2 Hz, 2H), 2.86 (d, J 2.6 Hz, 6H), 2.41 (m, 2H), 1.85 (m, 2H), 1.82 (m, 2H), 1.67 (m, 2H), 1.44 (t, J 7.2 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 422. $C_{25}H_{31}N_3O_3$.

2-((Dimethylamino)methyl)-5-hydroxy-6-(pyridin-3-yl)-1-phenyl-1*H*-indole-3-carboxylic acid ethyl ester dihydrochloride (7i). ^{1}H NMR (DMSO-d_6), δ, ppm: 10.27 (s, 1H), 9.51 (broad s, 1H), 8.96 (s, 1H), 8.74 (d, J 5.2 Hz, 1H), 8.53 (d, J 7.6 Hz, 1H), 7.92 (dd, J_1 7.6 Hz, J_2 5.2 Hz, 1H), 7.86 (s, 1H), 7.66 (m, 5H), 7.12 (s, 1H), 4.57 (s, 2H), 4.44 (q, J 6.8 Hz, 2H), 2.65 (s, 6H), 1.47 (t, J 6.8 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 416. $C_{25}H_{25}N_3O_3$.

2-Dimethylaminomethyl-5-hydroxy-1-methyl-6-fluoro-1*H*-indole-3-carboxylic acid ethyl ester hydrochloride (11). Acetic anhydride (0.358 ml, 3.74 mmol) was added to a solution of 470 mg (1.87 mmol) of 5-hydroxy-1,2-dimethyl-6-fluoro-1*H*-indole-3-carboxylic acid ethyl ester (compound **8**) in 5 ml of pyridine. The reaction was mixed for 12 h and poured into 10 ml of 5% $NaHCO_3$ solution; the resulting precipitate was collected by filtration, washed with water, and dried in vacuo. This produced 5-acetoxy-1,2-dimethyl-6-fluoro-1*H*-indole-3-carboxylic acid (compound 9), GC-MS (ESI) $[M + H]^+$ 294 ($C_{15}H_{16}FNO_4$). Compound 9 (380 mg, 1.3 mmol) was boiled with NBS (277 mg, 1.56 mmol) in 20 ml of CCl_4 for 4 h. After cooling, the precipitate was collected by filtration and washed with benzene. The filtrate was evaporated in vacuo and recrystallized from heptane. The produced 5-acetoxy-2-bromomethyl-1-methyl-6-fluoro-1*H*-indole-3-carboxylic acid (compound **10**), ^{1}H NMR ($CDCl_3$), δ, ppm: 7.86 (d, J 8.0 Hz, 1H), 7.13 (d, J 10.0 Hz, 1H), 5.11 (s, 2H), 4.40 (q, J 7.0 Hz, 2H), 3.76 (s, 3H), 2.35 (s, 3H), 1.43 (t, J 7.0 Hz, 3H). Dimethylamine (3.6 ml, 1 M) solution in benzene was added to a solution of compound 10 (335 mg, 0.9 mmol) in 15 ml of benzene. The reaction was mixed for 24 h and then evaporated in vacuo; the residue was dissolved in 25 ml of dichloromethane, washed with 10% potash solution, dried over Na_2SO_4, and evaporated in vacuo. This produced compound **11**, which was converted to the hydrochloride by addition of an equivalent quantity of 3 M HCl solution in dioxane to 5 ml of a solution of compound **11** in acetone; the resulting precipitate was collected by filtration, washed with ether, and dried in vacuo. This was recrystallized from absolute ethanol. The procedure produced 214 mg (72%) of compound 11

as the hydrochloride. ^{1}H NMR (DMSO-d_6), δ, ppm: 10.11 (broad s, 1H), 9.78 (s, 1H), 7.64 (d, J 8.8 Hz, 1H), 7.56 (d, J 11.6 Hz, 1H), 4.81 (d, J 5.6 Hz, 2H), 4.34 (q, J 7.2 Hz, 2H), 3.88 (s, 3H), 2.82 (d, J 4.8 Hz, 6H), 1.40 (t, J 7.2 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 295. $C_{15}H_{19}FN_2O_3$.

6-Acetamido-2-((dimethylamino)methyl)-5-hydroxy-1-methyl-1*H*-indole-3-carboxylic acid ethyl ester hydrochloride (16). A mixture of 5 g (18.2 mmol) of compound 2a and 5 ml of acetic anhydride was heated to dissolution and then cooled in an ice bath. A mixture of 1.260 g of fuming nitric acid and 1.7 ml of acetic anhydride previously prepared by cooling with ice and salt was then slowly added dropwise. At the end of the reaction (monitored by GC-MS), the mixture was poured into water, extracted with ethyl acetate, washed with water, dried over Na_2SO_4, evaporated in vacuo, and chromatographed on silica gel eluted with hexane:ethyl acetate:AcOH (2:1:0.01). This produced 2.4 g (41%) 5-acetoxy-1,2-dimethyl-6-nitro-1*H*-indole-3-carboxylic acid ethyl ester (compound **12**). ^{1}H NMR (DMSO-d_6), δ, ppm: 8.50 (s, 1H), 7.78 (s, 1H), 4.32 (q, J 7.2 Hz, 2H), 3.85 (s, 3H), 2.78 (s, 3H), 2.35 (s, 3H), 1.35 (t, J 7.0 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 321. $C_{15}H_{16}N_2O_6$. NBS (374 mg, 2.1 mmol) was added to a solution of 640 mg (2 mmol) of compound **12** in 15 ml of CCl_4. The reaction was mixed at room temperature for 3 h; the resulting succinimide precipitate was collected by filtration and washed with CCl_4; the filtrate was evaporated in vacuo. The resulting compound **13** was dissolved in 10 ml of benzene and supplemented with 6 ml of 1 M dimethylamine solution in benzene. The reaction was mixed for 0.5 h, filtered, and evaporated in vacuo. This produced 650 mg (90%) of unpurified 5-acetoxy-2-((dimethylamino)methyl)-1-methyl-6-nitro-1*H*-indole-3-carboxylic acid (compound **14**). GC-MS (ESI) $[M + H]^+$ 364. $C_{17}H_{21}N_3O_6$. Pd/C (10%, 65 mg) was added to a solution of 650 mg (1.79 mmol) of compound **14** in 20 ml of ethanol and the reaction was mixed under hydrogen to completion (monitored by GC/MS). On completion, the reaction mix was filtered through Celite and evaporated in vacuo. This produced 500 mg (96%) unpurified 6-amino-2-((dimethylamino)methyl)-5-hydroxy-1-methyl-1*H*-indole-3-carboxylic acid ethyl ester (compound **15**), GC-MS (ESI) $[M + H]^+$ 292. $C_{15}H_{21}N_3O_3$. Acetic anhydride (0.26 ml, 2.58 mmol) was added dropwise with intense mixing to a solution of 500 mg (1.72 mmol) of compound **15** in 20 ml of ethanol. On completion of the reaction, the solution was evaporated in vacuo and the residue was purified by HPLC. This produced compound **16**, which was converted to the hydrochloride by addition of the equivalent quantity of 3 M HCl in dioxane to a solution of base 16 in 5 ml of acetone; the resulting precipitate was collected by filtration, washed with ether, and dried in vacuo. This produced 260 mg (41%) of compound **16** as the hydrochloride. ^{1}H NMR (DMSO-d_6), δ, ppm: 9.93 (broad s, 1H), 9.82 (broad s, 1H), 9.24 (s, 1H), 8.21 (s, 1H), 7.57 (s, 1H), 4.81 (d, J 4.8 Hz, 2H), 4.35 (q, J 7.0 Hz, 2H), 3.84 (s, 3H), 2.83 (d, J 4.8 Hz, 6H), 2.15 (s, 3H), 1.41 (t, J 7.0 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 334. $C_{17}H_{23}N_3O_4$.

