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Synthesis, Structure, and Anti-influenza Activity of 2-(Adamantan-1-yl)-5-aryl-1,3,4-oxadiazoles and 2-(Adamantan-1-yl)-5-aryltetrazoles

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Abstract—Two series of new adamantyl derivatives of polynitrogen heterocycles, 2-(adamantan-1-yl)-5-aryl-1,3,4-oxadiazoles and 2-(adamantan-1-yl)-5-aryl-2*H*-tetrazoles, have been synthesized, and their structure has been determined by NMR spectroscopy, mass spectrometry, and X-ray analysis. Biological studies *in vitro* have revealed high inhibitory activity of some of the synthesized 2-(adamantan-1-yl)-5-aryl-2*H*-tetrazoles against H1N1 influenza A viruses in combination with a relatively low selectivity.

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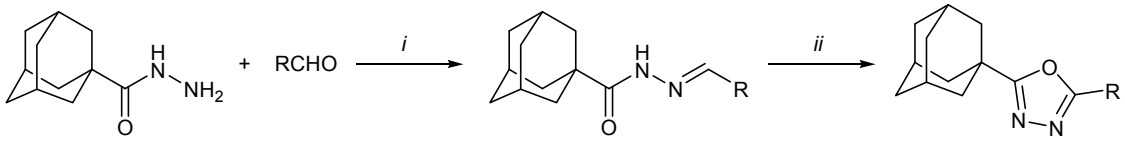
Amantadine and rimantadine [adamantan-1-amine and 1-(adamantan-1yl)ethanamine hydrochlorides] are traditional drugs used in the prevention and treatment of influenza A infections [1, 2]. The mechanism of action of these compounds is based on blocking of the M2 protein channel of influenza A viruses [3, 4]. These drugs have been developed several decades ago, and their efficiency appreciably decreased due to acquired resistance of some viral strains. Therefore, design of new drug analogs containing cage hydrocarbon fragments and active against influenza A viruses is a quite important problem. A promising way of creating new highly efficient anti-influenza drugs implies replacement of the amino group in amino adamantane derivatives by polynitrogen heterocyclic fragments [5–7]. Among the latter, of particular interest are 1,3,4-oxadiazoles and tetrazoles. 1,3,4-Oxadiazole derivatives exhibit a broad spectrum of biological activity, including antibacterial [8], antifungal [9], analgesic [10], anti-inflammatory [11], and antitumor [12]. Tetrazole ring is also known as a pharmacophore due to its high stability in metabolic processes, low toxicity, ability to form strong hydrogen bonds with proton donors, and other useful properties [13–16]. A large number of highly efficient drugs based on 1,3,4-oxadiazole and tetrazole derivatives are now used in the treatment of various diseases.

In the present work we synthesized by different methods two series of new adamantyl derivatives of polynitrogen heterocycles, 2-(adamantan-1-yl)-5-aryl-1,3,4-oxadiazoles and 2-(adamantan-1-yl)-5-aryltetrazoles, and studied their structure and biological activity.

2,5-Disubstituted 1,3,4-oxadiazoles can be obtained by oxidation with molecular iodine of condensation products of aldehydes and carboxylic acid hydrazides

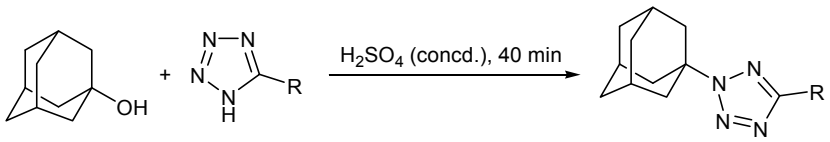
Scheme 1.



1 **2a–2c**

Reagents and conditions: *i*: EtOH, 6–11 h; *ii*: I_2, K_2CO_3, DMSO, 100°C, 1–4 h; R = Ph (**a**), naphthalen-1-yl (**b**), anthracen-9-yl (**c**).

Scheme 2.



