SYNTHESIS AND ANTIVIRAL ACTIVITY OF 3-CYANO-2(1H)PYRIDINE SELENONES

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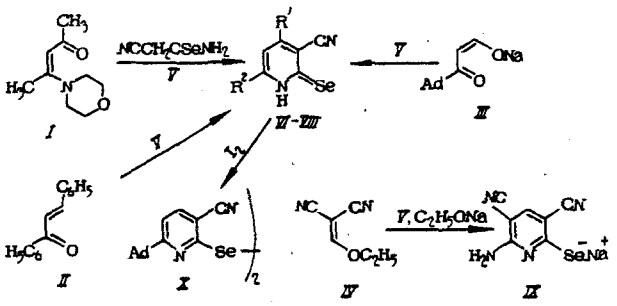
Selenium organic compounds are known to exhibit a high degree of antiviral activity [7, 10, 12]. Such simple and easily available compounds as alkyl (aryl) selenols, selenides, diselenides, selenium-containing aminocarboxylic acids as well as substituted selenophens and selenazoles have been broadly investigated [5, 6]. Many pyridine derivatives exhibit a high degree of biological activity. However, the physiological activity of selecium-containing pyridines has not been studied. We thought that the combination of both these functions into one compound and a study of their properties would be of interest. We therefore synthesized substituted 3-cyano-2(1H)-pyridine selenones and examined the antiviral activity of the resultant compounds.



R^1=CH_3(VI), C_6H_5(VII), H(VIII); R^2=CH_3(VI), C_6H_5(VII), Ad-1(VIII) (Ad – adamantyl)

Substituted 3-cyano-2(1H)-pyridine selenones (VI-VIII) and sodium-6-amino-3,5-dicyanopyridine-2-selenolate (IX) were synthesized by condensing 2-(morpholino)-2-pentenone-4 (I), chalcone (II), sodium-3-(1-adamantyl)-2-propene-3-olate (III), and ethoxymethylene malononitrile (IV) with cyanoselenoacetamide (V). The structure of pyridines VI-IX was confirmed by spectral characteristics (see Experimental).

Pyridine selenones are oxidized by atmospheric oxygen in ethanol or DMFA solutions to the corresponding diselenides. Diselenide X was preliminarily obtained by the iodine oxidation of pyridine selenone VIII in ethanol in the presence of the base.

TABLE 1. 3-Cyano-2(1H)-pyridine Selenones and Certain Derivatives

Compound	mp, °C (solvent)	Empirical formula
IX	>350	$C_7H_3N_4NaSe$
XI	114 (dec.) (methanol)	$C_{10}H_{10}N_2O_2Se$
XVI	28 n-pentane	$C_{11}H_{12}N_2Se$
XXIV	72—74 (methanol)	$C_{14}H_{16}N_2Se$
XXV	204—206 (nitromethane)	$C_{11}H_{12}I_4N_2Se$
XXVI	173—174 (nitromethane)	$C_{14}H_{16}I_4N_2Se$

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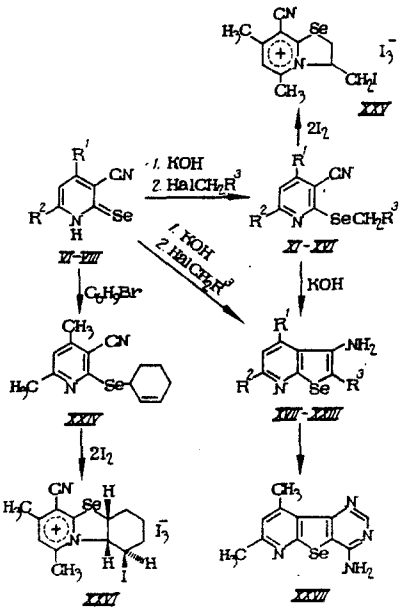

R^1=CH_3(XI—XIV, XVI—XIX), H(XV, XX—XXIII); R^2=CH_3 (XI—XIV, XVI—XIX), Ad(XV, XX—XXIII); R^3=COOH(XI, XXII), CN(XII, XVII), $COOC_2H_5$(XIII,XVIII,XXIII), COC_6H_5 (XIV,XIX,XXI), $CONH_2$(XV,XX), $CH=CH_2$(XVI)