Hydrochlorides of substitute ethyl esters of 2-((dimethylamino)methyl)-5-methoxy-1*H*-indole-3-carboxylic acid (17a – f), general method. A mixture of 0.5 mmol of the corresponding compound **4** or 7, 278 mg (1 mmol) of trimethylphenylammonium tosylate, and 154 mg (0.5 mmol) of Cs_2CO_3 in 2 ml of DMF was mixed at 100°C for 12 h. After cooling, the mixture was evaporated in vacuo and the residue was extracted with acetone, filtered, and evaporated in vacuo. This produced compounds **17a – f**, which were converted to the hydrochlorides by addition of the equivalent quantity of 3 M HCl solution in dioxane to solutions of bases **17a – f** in 5 ml of acetonitrile; the resulting precipitates were collected by filtration, washed with ether, and dried in vacuo. This yielded compounds **17a – f** as the hydrochlorides.

2-((Dimethylamino)methyl)-1-methyl-5-methoxy-6-(pyridin-3-yl)-1*H*-indole-3-carboxylic acid ethyl ester dihydrochloride (17a). ^{1}H NMR (DMSO-d_6), δ, ppm: 10.16 (broad s, 1H), 8.77 (s, 1H), 8.56 (d, J 4.8 Hz, 1H), 8.00 (d, J 8.0 Hz, 1H), 7.73 (s, 1H), 7.70 (s, 1H), 7.50 (dd, J_1 8.0 Hz, J_2 4.8 Hz, 1H), 4.87 (s, 2H), 4.40 (q, J 7.2 Hz, 2H), 3.99 (s, 3H), 3.84 (s, 3H), 2.84 (s, 6H), 1.44 (t, J 7.2 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 368. $C_{21}H_{25}N_3O_3$.

2-((Dimethylamino)methyl)-1-isopropyl-5-methoxy-6-(pyridin-3-yl)-1*H*-indole-3-carboxylic acid ethyl ester dihydrochloride (17b). ^{1}H NMR (MeOH-d 4), δ, ppm: 9.15 (s, 1H), 8.90 (d, J 8.0 Hz, 1H), 8.85 (d, J 5.6 Hz, 1H), 8.19 (dd, J_1 8.0 Hz, J_2 5.6 Hz, 1H), 8.00 (s, 1H), 7.89 (s, 1H), 5.15 (m, 1H), 4.97 (s, 2H), 4.55 (q, J 7.2 Hz, 2H), 3.96 (s, 3H), 3.04 (s, 6H), 1.79 (d, J 6.8 Hz, 6H), 1.55 (t, J 7.2 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 396. $C_{23}H_{29}N_3O_3$.

2-((Dimethylamino)methyl)-5-methoxy-6-(pyridin-3-yl)-1-cyclopropyl-1*H*-indole-3-carboxylic acid ethyl ester dihydrochloride (17c). ^{1}H NMR (DMSO-d_6), δ, ppm: 10.21 (broad s, 1H), 9.04 (s, 1H), 8.82 (d, J 5.6 Hz, 1H), 8.70 (d, J 8.0 Hz, 1H), 7.98 (dd, J_1 8.0 Hz, J_2 5.6 Hz, 1H), 7.82 (s, 1H), 7.74 (s, 1H), 4.92 (d, J 3.6 Hz, 2H), 4.40 (q, J 7.0 Hz, 2H), 3.86 (s, 3H), 3.74 (m, 1H), 2.88 (d, J 3.6 Hz, 6H), 1.43 (t, J 7.0 Hz, 3H), 1.36 (m, 2H), 1.13 (m, 2H). GC-MS (ESI) $[M + H]^+$ 394. $C_{23}H_{27}N_3O_3$.

2-((Dimethylamino)methyl)-5-methoxy-6-(pyridin-3-yl)-1-cyclohexyl-1*H*-indole-3-carboxylic acid ethyl ester dihydrochloride (17d). ^{1}H NMR (DMSO-d_6), δ, ppm: 9.92 (broad s, 1H), 9.05 (s, 1H), 8.84 (d, J 4.8 Hz, 1H), 8.62 (d, J 7.6 Hz, 1H), 8.03 (dd, J_1 7.6 Hz, J_2 4.8 Hz, 1H), 7.96 (s, 1H), 7.78 (s, 1H), 4.96 (s, 2H), 4.68 (broad m, 1H), 4.41 (q, J 6.8 Hz, 2H), 3.86 (s, 3H), 2.86 (s, 6H), 2.40 (m, 2H), 1.84 (m, 4H), 1.65 (m, 2H), 1.45 (t, J 6.8 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 436. $C_{26}H_{33}N_3O_3$.