3a–3j **4a–4j**

R = Ph (**a**), 2-MeC_6H_4 (**b**), 3-MeC_6H_4 (**c**), pyridin-4-yl (**d**), pyridin-2-yl (**e**), 4-$CF_3C_6H_4$ (**f**), 3-$CF_3C_6H_4$ (**g**), 2-ClC_6H_4 (**h**), 4-ClC_6H_4 (**i**), 4-$O_2NC_6H_4$ (**j**).

(Scheme 1). Aldehydes containing both electron-donating and electron-withdrawing substituents and both aromatic and aliphatic carboxylic acid hydrazides can be used as starting compounds [17]. However, analogous reactions with sterically hindered carboxylic acid hydrazides have not been discussed previously. We have found that the cyclization stage with the formation of 1,3,4-oxadiazoles **2a**–**2c** is slow and that the presence of excess iodine is necessary for the reaction to occur. By optimizing the reaction conditions we succeeded in obtaining target compounds **2a**–**2c** in fairly high yields (84–89%; Scheme 1). Initial adamantane-1-carboxylic acid hydrazide (**1**) was synthesized by treatment of the corresponding methyl ester with hydrazine hydrate in ethanol under isothermal conditions in a microwave reactor, which considerably improved the yield and accelerated the process.

5-Substituted NH-tetrazoles **3a**–**3j** were synthesized by azidation of the corresponding nitriles with dimethylammonium azide in DMFA at 110–115°C [13, 14]. 2-(Adamantan-1-yl)-2*H*-tetrazoles **4a**–**4j** were prepared by alkylation of tetrazoles **3a**–**3j** with adamantan-1-ol in the presence of concentrated sulfuric acid (Scheme 2). It is presumed that carbenium ions generated from primary, secondary, and tertiary alcohols by the action of mineral acids react with protonated tetrazole, which is responsible for the high selectivity of the alkylation involving exclusively the N^2 atom of the tetrazole ring [5, 14, 18].

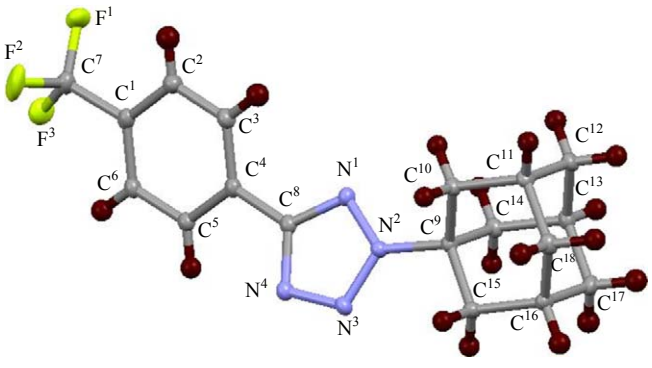


Fig. 1. Structure of the molecule of 2-(adamantan-1-yl)-5-[4-(trifluoromethyl)phenyl]-2*H*-tetrazole (**4f**) according to the X-ray diffraction data.

The structure of the isolated compounds was reliably proved by ^1H and ^{13}C NMR spectroscopy, high-resolution mass spectrometry, and X-ray analysis. Their ^1H NMR spectra contained signals typical of the adamantane fragment and aromatic substituents. In the ^{13}C NMR spectra of oxadiazoles **2a**–**2c**, carbon atoms of the heterocycle resonated at δ_C 162–164 and 172–174 ppm, and in the C^5 signal in the spectra of tetrazoles **4a**–**4j** was located at δ_C 162–164 ppm. According to the X-ray diffraction data for compounds **4f** and **4j**, the 2*H*-tetrazole ring is a planar highly aromatic system (Figs. 1, 2). The dihedral angle between the tetrazole ring and the aromatic substituent on C^5 is not large (≤30°), which allows some π-conjugation between these fragments. The tetrazole ring is partially sterically shielded by the bulky adamantyl substituent.