The pyridine selenones VI [8] and VIII were utilized for the synthesis of 2-alkylselenone pyridines, 3-aminoselenonepheno[2,3-b]pyridines, the salts of seleneazolo[3,2-a]-pyridinium and other annelated selenium-containing pyridines. The alkylation of pyridine selenones VI and VIII by halogen alkanes in the presence of an equimolar quantity of KOH and DMFA proceeded selectively on the selenium atom with the formation of 2-alkylselenopyridines (XI-XVI, XXIV). Compounds XII-XV were cyclated in the presence of an aq. KOH solution in DMFA by the Thorpe—Ziegler reaction to substituted 3-aminoselenopheno[2,3-b]pyridines (XVII-XX). In addition, compounds XVII-XXIII were obtained in one stage without removing the 2-alkylselenopyridines. The reaction between the pyridine selenones VI and VIII with alkylhalogenides took place in DMFA in an excess of aq. KOH solution. There was no significant difference between the yields of compounds XVII-XVIII obtained from the pyridine selenones and halogen derivatives of methylene-active nitriles or by the cyclation of 2-(R^3-methylseleno)pyridines XI-XVI. We found that the yields and formation rates for the 3-aminoselenopheno[2,3-b]pyridines are directly proportional to the electron-acceptor properties of the R^3 substituent. This corresponds to the data for synthesizing the 3-aminothiene[2,3-b]-pyridines [2, 9]. The structure of the 3-aminoselenopheno[2,3-b]pyridines XVII-XXIII was confirmed by spectral tests. Their IR-spectra were characterized by stretching and deformation vibrations of the free and bound amino groups in the 3150-3450 and 1605-1670 cm^{-1} regions respectively. The PMR spectra exhibit a broadened amino group proton signal in the δ 6.3-7.2 ppm region.

Compounds XVI and XXIV readily react with 2 equivalents of iodine in dry chloroform to form triiodides of selenazo[3,2-a]pyridinium (XXV, XXIV). In accordance with physicochemical analysis, quaternization of compounds XVI and XXIV to the salts XXV and XXVI proceeds stereoselectively [11].

During the transition of compounds XVI and XXIV to XXV and XXVI their IR spectra exhibit a diminished nitrile group absorption band intensity and a shift toward the high-frequency region to 2234-2235 cm^{-1}. This is associated with the dislocation of the positive charge in the pyridine fragment of compounds XXV and XXVI. The PMR spectra of the thiazolo[3,2-a]-pyridinium salts XXV and XXVI exhibit a shift in the proton signals of the pyridine ring and associated alkyl substituents to the weak fields $\Delta\delta$ 0.60-0.69 ppm, $\Delta\delta$ 0.17, and $\Delta\delta$ 0.33-0.69 ppm respectively. This also confirms the dislocation of the positive charge in the pyridine ring. The proton signal of the NCH group in compound XXVI is manifested in the form of a double doublet in the δ 6.13 ppm region with a SSCC of $^3J_{cis}$ 4.7 Hz and $^3J_{trans}$ 10.6 Hz.

This kind of NCH proton multiplicity indicates that the quaternization of azine XXIV to XXVI is highly stereoselective.

Pyridoselenophenopyrimidine (XXVII) was synthesized from 3-amino-4,6-dimethyl-2-cyanoselenopheno[2,3-b]pyridine (XVII).

EXPERIMENTAL (CHEMICAL)

The IR-spectra of the compounds were recorded on a UR-20 instrument in KBr pellets. The PMR spectra were recorded on a Bruker WM-250 (250 MHz) instrument in DMSO-d_6 solutions with TMS as the standard. Reaction progress and purity of synthesized substances were controlled by TLC on Silufol UV-250 plates in a 3:5 acetone—hexane system. The selenoorganic compounds were synthesized, separated, and purified in argon. Element analysis data corresponded to the calculated values.

4,6-Dimethyl-3-cyano-2(1H)-pyridine Selenone (VI). A mixture of 3.4 g (20 mmol) of β-enaminoketone I, 2.9 g (20 mmol) of cyanoselenoacetamide V, and 0.2 ml of morpholine in 40 ml of abs. ethanol was stirred at 50°C for 2 h. The reaction mixture was acidified with 3 ml of AcOH, and the precipitate was filtered off and subsequently washed with ethanol and hexane. Yield of VI was 88%, mp 214-216°C (decomp.) (lit: mp 214-216°C [6]). IR spectrum, ν, cm^{-1}: 2223 (C≡N). PMR spectrum, δ, ppm: 2.36 s (3H, 4-CH_3); 2.40 s (3H, 6-CH_3); 6.68 s (1H, C^5H); 14.23 s (1H, NH).