2-((Dimethylamino)methyl)-5-methoxy-6-(pyridin-3-yl)-1-phenyl-1*H*-indole-3-carboxylic acid ethyl ester dihydrochloride (17e). ^{1}H NMR (DMSO-d_6), δ, ppm: 9.58 (broad s, 1H), 8.89 (s, 1H), 8.74 (d, J 4.8 Hz, 1H), 8.41 (d, J 7.6 Hz, 1H), 7.87 (dd, J_1 7.6 Hz, J_2 4.8 Hz, 1H), 7.82 (s, 1H), 7.67 (m, 5H), 7.15 (s, 1H), 4.60 (d, J 3.2 Hz, 2H), 4.47 (q, J 7.0 Hz, 2H), 3.90 (s, 3H), 2.65 (d, J 3.2 Hz, 6H), 1.48 (t, J 7.0 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 430. $C_{26}H_{27}N_3O_3$.

2-((Dimethylamino)methyl)-1-methyl-5-methoxy-6-fluoro-1*H*-indole-3-carboxylic acid ethyl ester hydrochloride (17f). ^{1}H NMR (DMSO-d_6), δ, ppm: ^{1}H NMR (DMSO-d_6), δ, ppm: 9.76 (broad s, 1H), 7.69 (d, J 11.6 Hz, 1H), 7.67 (d, J 8.4 Hz, 1H), 4.83 (s, 2H), 4.37 (q, J 7.0 Hz, 2H), 3.90 (s, 3H), 3.89 (s, 3H), 2.84 (s, 6H), 1.42 (t, J 7.0 Hz, 3H). GC-MS (ESI) $[M + H]^+$ 309. $C_{16}H_{21}FN_2O_3$.

Hydrochlorides of substituted ethyl esters of 2-((dimethylamino)methyl)-5-isopropyloxy-1*H*-indole-3-carboxylic acids (17g, h), general method. Cesium carbonate (195 mg, 0.6 mmol) and isopropyl bromide (156 mg. 0.63 mmol) were added to a solution of 0.4 mmol of compound 4o or 7a (70 mg, 0.2102 mmol) in 3 ml of DMF. The reaction was mixed at room temperature to completion of the reaction (2 – 4 days, monitored by GC-MS). At completion, the reaction mix was poured into 20 ml of 5% potash solution, extracted with ethyl acetate, dried over Na_2SO_4, and evaporated in vacuo. Product was purified by HPLC. This yielded compounds **17g, h** with yields of 33 – 46%, which were converted to hydrochlorides by addition of the equivalent quantity of 3 M HCl solution in dioxane to solutions of bases of **17g, h** in 5 ml of acetone; the resulting precipitates were collected by filtration, washed with ether, and dried in vacuo. Compounds **17g, h** were obtained as the hydrochlorides.

2-((Dimethylamino)methyl)-5-isopropoxy-1-methyl-6-(pyridin-3-yl)-1*H*-indole-3-carboxylic acid ethyl ester dihydrochloride (17g). ^{1}H NMR (AcOH-d_4), δ, ppm: 9.15 (d, J 1.6 Hz, 1H), 8.88 (dt, J_1 8.0 Hz, J_2 1.6 Hz, 1H), 8.82 (d, J 5.6 Hz, 1H), 8.15 (dd, J_1 8.0 Hz, J_2 5.6 Hz, 1H), 7.87 (s, 1H), 7.83 (s, 1H), 4.93 (s, 2H), 4.73 (m, 1H), 4.52 (q, J 7.2 Hz, 2H), 4.03 (s, 3H), 3.03 (s, 6H), 1.53 (t, J 7.2 Hz, 3H), 1.36 (d, J 6.0 Hz, 6H). GC-MS (ESI) $[M + H]^+$ 396. $C_{23}H_{29}N_3O_3$.

6-Acetamido-2-((dimethylamino)methyl)-5-isopropoxy-1-methyl-1*H*-indole-3-carboxylic acid ethyl ester hydrochloride (17h). ^{1}H NMR (MeOH-d_4), δ, ppm: 8.33 (s, 1H), 7.66 (s, 1H), 4.85 (s, 2H), 4.68 (m, 1H), 4.49 (q, J 7.2 Hz, 2H), 3.90 (s, 3H), 2.99 (s, 6H), 2.25 (s, 6H), 1.51 (t, J 7.2 Hz, 3H), 1.44 (d, J 6.0 Hz, 6H). GC-MS (ESI) $[M + H]^+$ 376. $C_{20}H_{29}N_3O_4$.