All oxadiazole and tetrazole derivatives **2a**–**2c** and **4a**–**4j** were tested for antiviral activity against influenza A/Puerto Rico/8/34 viruses (H1N1) (Table 1). Some adamantyltetrazoles showed a high inhibitory activity, namely compounds **4a** (IC_{50} 0.6 μg/mL), **4b** (IC_{50} 5 μg/mL), **4c** (IC_{50} 2 μg/mL), and **4h** (IC_{50} 2 μg/mL). However, these compounds also showed appreciable cytotoxicity, which considerably reduced the selectivity of their action. Thus, the synthesized compounds can be used as basic structures for the design of new antiviral agents.

EXPERIMENTAL

The mass spectra (electrospray ionization) were recorded on a Bruker MicrOTOF mass spectrometer. The ^1H and ^{13}C NMR spectra were measured on a Bruker Avance III 400 spectrometer at 400.13 and 100.61 MHz, respectively, using tetramethylsilane as internal standard. Thin-layer chromatography was performed on Merck TLC Silica gel 60 F254 plates using eluent systems individually selected for each

particular case; spots were visualized under UV light (λ 254, 365 nm) or by treatment with iodine vapor. The melting points were determined with a Wagetechnik Rapido PHMK micro hot stage. The synthesis of adamantane-1-carbohydrazide (**1**) was carried out in a Biotage Initiator+ microwave reactor (maximum pressure 30 bar).

Adamantane-1-carbohydrazide (1). A mixture of 25.74 mmol of methyl adamantane-1-carboxylate, 128.68 mmol of hydrazine hydrate, and 5 mL of ethanol was heated for 8 h at 150°C in a microwave reactor. The mixture was poured into 50 mL of water, and the precipitate was filtered off, washed with water, and dried in a thermostat. Yield 4.8 g (96%), white crystals, mp 153–155°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.57–1.73 m (6H, Ad), 1.76 d (6H, Ad, J = 2.6 Hz), 1.94 s (3H, Ad), 4.11 s (2H, NH_2), 8.67 s (1H, NH). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 28.06, 36.62, 39.06, 39.51 (Ad), 176.79 (C=O). Mass spectrum: m/z 195.1491 $[M + H]^+$. $C_{11}H_{18}N_2O$. Calculated: M 194.2734.

2-(Adamantan-1-yl)-1,3,4-oxadiazoles 2a–2c (*general procedure*). A mixture of 1 mmol of the corresponding aldehyde, 1 mmol of hydrazide **1**, and 20 mL of ethanol was refluxed for 4–11 h (TLC, CCl_4–*i*-PrOH–AcOH, 85:15:5). The solvent was evaporated under reduced pressure, the residue was dissolved in 5 mL of DMSO, and 1.7 mmol of iodine and 4.25 mmol of potassium carbonate were added. The mixture was heated at 100°C until the reaction was complete (TLC, CH_2Cl_2–MeOH, 9:1) and treated with 20 mL of a 5% solution of sodium thiosulfate, and the precipitate was filtered off and recrystallized from aqueous ethanol.

2-(Adamantan-1-yl)-5-phenyl-1,3,4-oxadiazole (2a). Yield 0.249 g (89%), white powder, mp 105–107°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.78 s (6H, Ad), 2.07 s (9H, Ad), 7.61 d.d (3H, H_{arom}, J = 5.6, 3.6 Hz), 8.00 d.d (2H, H_{arom}, J = 7.8, 1.7 Hz). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 27.64, 34.34, 36.18, 39.86 (Ad); 124.11, 126.89, 129.84, 132.23 (C_{arom}); 163.96 and 172.51 (C^2, C^5). Mass spectrum: m/z 303.1469 $[M + Na]^+$. $C_{18}H_{20}N_2O$. Calculated: M 280.3710.

2-(Adamantan-1-yl)-5-(naphthalen-1-yl)-1,3,4-oxadiazole (2b). Yield 0.285 g (86%), white powder, mp 151–153°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.80 t (6H, Ad, J = 2.8 Hz), 2.09–2.16 m (9H, Ad), 7.59–7.76 m (3H, H_{arom}), 8.05 d.d (1H, H_{arom}, J = 8.2, 1.4 Hz), 8.18 t (2H, H_{arom}), 9.06 d (1H, H_{arom}). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 27.70, 34.33, 36.25, 39.98 (Ad); 120.56, 125.62, 125.90, 127.08, 128.43, 128.71, 129.21, 129.74, 132.72, 133.90 (C_{arom}); 163.88 and 172.01 (C^2, C^5). Mass spectrum: m/z 353.1623 $[M + Na]^+$. $C_{22}H_{22}N_2O$. Calculated: M 330.4310.