4,6-Diphenyl-3-cyano-2-(1H)-pyridine Selenone (VII). A mixture of 1.04 g (15 mmol) of compound II and 0.74 g (5 mmol) of V in a solution of 0.09 g (4 mmol) of sodium in 20 ml of abs. ethanol was boiled for 2 h. The reaction mixture was cooled and washed with ethanol and hexane. Yield of VII was 52%. mp 280-283°C (decomp.) (lit:mp 280-283°C [3]). IR spectrum, ν, cm^{-1}: 2207 (C≡N). PMR spectrum, δ, ppm: 7.10 s (1H, C^5H), 7.3-8.0 m (10H, $[C_6H_5]_2$).

6-(1-Adamantyl)-3-cyano-2(1H)-pyridine selenone (VIII) was obtained by method [11]. Yield of VIII was 52%, mp 185-190°C (decomp.) IR spectrum, ν, cm^{-1}: 2230 (C≡N). PMR spectrum, δ, ppm: 1.80-2.13 m (15H, Ad), 6.85 d (1H, C^5H). 7.75 d (1H, C^4H, ^3J8 Hz), 8.88 s (1H, NH).

Sodium-6-amino-3,5-dicyanopyridine-2-selenolate (IX). A 1.47 g (10 mmol) portion of compound V and 1.22 g (10 mmol) of ethoxymethylene malononitrile IV was sequentially added to a solution of 0.23 g(10 mmol) of sodium in 20 ml of abs. ethanol. The reaction mixture was stirred at 25°C for 2 h after which the precipitate was separated and subsequently washed with ethanol and hexane. Yield of IX was 67%. IR spectrum, ν, cm^{-1}: 3228, 3343, 3450 (NH_2), 2220 sh. (5-C-N), 2214 (3-C-N), 1630 (δ NH_2). PMR spectrum, δ, ppm: 7.92 s (1H, C^4H), 8.48 s (2H, NH_2) (Table 1).

Bis[2,2'-(1-adamantyl-3-cyanopyridyl-1)]diselenide (X) was obtained by method (1). Yield was 80%, mp 249-255°C.

Substituted 2-Alkylseleno-3-cyanopyridines (XI-XVI). General method. A 5.6 ml portion of a 10% KOH solution and 10 mmol of the corresponding alkylhalogenide was added while stirring to a suspension of 10 mmol of the corresponding pyridine selenone VI and VIII in 20 ml of DMFA. The reaction mixture was then stirred for 30 min at 25°C, and then diluted with 10 ml of water and cooled to 0°. The precipitate was filtered, washed with water, ethanol, and hexane. The mp of compounds XII-XV and their IR spectra were identical to that of 2-alkylselenopyridines, described in [1, 8].

4,6-Dimethyl-3-cyanopyridine-2-selenoacetic Acid (XI). Yield of XI was 84%. IR spectrum, ν, cm^{-1}: 1698 (C=O), 2227 (C≡N). PMR spectrum, δ, ppm: 2.38 s (3H, 4-CH_3), 2.43 s (3H, 6-CH_3), 4.03 s (2H, CH_2), 7.16 s (1H, C^5H) (Table 1).

2-Allylseleno-4,6-dimethyl-3-cyanopyridine (XVI). Yield of XVI was 73% (from n-pentane). IR spectrum, ν, cm^{-1}: 2220 (C≡N). PMR spectrum, δ, ppm: 2.40 s (3H, 4-CH_3), 2.52 s (3H, 6-CH_3), 3.96 d (2H, $SeCH_2$, ^3J=7 Hz), 5.04 d (1H, CH_2=C, $^3J_{cis}$ 10.5 Hz), 5.20 d (1H, CH_2=C, $^3J_{trans}$ 18 Hz), 5.97 m (1H, CH=C), 7.13 s (1H, C^5H) (Table 1).

Substituted 3-aminoselenopheno[2,3-b]pyridines (XVII-XXIII). General method A. A 5.6 ml portion of a 10% KOH solution, 10 mmol of alkylhalogenide, and 3-4 ml of a 10% KOH were sequentially added to a suspension of 10 mmol of the corresponding 3-cyanopyridine-2(1H)-selenone (V, VII) in 20 ml of DMFA. The reaction mixture was stirred for 2-3 h, then diluted

TABLE 2. Effect of Synthesized Compounds on Influenza and Herpes Infection in Cell Cultures