2-((Dimethylamino)methyl)-5-hydroxy-1-methyl-6-(pyridin-3-yl)-1*H*-indole dihydrochloride (18). A solution of 500 mg (1.4 mmol) of compound 7a in 10 ml of concentrated HCl was mixed at 100-110°C to complete conversion of the starting material to the target product, monitored by GC-MS (12 – 14 h). The resulting indigo-blue solution was held in an ultrasonic bath on ice for 2 h; the resulting precipitate was collected by filtration, washed with cooled concentrated HCl to complete decolorization, washed with ether, and dried in vacuo. This produced 420 mg (84%) of compound **18**, ^{1}H NMR (DMSO-d_6), δ, ppm: 10.92 (broad s, 1H), 9.69 (s, 1H), 9.06 (s, 1H), 8.73 (d, J 6.0 Hz, 1H), 8.62 (d, J 8.4 Hz, 1H), 7.92 (dd, J_1 8.4 Hz, J_2 6.0 Hz, 1H), 7.66 (s, 1H), 7.17 (s, 1H), 6.66 (s, 1H), 4.52 (s, 2H), 3.85 (s, 3H), 2.76 (s, 6H). GC-MS (ESI) $[M + H]^+$ 326. GC-MS (ESI) $[M + H]^+$ 282. $C_{17}H_{19}N_3O$. **2-((Dimethylamino)methyl)-5-hydroxy-1-methyl-6-(pyridin-3-yl)-1*H*-indole-3-carboxylic acid dihydrochloride (19)**. A solution of 500 mg (1.4 mmol) of compound 7a in 15 ml of 10% NaOH solution was boiled with mixing to complete conversion of the ester to acid **19** (2 – 3 h), cooled in an ice bath, and neutralized with 2.22 ml of acetic acid. The mixture was held in a refrigerator for 12 h and the resulting precipitate was collected by filtration, washed with iced water, and dried in vacuo. This produced 150 mg (33%) of acid **19**, ^{1}H NMR (DMSO-d_6), δ, ppm: 13.45 (broad s, 1H), 9.25 (s, 1H), 8.78 (s, 1H), 8.45 (d, J 3.6 Hz, 1H), 8.00 (d, J 7.6 Hz, 1H), 7.71 (s, 1H), 7.38 (m, 2H), 4.04 (s, 2H), 3.81 (s, 3H), 2.34 (s, 6H). GC-MS (ESI) $[M + H]^+$ 326 $C_{18}H_{19}N_3O_3$. Acid **19** was dissolved in a minimal volume of glacial acetic acid, cooled, and supplemented, with intense mixing and cooling, with 5 ml of 6 M HCl/EtOAc-EtOH solution (prepared in anhydrous conditions by slow dropwise addition of the required quantity of AcCl to anhydrous ethanol with cooling, followed by mixing at 10 – 15°C in a closed vessel for 5 h and holding at least overnight in a refrigerator). When required, compound **19** dihydrochloride was re-precipitated with ether (or acetone), the precipitate was collected by filtration, thoroughly washed with acetone and ether, and dried to complete removal of traces of solvents. This produced 150 mg of compound **19** as the dihydrochloride. ^{1}H NMR (DMSO-d_6), δ, ppm: 10.11 (broad m, 2H), 9.13 (s, 1H), 8.80 (d, J 5.6 Hz, 1H), 8.75 (d, J 8.0 Hz, 1H), 8.02 (dd, J_1 8.0 Hz, J_2 5.6 Hz, 1H), 7.87 (s, 1H), 7.74 (s, 1H), 4.86 (s, 2H), 3.98 (s, 3H), 2.84 (s, 6H). GC-MS (ESI) $[M + H]^+$ 326. $C_{18}H_{19}N_3O_3$.

Hydrochlorides of 2-((dimethylamino)methyl)-5-hydroxy-1-methyl-6-(pyridin-3-yl)-1*H*-indole-3-carboxamides (20a-f), general method. A solution of 80 mg (0.24 mmol) of acid 19, 71 mg (0.35 mmol) of N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride, and 50 mg (0.35 mmol) of N-hydroxybenzotriazole in 2 ml of DMF was supplemented with 0.27 mmol of the corresponding amine (ammonia and methylamine were used as the hydrochlorides) and 0.07 ml (0.27 mmol, doubled for hydrochlorides) of triethylamine. Reactions were mixed for 24 h and evaporated in vacuo, and target products **20a – f** were extracted by HPLC. Hydrochlorides were prepared by addition of the equivalent quantity of 3 M HCl solution in dioxane to solutions of the bases in 5 ml of acetone; the resulting precipitates were collected by filtration, washed with ether, and dried in vacuo. This yielded compounds **20a – f** as the hydrochlorides with yields of 43 – 74%.

2-((Dimethylamino)methyl)-5-hydroxy-1-methyl-6-(pyridin-3-yl)-1*H*-indole-3-carboxamide dihydrochloride (20a). ^{1}H NMR (DMSO-d_6), δ, ppm: 10.53 (broad s, 1H), 10.12 (broad s, 1H), 9.14 (s, 1H), 8.81 (m, 2H), 8.06 (dd, J_1 8.0 Hz, J_2 5.6 Hz, 1H), 7.89 (s, 1H), 7.71 (broad s, 2H), 7.51 (s, 1H), 4.73 (d, J 3.2 Hz, 2H), 3.95 (s, 3H), 2.82 (d, J 3.2 Hz, 6H). GC-MS (ESI) $[M + H]^+$ 325. $C_{18}H_{20}N_4O_2$.

2-((Dimethylamino)methyl)-5-hydroxy-*N*,1-dimethyl-6-(pyridin-3-yl)-1*H*-indole-3-carboxamide dihydrochloride (20b). ^{1}H NMR (DMSO-d_6), δ, ppm: 10.34 (broad s, 1H), 9.60 (s, 1H), 8.82 (s, 1H), 8.52 (broad s, 1H), 8.21 (broad s, 1H), 8.04

(broad m, 1H), 7.63 (s, 1H), 7.47 (broad m, 1H), 7.41 (s, 1H), 4.68 (s, 2H), 3.91 (s, 3H), 2.88 (s, 3H), 2.81 (s, 6H). GC-MS (ESI) $[M + H]^+$ 339. $C_{19}H_{22}N_4O_2$.

2-((Dimethylamino)methyl)-5-hydroxy-*N*,*N*,1-trimethyl-6-(pyridin-3-yl)-1*H*-indole-3-carboxamide dihydrochloride (20c). ^{1}H NMR (DMSO-d_6), δ, ppm: 10.60 (broad s, 1H), 10.20 (broad s, 1H), 9.16 (s, 1H), 8.82 (m, 2H), 8.08 (m, 1H), 7.88 (s, 1H), 7.20 (s, 1H), 4.64 (s, 2H), 3.96 (s, 3H), 3.07 (s, 6H), 2.79 (s, 6H). GC-MS (ESI) $[M + H]^+$ 353. $C_{20}H_{24}N_4O_2$.

(2-((Dimethylamino)methyl)-5-hydroxy-1-methyl-6-(pyridin-3-yl)-1*H*-indole-3-yl)(pyrrolidin-1-yl)methanone dihydrochloride (20d). ^{1}H NMR (DMSO-d_6), δ, ppm: 10.61 (broad s, 1H), 10.14 (broad s, 1H), 9.15 (s, 1H), 8.81 (m, 2H), 8.06 (dd, J_1 8.0 Hz, J_2 5.6 Hz, 1H), 7.87 (s, 1H), 7.20 (s, 1H), 4.65 (s, 2H), 3.96 (s, 3H), 3.58 (broad m, 2H), 3.46 (broad m, 2H), 2.79 (s, 6H), 1.89 (broad m, 4H). GC-MS (ESI) $[M + H]^+$ 379. $C_{22}H_{26}N_4O_2$.