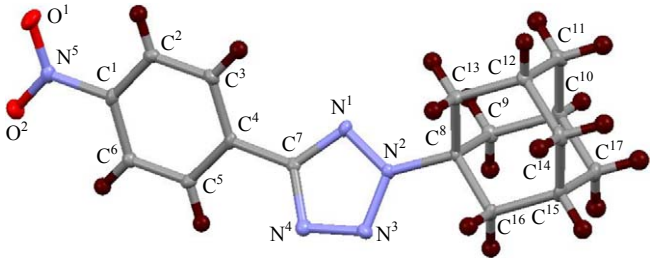


Fig. 2. Structure of the molecule of 2-(adamantan-1-yl)-5-(4-nitrophenyl)-2*H*-tetrazole (**4j**) according to the X-ray diffraction data.

2-(Adamantan-1-yl)-5-(anthracen-9-yl)-1,3,4-oxadiazole (2c). Yield 0.175 g (84%), yellow powder, mp 175–177°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.92–1.80 m (6H, Ad), 2.19 s (3H, Ad), 2.27 d (6H, Ad, J = 2.3 Hz), 7.52–7.60 m (4H, H_{arom}), 7.96 d (2H, H_{arom}, J = 9.2 Hz), 8.06–8.12 m (2H, H_{arom}), 8.68 s (1H, H_{arom}). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 27.82, 34.69, 36.34, 40.05 (Ad); 117.86, 125.12, 125.63, 127.55, 128.72, 131.04, 131.13, 131.35 (C_{arom}); 162.73, 173.74 (C^2, C^5). Mass spectrum:

Table 1. Cytotoxicity (CC_{50}), antiviral activity (IC_{50}), and selectivity indices (SI) of adamantyl-substituted 1,3,4-oxadiazoles **2a**–**2c** and tetrazoles **4a**–**4j** against influenza A viruses (H1N1)

Compound no.	CC_{50}, μg/mL	IC_{50}, μg/mL	SI
2a	89	26	3
2b	270	130	2
2c	>300	66	5
4a	4.1	0.6	7
4b	12.6	5	3
4c	14.1	2	7
4d	>300	200	2
4e	>300	36	8
4f	>300	>300	1
4g	>300	200	2
4h	3.7	2	2
4i	45.4	16	3
4j	>300	>300	1
Rimantadine[a]	60	12	5

[a] Reference drug.

m/z 403.1772 $[M + Na]^+$. $C_{26}H_{24}N_2O$. Calculated: *M* 380.4910.

5-Substituted tetrazoles 3a–3j (*general procedure***).** Sodium azide, 11.4 mmol, and dimethylamine hydrochloride, 11.2 mmol, were dissolved in 10 mL of DMF, 10 mmol of the corresponding nitrile and 5 mL of DMF were added, and the resulting suspension was heated for 8 h at 110–115°C. The mixture was cooled and filtered from the precipitate of sodium chloride, and the precipitate was washed with 10 mL of DMF on a filter. The filtrate was diluted with 30 mL of cold water and acidified with 10% aqueous HCl to pH 2–3. The precipitate was filtered off, thoroughly washed with cold water (3×30 mL), and dried in air. The physical constants and spectral parameters of **3a–3j** were in agreement with published data [13, 14].

2-(Adamantan-1-yl)-2*H*-tetrazoles 4a–4j (*general procedure***).** Compound **3a–3j**, 10 mmol, and adamantan-1-ol, 10 mmol, were dissolved in 25 mL of 94% sulfuric acid. The mixture was kept for 40 min at room temperature and poured into 150 mL of an ice–watter mixture. The product was extracted with chloroform (2×20 mL), the extract was washed with a 5% aqueous solution of Na_2CO_3 and with water and dried over anhydrous $MgSO_4$, and the solvent was evaporated under reduced pressure.