Compound	Influenza A virus		Herpes simplex-1			
	toxicity zone, mm	activity zone, mm	TK+ strain		TK− strain	
			solution concn., μg/ml			
			50	25	50	25
VI	—	—	—	—	—	—
IX	15	18	+	—	+	—
X	—	—	—	—	—	—
XI	10	—	+	—	+	+
XII	—	—	—	—	—	—
XIII	30	—	—	—	—	—
XIV	—	—	—	—	—	—
XV	5	20	+	+	+	+
XVI	10	—	—	—	—	—
XVII	10	20	+	+	+	+
XVIII	—	—	—	—	—	—
XIX	—	—	—	—	—	—
XX	—	—	—	—	—	—
XXI	—	—	—	—	—	—
XXII	20	—	—	—	—	—
XXIII	15	—	—	—	—	—
XXV	22	—	—	—	—	—
XXVI	—	—	—	—	—	—
XXVII	15	26	—	—	—	—

Note. Count 48 h after adsorption at which period the cytopathological effect was expressed by 4+.

with 10 ml of water. The precipitate was filtered and washed with water, ethanol, and hexane. Compounds XVII-XXIII are identical to the 3-aminoselenopheno[2,3-b]pyridine, described in [1,3] with respect to mp and IR spectra.

6-(1-Adamantyl)-3-amino-2-carbamoylselenopheno[2,3-b]pyridine (XX)B. A 4 ml portion of a 10% KOH solution was added to a suspension of 10 mmol of compound XV in 20 ml of DMFA. The reaction mixture was stirred for 3 h, then diluted with 10 ml of water. The precipitate was filtered off and washed with water, ethanol, and hexane. Yield of XX was 90%, mp 292-297°C (from AcOH). IR spectrum, ν, cm^{-1}: 3440–3170(NH_2), 1650 ($CONH_2$).

Compound XX exhibited the same mp and IR spectrum as selenopheno[2,3-b]pyridine, described in [1]. Compounds XXI-XXIII were obtained by method [1].

4,6-Dimethyl-3-cyano-2-(2-cyclohexane-1-ylseleno)pyridine (XXIV). Yield of XXIV was 81% (from methanol). IR spectrum, ν, cm^{-1}: 2217 (C≡N). PMR spectrum, δ, ppm: 1.72, 1.98, 2.08 m (6H, $[-CH_2-]_3$), 2.39 s (3H, 4-CH_3), 2.28 s (3H, 6-CH_3), 4.75 m (1H, CH–Se), 5.86 m (2H, CH=CH), 7.14 s (1H, C^5H) (Table 1).

5,7-Dimethyl-3-iodomethyl-8-cyano-2,3-dihydroselenazolo [3,2-a]pyridinium Triiodide (XXV). A solution of 1 g (4 mmol) of iodine in 25 ml of chloroform was added dropwise with stirring for 10 min to a solution of 0.5 g (2 mmol) of compound XVI in 5 ml of dry chloroform. The reaction mixture was stirred at 25°C for 1 h. The precipitate was filtered off and washed with chloroform. Yield of XXV was 86%. IR spectrum, ν, cm^{-1}: 2235 (C≡N). PMR spectrum, δ, ppm: 2.57 s (3H, 7-CH_3), 2.85 s (3H, 5-CH_3), 3.65 m (2H, CH_2), 3.93-4.26 m (2H, $SeCH_2$), 5.96 m (1H, NCH); 7.73 s (1H, C^6H). (Table 1).

6,8-Dimethyl-4-iodo-9-cyano-4a,10a-cis-4,4a-trans-1,2,3,4,4a,10a-hexahydrobenzoselenazolo[3,2-a]pyridinium Triiodide (XXVI). A solution of 1 g (4 mmol) of iodine in 25 ml of chloroform was added with stirring at 25°C over a period of 10 min to a solution of 0.58 g (10 mmol) of compound XXIV in 5 ml of dry chloroform. The reaction mixture was stirred for 1 h at 25°C. The precipitate was filtered off and washed with chloroform. Yield of XXVI was 84%. IR spectrum, ν, cm^{-1}: 2334 (C≡N). PMR spectrum, δ, ppm: 1.57, 1.76, 2.04,

TABLE 3. Effect of Compounds XV and XXVII on Influenza A Virus Reproduction

Compound	Concentration μg/ml	GA titer, $\log_2$	Infectious titer, log pfu/cell
XV	50	1	5,0
	25	3	5,9
	12,5	4	6,2
XXVII	50	1	5,2
	25	3	6,0
	12,5	5	6,7
Control	—	6	7,4

2.28 m (6H, $[-CH_2-]_3$), 2.66 s (3H, 8—CH_3), 2.17 s (3H, 6-CH_3), 4.92 m (1H, CH), 5.2 m (1H, CHSe), 6.13 d.d. (1H, CHN, $^3J_{cis}$ 4.7 Hz, $^3J_{trans}$ 10.6 Hz). (Table 1).