(2-((Dimethylamino)methyl)-5-hydroxy-1-methyl-6-(pyridin-3-yl)-1*H*-indole-3-yl)(morpholino)methanone dihydrochloride (20e). GC-MS (ESI) $[M + H]^+$ 395. $C_{22}H_{26}N_4O_3$.

2-((Dimethylamino)methyl)-*N*-(3-(dimethylamino)propyl)-5-hydroxy-*N*,1-dimethyl-6-(pyridin-3-yl)-1*H*-indole-3-carboxamide trihydrochloride (20f). ^{1}H NMR (DMSO-d_6), δ, ppm: 10.76 (broad s, 1H), 10.54 (broad s, 1H), 10.29 (broad s, 1H), 9.14 (s, 1H), 8.81 (d, J 5.6 Hz, 1H), 8.78 (d, J 8.0 Hz, 1H), 8.04 (dd, J_1 8.0 Hz, J_2 5.6 Hz, 1H), 7.86 (s, 1H), 7.36 (broad s, 1H), 4.66 (s, 2H), 3.97 (s, 3H), 3.60 (broad m, 4H), 3.08 (s, 3H), 2.80 (s, 12H), 2.10 (broad m, 2H). GC-MS (ESI) $[M + H]^+$ 424. $C_{24}H_{33}N_5O_2$.

2-((Dimethylamino)methyl)-5-hydroxy-1-methyl-6-(pyridin-3-yl)-3-cyano-1*H*-indole dihydrochloride (24).

A solution of 0.252 ml (4.88 mmol) of bromine in 5 ml of CCl_4 was added dropwise to a boiling solution of 750 mg (2.44 mmol) of 1,2-dimethyl-3-cyano-1*H*-indole-5-yl acetate **21** in 75 ml of CCl_4 and the mixture was boiled for 12 h. The cooled reaction was filtered and evaporated in vacuo; the precipitate of compound **22** was dissolved in 15 ml of THF and 7.3 ml of 1 M dimethylamine solution in benzene was added and the reaction was mixed for 0.5 h. After evaporation in vacuo, the residue was chromatographed on silica gel impregnated with triethylamine with hexane:ethyl acetate:triethylamine (3:1:0.1) as eluant. This produced 419 mg (49%) of 6-bromo-2-((dimethylamino)methyl)-1-methyl-3-cyano-1*H*-indol-5-yl acetate (compound 23). ^{1}H NMR ($CDCl_3$), δ, ppm: 7.67 (s, 1H), 7.49 (s, 1H), 4.70 (s, 2H), 3.82 (s, 3H), 2.40 (s, 3H). GC-MS (ESI) $[M + H]^+$ 350, 352. $C_{15}H_{16}BrN_3O_2$. Product **23** was converted to 2-((dimethylamino)methyl)-5-hydroxy-1-methyl-6-(pyridin-3-yl)-1*H*-indole-3-carbonitrile dihydrochloride (compound **24**) by analogy with the synthesis of compounds **7a – i**. The yield was 76%. ^{1}H NMR (MeOH-d_4), δ, ppm: 9.24 (s, 1H), 8.97 (d, J 8.0 Hz, 1H), 8.82 (d, J 5.6 Hz, 1H), 8.17 (dd, J_1 8.0 Hz, J_2 5.6 Hz, 1H), 7.91 (s, 1H), 7.27 (s, 1H), 4.82 (s, 2H), 4.05 (s, 3H), 3.08 (s, 6H). GC-MS (ESI) $[M + H]^+$ 307. $C_{18}H_{18}N_4O$.

EXPERIMENTAL BIOLOGICAL SECTION

***In vitro* hepatotoxicity of the compounds synthesized here** was determined by comparison of their abilities to kill primary human hepatocytes (obtained from Life Technologies, USA) in cell culture [9]. Hepatocytes were grown in the wells of 96-well plates (20,000 cells/well) and incubated for 1 day at 37°C in an incubator at 100% humidity in an atmosphere of 5% O_2/95% CO_2. After incubation for 1 day, wells were supplemented with compounds at different concentrations (from 100 μM to 0.1 μM with semilogarithmic steps) for assessment of hepatotoxicity. Hepatocytes were incubated in compound-containing medium for 48 h in the same incubation conditions. After incubation, the numbers of live cells were measured using the AlamarBlue fluorescence test as recommended by the manufacturer (Life Technologies, USA). Table 1 shows in vitro hepatotoxicity data for Arbidol and several of the compounds synthesized here as CC_{50} values (concentrations of substance lethal to 50% of cells).

Antiviral activity of compounds synthesized here against bovine viral diarrhea virus (BVDV) was assessed in cultured Madin Darby bovine kidney cell (MDBK cells) infected with BVDV strain NADL (ATCC) using a method described in [4, 10] over the concentration range 0.05 – 100 μM (total of eight serial three-fold dilutions). Synthesized compounds were inactive against BVDV, for example the EC_{50} values (concentrations of substances inhibiting virus reproduction by 50%) and CC_{50} values for compound 6 were >61 μM and 61 μM respectively, compared with >21 μM and 21 μM for compound 7b.

TABLE 1. *In Vitro* Hepatotoxicity[a] of Compounds Synthesized here

Compound	CC_{50}, μM	Compound	CC_{50}, μM	Compound	CC_{50}, μM
Arbidol	3.0	**7a**	> 100	**17f**	> 100
4a	16.1	**7b**	> 100	**18**	18.8
6	> 100	**11**	25.7	**19**	> 100

[a] Measurements from three independent experiments (each in triplicate).