2-(Adamantan-1-yl)-5-phenyl-2*H*-tetrazole (4a). Yield 1.797 g (64%), white crystals, mp 89–91°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.78 q (6H, Ad, *J* = 2.2 Hz), 2.25 t (3H, Ad, *J* = 3.1 Hz), 2.34 d (6H, Ad, *J* = 2.9 Hz), 7.45–7.66 m (3H, H_{arom}), 8.01–8.15 m (2H, H_{arom}). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 29.31, 35.63, 42.11, 64.16 (Ad); 126.78, 127.69, 129.66, 130.84 (C_{arom}), 163.89 (C^5). Mass spectrum: *m/z* 303.1565 $[M + Na]^+$. $C_{17}H_{20}N_4$. Calculated: *M* 280.3750.

2-(Adamantan-1-yl)-5-(2-methylphenyl)-2*H*-tetrazole (4b). Yield 1.90 g (65%), white crystals, mp 84–86°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.72–1.85 m (6H, Ad), 2.22–2.29 m (3H, Ad), 2.34 d (6H, Ad, *J* = 3.4 Hz), 2.56 s (3H, CH_3), 7.31–7.45 m (3H, H_{arom}), 7.92 d.d (1H, H_{arom}, *J* = 7.7, 1.3 Hz). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 21.56 (CH_3), 29.31, 35.65, 42.11, 63.97 (Ad); 126.63, 126.92, 129.42, 130.36, 131.81, 137.09 (C_{arom}); 164.24 (C^5). Mass spectrum: *m/z* 317.1731 $[M + Na]^+$. $C_{18}H_{22}N_4$. Calculated: *M* 294.4020.

2-(Adamantan-1-yl)-5-(3-methylphenyl)-2*H*-tetrazole (4c). Yield 2.559 g (87%), white crystals, mp 83–85°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.72–1.85 m (6H, Ad), 2.22–2.28 m (3H, Ad), 2.33 d (6H, Ad, *J* = 2.9 Hz), 2.41 s (3H, CH_3), 7.35 d (1H, H_{arom}, *J* = 7.4 Hz), 7.44 t (1H, H_{arom}, *J* = 7.6 Hz), 7.83–7.91 m (2H, H_{arom}). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 21.39 (CH_3); 29.31, 35.63, 42.10, 64.11 (Ad); 123.95, 127.21, 127.63, 129.55, 131.47, 138.99 (C_{arom}); 163.96 (C^5). Mass spectrum: *m/z* 317.1730 $[M + Na]^+$. $C_{18}H_{22}N_4$. Calculated: *M* 294.4020.

4-[2-(Adamantan-1-yl)-2*H*-tetrazol-5-yl]pyridine (4d). Yield 2.364 g (84%), white crystals, mp 132–134°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.79 t (6H, Ad, *J* = 3.1 Hz), 2.27 q (3H, Ad, *J* = 2.8 Hz), 2.34 d (6H, Ad, *J* = 2.9 Hz), 7.93–8.00 m (2H, Py), 8.70–8.76 m (2H, Py). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 29.32, 35.68, 42.16, 64.55 (Ad); 120.75, 134.95, 150.91 (Py); 162.02 (C^5). Mass spectrum: *m/z* 304.1538 $[M + Na]^+$. $C_{16}H_{19}N_5$. Calculated: *M* 281.3630.

2-[2-(Adamantan-1-yl)-2*H*-tetrazol-5-yl]pyridine (4e). Yield 2.153 g (77%), white crystals, mp 118–120°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.72–1.85 m (6H, Ad), 2.22–2.29 m (3H, Ad), 2.34 d (6H, Ad, *J* = 2.9 Hz), 7.51–7.59 m (1H, Py), 8.01 t.d (1H, Py, *J* = 7.7, 1.8 Hz), 8.15 d (1H, Py, *J* = 7.8 Hz), 8.72–8.78 m (1H, Py). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 29.32, 35.60, 42.11, 64.41 (Ad); 122.80, 125.57, 138.00, 146.95, 150.55 (Py); 163.98 (C^5). Mass spectrum: *m/z* 304.1537 $[M + Na]^+$. $C_{16}H_{19}N_5$. Calculated: *M* 281.3630.