4,Amino-7,9-dimethylpyrido[2,3:2^1,3^1] selenopheno[4,5-d]pyrimidine (XXVI) was obtained by method [8]. Yield of XXVII was 79%, mp 274-276°C. IR spectrum, ν, cm^{-1}: 3410, 3330, 3200 (NH_2), 1638 (δ NH_2).

EXPERIMENTAL (BIOLOGICAL)

We employed the influenza A virus (classic fowl plague virus, Waybridge strain) obtained from the museum strain laboratory of the D. I. Ivanovskii Institute of Virology of the Academy of Medical Sciences of the USSR, and the herpes simplex virus (HSV) type I, strains α_2 (TK^+) and α_2 (TK^-), clone σ_{11} which were kindly provided by R. A. Gibadulin (D. I. Ivanovskii Institute of Virology).

The compounds were tested for anti-influenza activity in a chick embryo fibroblast (CEF) cell culture by method [4] by observing patch formation suppression caused by applying the compounds to a paper disk (diffusion test in agar). A two-day old monolayer CEF was inoculated with an appropriate virus employing a multiple infection of 0.01 biological units/cell. One hour after the virus was adsorbed a single layer of CEF was coated by the Dulbbek method. After the latter congealed paper disks impregnated with a solution of the test compound at a concentration of 125 μg/ml per disk were applied. Inhibiting activity was measured after 48 h and was interpreted as the difference between the patch suppression zone and the toxicity zone. Data on anti-influenza activity are given in Table 2.

An analysis of the research results shows that compounds IX, XI, XV, XVII, and XXVII exhibit activity against influenza virus A. The most promising anti-influenza activity was exhibited by compounds XV and XXVII in the diffusion test in agar.

Table 3 shows the test results for GA synthesis inhibition in CEF and infectious virus titer suppression by compounds XV and XXVII in multicyclic experiments (multiple infection by 0.01 pfu/cell). Thus, the indicated compounds XV and XXVII exhibit pronounced anti-influenza activity at concentrations of 50 and 25 μg/ml and inhibit GA synthesis by 5 and 3 $\log_2$ and viral infectiousness by 2.2 and 1.5 log pfu/cell respectively.

The following toxicity series was plotted upon screening the anti-influenza efficacy of the compounds as measured on the compounds' toxicity in agar: XIII > XXII > XXIII > XXV > IX > XXVII > XI > XVII > XVI > XV.

Compounds VII, X, XII, XIV, XVIII—XXI, and XXVI were not of interest because of their poor solubility and because they exhibited neither antiviral nor toxic properties.

Concentrations at nontoxic ranges were selected in the Vero cell culture experiments. The compounds were selected on the basis of a tentative structural series of toxicity. A cell culture was inoculated with 0.01 $TCID_{50}$/cell [sic] of herpes simplex-1. Maximum cytopathic activity manifested by complete degeneration of the monolayer (on a 4-point scale) occurred after 48 h. The compounds' antiviral action was evaluated by method [4]. Table 2 gives the study data on herpes infection inhibition in a Vero cell culture. The preparations' efficacy was evaluated by their ability to suppress cytopathic activity induced by the herpes simplex-1 virus (strains TK^+ and TK^-). One can see from Table 2 that anti-herpes activity was exhibited by compounds IX, XI, XV, and XVII. Compounds XV and XVII should be noted for their pronounced antiviral activity against both the TK^+ and TK^-strains.

Thus, in analyzing the results of the experimental data on the screening of selenium-containing compounds, one can see that they manifest antiviral activity. One should note that compounds XV and XVII exhibit antiviral properties both against influenza and herpes infection.

Remantadin was used as the reference standard for the influenza infection tests and acyclovir was used for the herpes tests. Remantadin exhibits antiviral action against influenza A, and acyclovir exhibited activity against the TK^+ strains of the herpes simplex virus. The selenium-containing compounds (e.g., XV and XVII) were less effective in their antiviral action than the reference standards, but were effective in all of the tested models, i.e., the RNA- and DNA-containing viruses thereby demonstrating their clear advantage.

One might note that the compounds with different chemical structures exhibit antiviral activity. The results of our tests and analysis of literature data [4] indicate that further screenings of selenium-containing compounds are warranted as well as continued efforts at developing goal-oriented synthesis of selenium compounds that can be utilized as inhibitors of broad spectrum viral reproduction.

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