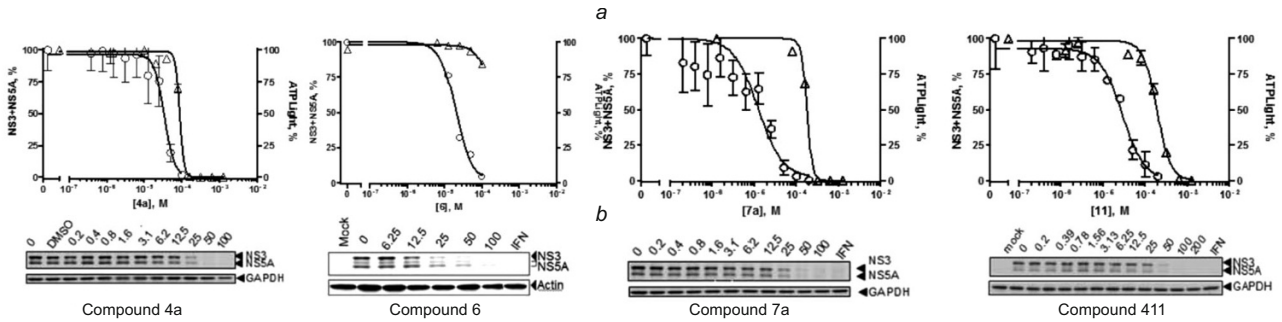


Fig. 1. Concentration relationships of (*a*) anti-HCV activity (0) and cytotoxicity (Δ) of compounds 4a, 6, 7a, and 11 and protein bands obtained by Western blots (*b*). Virus control – Arbidol [8].

Cytotoxicity and antiviral activity against hepatitis C virus (HCV)in Huh7.3 human hepatoma cells by Western blot analysis. The antiviral activity of the compounds synthesized here was assessed in Huh7.3 human hepatoma cells, which are highly sensitive to infection of hepatitis C virus [11]. Cells were cultured in modified Eagle-Dulbecco medium (DMEM) containing 9% calf serum and 1% vital amino acids. Cells were added to each well in six-well plates (200,000 cells/well) and were incubated for one day, after which test compounds at different concentrations were added to cells. Cells were incubated with test compounds for 24 h, after which hepatitis C virus strain JFH-1 (genotype 2a) was added, as described in [12]. Cells were incubated at 37°C for 72 h. When incubation was complete, medium was removed and cells were lysed with buffer containing 50 mM Tris-HCl pH 7.2, 150 mM NaCl, 0.1% sodium dodecylsulfate, 0.1% sodium deoxycholate, 1% Triton X-100, and 17.4 mg/ml phenylmethylsulfonylfluoride (PMSF). Protein contents were assayed using a BCA protein assay kit following the manufacturer's instructions (Pierce Biotechnology, USA) using BSA (bovine serum albumin) as standard. Before loading samples onto gels, the concentrations of each sample were corrected such that each well was loaded with 10 μg protein. Samples were mixed with an equal volume of reducing buffer, heated at 95°C for 7 min, and then subjected to electrophoresis in 4 – 20% tris-glycine buffer (Invitrogen, USA). Separated proteins were transferred to 0.45 μm nitrocellulose membranes (Pierce, USA), by semidry transfer. After transfer, membranes were blocked with Superblock buffer (Pierce, USA) and incubated with primary mouse antibodies specific for viral protein NS5A (Biodesign International, USA; diluted 1:1000) and core antigen (Affinity Bioreagents, USA; diluted 1:1000) and additionally incubated at room temperature for 1 h. Membranes were then incubated with secondary antibodies to mouse immunoglobulins (IgG) conjugated with horseradish peroxidase (Pierce, USA; diluted 1:10,000). Protein bands were detected using the chemiluminescent reagent LumiGlo (Cell Signaling, USA) and exposure of membranes to X-ray film. Protein bands were analyzed quantitatively using ImageJ [http://rsb.info.nih.gov/ij].

The cytotoxicity of the compounds synthesized here was assessed using the MTT test as described in [13]. Cells were seeded in 96-well plates and test compounds were then added. Plates were incubated at 37°C for 72 h. MTT solution was added to each well (10 μg/well of 5 mg/ml solution) and plates were incubated for a further 4 h at 37°C. At completion of incubation, 100 μl of solubilizing buffer was added to each well (0.01 N HCl, 10% sodium dodecylsulfate). Staining was measured spectrophotometrically at a wavelength of 570 nm. Percentage cytotoxicity was calculated as:

TABLE 2. Antiviral Activity[a] and Selectivity of Compounds against HCV in Huh7.3 Human Hepatoma Cells

Compound	EC_{50}, μM	CC_{50}, μM	TI_{50}
Arbidol	3.9	18.9	4.8
4a	34.2*	87.5*	2.6
6	17.6	235.4**	13.3*
7a	19.6	175.4*	8.9
7b	32.2*	112.5*	3.5
11	28.6*	190.0*	6.6

[a] Measurements from three independent experiments (each in triplicate). Significant differences from Arbidol: * p 0.95; ** p 0.99.

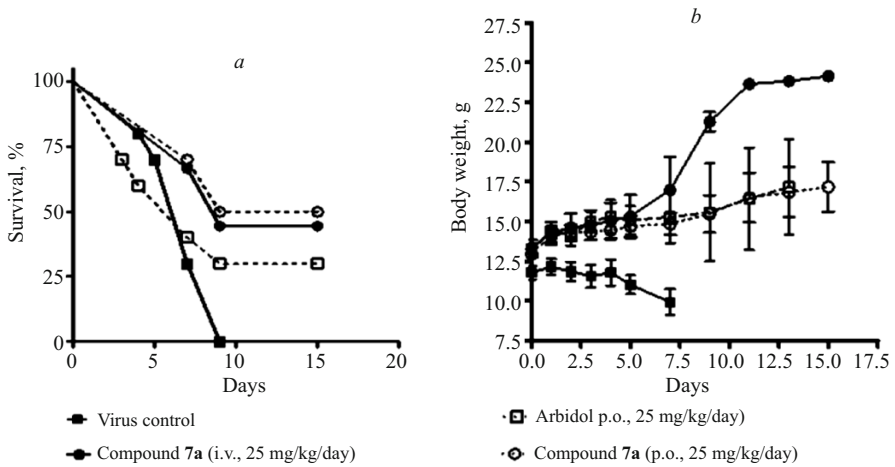


Fig. 2. Effects of Arbidol and compound 7a on the survival (*a*) and body weight (*b*) of mice infected with influenza A/Aichi/2/69 (H3N2) virus after oral and intravenous dosage (experiment 1). The MST of mice in the virus control group was 5.5 days, compared with 7.3 days in the Arbidol group, 10.4 days in the compound 7a (i.v.) group, and 10.9 days in the compound 7a (p.o.) group.

$$\text{Toxicity\%} = \frac{\text{OD}_{570}^{0} - \text{OD}_{570}^{\text{cpd}}}{\text{OD}_{570}^{0}} \cdot 100,$$

where OD_{570} is the optical density measured at 570 nm; the superscript identifies control cells (0) or cells in the presence of test compound (cpd).