2-(Adamantan-1-yl)-5-(4-trifluoromethylphenyl)-2*H*-tetrazole (4f). Yield 2.938 g (84%), white crystals, mp 120–122°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.79 t (6H, Ad, *J* = 3.0 Hz), 2.23–2.29 m (3H, Ad), 2.35 d (6H, Ad, *J* = 2.9 Hz), 7.93 d (2H, H_{arom}, *J* = 8.2 Hz), 8.28 d (2H, H_{arom}, *J* = 8.0 Hz). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 29.31, 35.58, 42.08, 64.57 (Ad), 123.07, 125.78 (CF_3), 126.67, 126.71, 127.55, 131.47 (C_{arom}), 130.36, 130.68, 130.99, 131.31 ($C^{4'}$), 162.74 (C^5). Mass spectrum: *m/z* 371.1460 $[M + Na]^+$. $C_{18}H_{19}F_3N_4$. Calculated: *M* 348.3732.

2-(Adamantan-1-yl)-5-(3-trifluoromethylphenyl)-2*H*-tetrazole (4g). Yield 3.164 g (91%), white crystals, mp 129–131°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.79 d (6H, Ad, *J* = 3.3 Hz), 2.24–2.31 m (3H, Ad), 2.35 d (6H, Ad, *J* = 2.8 Hz), 7.72 d.d (2H, H_{arom}, *J* = 16.4, 8.2 Hz), 8.27–8.35 m (2H, H_{arom}). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 29.33, 35.72, 42.20, 64.31 (Ad), 122.72, 125.43 (CF_3), 123.15, 126.82, 128.87, 131.11, 130.27 (C_{arom}), 130.60,

130.92 ($C^{3'}$), 162.67 (C^5). Mass spectrum: *m*/*z* 371.1447 $[M + Na]^+$. $C_{18}H_{19}F_3N_4$. Calculated: *M* 348.3732.

2-(Adamantan-1-yl)-5-(2-chlorophenyl)-2*H*-tetrazole (4h). Yield 2.469 g (78%), white crystals, mp 101–103°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.78 q (6H, Ad, *J* = 2.3 Hz), 2.22–2.28 m (3H, Ad), 2.34 d (6H, Ad, *J* = 3.0 Hz), 7.55 d.t.d (2H, H_{arom}, *J* = 19.2, 7.4, 1.6 Hz), 7.67 d.d (1H, H_{arom}, *J* = 7.9, 1.4 Hz), 7.89 d.d (1H, H_{arom}, *J* = 7.5, 2.0 Hz). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 29.31, 35.60, 42.10, 64.35 (Ad); 126.86, 128.04, 131.12, 131.82, 132.20, 132.36 (C_{arom}); 162.14 (C^5). Mass spectrum: *m*/*z* 337.1199 $[M + Na]^+$. $C_{17}H_{19}ClN_4$. Calculated: *M* 314.8170.

2-(Adamantan-1-yl)-5-(4-chlorophenyl)-2*H*-tetrazole (4i). Yield 2.965 g (94%), white crystals, mp 131–133°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.79 t (6H, Ad, *J* = 3.1 Hz), 2.23–2.36 m (9H, Ad), 7.49–7.57 m (2H, H_{arom}), 8.01–8.09 m (2H, H_{arom}). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 29.32, 35.71, 42.18, 64.13 (Ad); 126.61, 128.33, 129.48, 135.55 (C_{arom}); 162.97 (C^5). Mass spectrum: *m*/*z* 337.1191 $[M + Na]^+$. $C_{17}H_{19}ClN_4$. Calculated: *M* 314.8170.