Western blot results on the anti-HCV activity of the compounds synthesized here and *in vitro* cytotoxicity results yielded protein bands and plots of concentration vs. anti-HCV activity for compounds (Fig. 1), which were used to determine EC_{50}, CC_{50}, and TI_{50} (therapeutic index) values as shown in Table 2.

Antiviral activity of the compounds synthesized here against influenza A/Aichi/2/69 (H3N2) was determined by immunoenzyme analysis (IEA) in MDCK (Madin-Darby canine kidney) cell cultures as described in [4, 14]. A total of 8 – 10 serial three-fold dilutions from concentrations of 100 to 0.1 μM were prepared. Four repeats were used for each point in this experiment. Deviations from the mean were by no more than 10 – 15%. The results were plotted as dose-response curves, which were used to determine EC_{50} values. Table 3 shows data on the antiviral activity of the most active of the novel compounds.

Cytotoxicity of compounds synthesized here in MDCK cell cultures was determined as described in [4]. A total of five serial three-fold dilutions with concentrations from 15 to 1 μM were prepared. Cytotoxicity data for the compounds most active against influenza A/Aichi/2/69 (H3N2) are shown in Table 3.

Effectiveness of the compounds most active against influenza A/Aichi/2/69 (H3N2) virus in a mouse influenza pneumonia model. The efficacies of compounds in a mouse influenza pneumonia model (mongrel females, mean weight 12 – 15 g) were tested in mice intranasally infected with influenza A/Aichi/2/69 (H2N2) under light anesthesia (10 LD_{50} in 50 μl) as described in [15]. Study compounds **7a** and **11** at doses of 25 and 60 mg/kg/day and reference agent Arbidol were given 24 h before infection, 1 h before infection, 24 h after infection, and then once daily for five days. Oral administration was with single-use insulin syringes with a special needle (lavage). The virus control group consisted of 10 animals, as did the compounds **7a**

TABLE 3. Antiviral Activity[a] of Some of the Compounds Synthesized Here against Influenza A/Aichi/2/69 (H3N2) Virus and their Cytotoxicities and Therapeutic Indexes

Compound	EC_{50}, μM	CC_{50}, μM	TI_{50}
Arbidol	12.9	97	7.5
7a	12.7	188	14.8
7b	18.6	110	5.9
11	4.3*	151	35.1*
17f	> 50	203	

[a] Measurements from three independent experiments (each in triplicate). Significant differences from Arbidol: *p 0.95; **p 0.99.

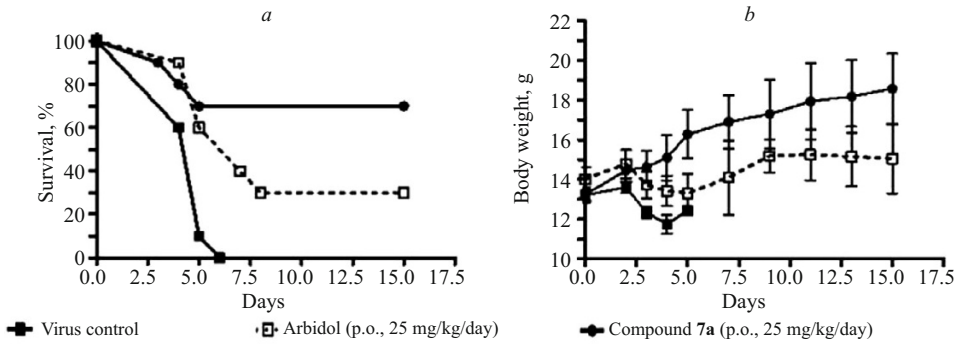


Fig. 3. Effects of Arbidol and compound 7a on the survival (*a*) and body weight (*b*) of mice infected with influenza A/Aichi/2/69 (H3N2) virus after oral dosage (experiment 2). Mean survival time in the virus control group was 3.7 days, compared with 7.9 days in the Arbidol group and 11.4 days for the compound 7a (p.o.) group.

and **11** and Arbidol treatment groups. Treated and control animals were observed daily; mice were weighed daily for the five days after infection and then every second day. The chemotherapeutic activity of compounds in the influenza pneumonia model was evaluated in terms of three criteria: protection from lethal viral infection, increased mean survival time, and changes in weight in groups of animals treated with agents as compared with the control group. The mean survival time (MST) of the mice was calculated as:

$$\text{MDL} = \Sigma f(d-1)/n,$$

where f is the number of mice dying on day d, surviving mice were also included in f and d in this case was 16; n was the number of mice in the group. Decreases or increases in weight were calculated separately for each mouse and expressed as percentages, taking the animal's pre-infection weight as 100%. Mean percentage weight loss or gain was calculated for all mice of each group. These results are presented in Figs. 2 – 4.

RESULTS AND DISCUSSION

Novel 2-aminomethyl-5-hydroxy-1*H*-indole-3-carboxylic acid derivatives **4 – 7**, **11**, **16 – 20**, and **24**, in contrast to Arbidol, generally had low potential hepatotoxicity (CC_{50}). The in vitro hepatotoxicity of compounds **4a**, **11**, and **18** (CC_{50} 16.1, 25.7, and 18.8 μM respectively) was 5.4 – 8.6 times lower than that of Arbidol (CC_{50} = 3 μM). The remaining study compounds had virtually no cytotoxic actions on primary hepatocytes (CC_{50} > 100 μM).

Assessment of antiviral activity showed that the compounds synthesized here were inactive against bovine viral diarrhea virus and had weak activity in relation to the human hepatoma cell line Huh7.3 with high sensitivity to HCV (strain JFH-1, genotype 2a) infection [11, 12, 16, 17]. Only some compounds had activity at micromolar concentrations (Fig. 1, Table 2), though

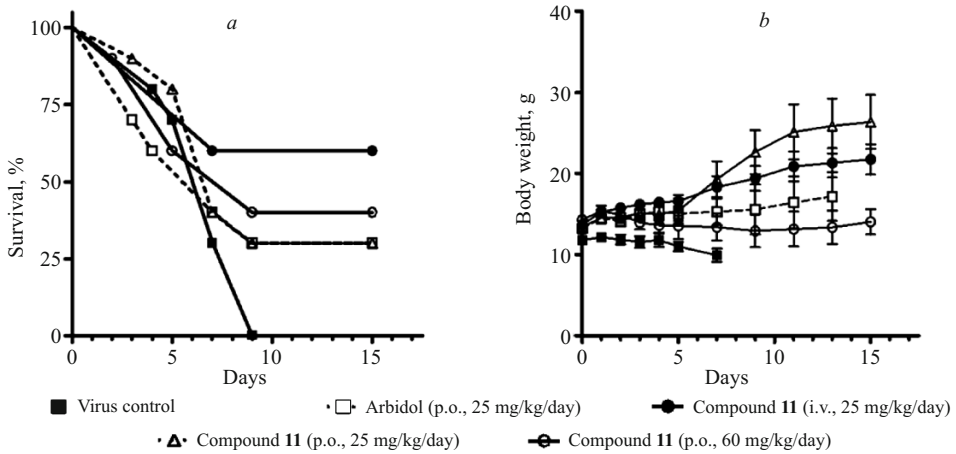


Fig. 4. Effects of Arbidol and compound 11 on the survival (*a*) and body weight (*b*) of mice infected with influenza A/Aichi/2/69 (H3N2) virus after oral and intraperitoneal dosage. Mean survival time in the virus control group was 5.5 days, compared with 7.3 days in the Arbidol (p.o.,

their therapeutic indexes (TI, defined as CC_{50}/E_{C5}) were low (TI_{50} = 2.6 – 13.3). Compound **6** had the greatest therapeutic index, with EC_{50} = 17.6 μM. We note that all test compounds (Table 2) were less active than Arbidol, with EC_{50} = 3.9 μM, while compounds **4a** and **7b** were also less active than Arbidol in terms of therapeutic index (TI_{50} = 2.6 for compound **4a**, TI_{50} = 3.5 for compound **7b**, TI_{50} = 4.8 for Arbidol).

The vast majority of the compounds synthesized here were also shown to be inactive against influenza A/Aichi/2/69 (H3N2) in cell cultures. The only exceptions were compounds **7a**, **7b**, and **11**, compound 11 being more active (EC_{50} = 4.3 μM) and having a larger therapeutic index (TI_{50} = 35.1) than compound 7a (TI_{50} = 14.8); the activity of this compound was essentially the same as that of Arbidol, though its TI was twice that of Arbidol (Table 3).

Compounds **7a** and **11** were then compared with Arbidol in a mouse influenza pneumonia model using animals infected with A/Aichi/2/69 (H3N2) virus. Compound **7a** at a dose of 25 mg/kg/day given i.p. and p.o. was more effective than Arbidol (Fig. 1). Treatment with compound **7a** prolonged the survival time of the mice to 10.4 days with i.v. and 10.9 days with p.o. treatment, as compared with mean survival times of 5.5 days in virus controls and 7.3 days with Arbidol treatment. In another experiment, use of a dose of 25 mg/kg/day (p.o.) of compound 7a showed it to be more effective than Arbidol (Fig 2). In this experiment, compound 7a prolonged survival time to 11.4 days when given p.o., as compared with 3.7 days in the virus control and 7.9 days with Arbidol treatment. It should be noted that prophylaxis and treatment of mice with compound **7a** did not reduce body weight at any point in the observation period (Fig. 1, *b* and Fig. 2, *b*). It should also be noted that the i.v. and p.o. efficacies of compound 7a were similar (Fig. 1, *a* and Fig. 2, *a*), which may point to high bioavailability.

Compound **11** was also effective in the treatment of mice infected with influenza A/Aichi/2/69 (H3N2) virus (Fig. 3). Thus, at a dose of 25 mg/kg/day, mean survival times in mice given this compound i.v. and p.o. were 11.4 and 8.3 days, compared with virus control and treatment with Arbidol, for which mean survival times were 5.5 and 7.3 days respectively. An increase in the dose of compound **11** to 60 mg/kg/day led to some decrease in treatment efficacy, as mean survival time decreased to 9.8 days, which can evidently be explained on the basis that this dose produced some degree of toxicity.

It should also be noted that the efficacy of compound **11**, in contrast to compound 7a, when given i.v. was 1.37 times greater than when given p.o. (Fig. 3, *a*), which may be evidence that it has lower bioavailability than compound **7a**.

Thus, we report the synthesis of a series of novel 2-aminomethyl-5-hydroxy-1*H*-indole-3-carboxylic acid derivatives and studies of their antiviral activity against BVDV, HCV, and influenza A/Aichi/2/69 (H3N2) viruses. Micromolar concentrations of compounds **7a** and **11** were found to suppress the replication of influenza A/Aichi/2/69 (H3N2) virus in MDCK cell cultures. In addition, these compounds were highly effective in the prophylaxis and treatment of mice infected with influenza A/Aichi/2/69 virus, their efficacies being markedly greater than that of the known antiviral drug Arbidol. Our future studies will provide detailed assessment of the pharmacology and toxicology of compounds **7a** and **11**, as well as the antiviral properties of novel 2,4-bis-aminomethyl-5-hydroxy-1*H*-indole-3-carboxylic acid derivatives.

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