2-(Adamantan-1-yl)-5-(4-nitrophenyl)-2*H*-tetrazole (4j). Yield 2.537 g (78%), white crystals, mp 184–186°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.77–1.84 m (6H, Ad), 2.25–2.32 m (3H, Ad), 2.36 d (6H, Ad, *J* = 3.0 Hz), 8.32 d (2H, H_{arom}, *J* = 8.9 Hz), 8.39 d (2H, H_{arom}, *J* = 8.6 Hz). ^{13}C NMR spectrum (DMSO-d_6), δ_C, ppm: 29.33, 35.66, 42.16, 64.62 (Ad); 124.76, 127.90, 133.65, 148.83 (C_{arom}); 162.27 (C^5). Mass spectrum: *m*/*z* 348.1427 $[M + Na]^+$. $C_{17}H_{19}N_5O_2$. Calculated: *M* 325.3720.

The X-ray diffraction data for compounds **4f** and **4j** were obtained at 100(2) K on SuperNova and Xcalibur diffractometers (monochromatized Cu K_α and Mo K_α radiation, respectively). The structures were solved by the direct method and by the charge flipping method using SHELXS [19] and Superflip [20], respectively, and were refined by the least-squares method in anisotropic approximation for non-hydrogen atoms (SHELXL [21] implemented in Olex2 [22]). Mechanical twinning in the crystal structure of **4j** was taken into account using CrysAlisPro (Agilent Technologies).

A single crystal of **4f** was obtained by crystallization from methanol; $C_{18}H_{19}F_3N_4$, *M* 348.37. Triclinic crystal system, space group *P*-1; unit cell parameters: *a* = 6.6619(2), *b* = 10.2870(4), *c* = 12.6694(4) Å; α = 109.070(3), β = 97.826(3), γ = 96.119(3)°; *V* = 802.31(5) Å^3; *Z* = 2; μ(Cu K_α) = 0.951 mm^{-1}; d_{calc} = 1.442 g/cm^3; $-8 \le h \le 7$, $-12 \le k \le 12$, $-15 \le l \le 15$. Total of 13842 reflection intensities were measured in the range $7.508 \le 2\theta \le 152.652°$, including 3306 independent reflections (R_{int} = 0.0291, R_σ = 0.0185). Final divergence factors: R_1 = 0.0335, wR_2 = 0.1171 [reflections with $I > 2\sigma(I)$]; R_1 = 0.0362, wR_2 = 0.1211 (all independent reflections). Residual electron density ρ_{min}/ρ_{max} = 0.35/–0.20 $\bar{e}$/Å^3. CCDC entry no. 1839190.

A single crystal of **4j** was obtained by crystallization from methanol; $C_{17}H_{19}N_5O_2$, *M* 325.37. Triclinic crystal system, space group *P*-1; unit cell parameters: *a* = 6.7879(9), *b* = 8.4931(11), *c* = 13.935(4) Å; α = 104.165(17), β = 101.222(17), γ = 90.121(10)°; *V* = 763.0(3) Å^3; *Z* = 2; μ(Mo K_α) = 0.097 mm^{-1}; d_{calc} = 1.416 g/cm^3; $-8 \le h \le 6$, $-11 \le k \le 11$, $-18 \le l \le 18$. Total of 3701 reflection intensities were measured in the range $6.128 \le 2\theta \le 54.99°$, all of which were independent (R_σ = 0.0568). Final divergence factors: R_1 = 0.0530, wR_2 0.1158 [reflections with $I > 2\sigma(I)$]; R_1 = 0.0675, wR_2 = 0.1216 (all independent reflections). Residual electron density ρ_{min}/ρ_{max} = 0.33/–0.24 $\bar{e}$/Å^3. CCDC entry no. 1839189.

***In vitro* MTT cytotoxicity assay.** The cytotoxicity of the synthesized compounds was evaluated on MDCK cell culture which was incubated for 48 h at 36°C in a 5% CO_2 atmosphere. Triple dilution series with concentrations of 300 to 4 μg/mL were prepared from the compounds on Eagle's minimal essential medium (MEM), and MTT assay was performed in 96-well microplates [23, 24]. The cells were washed twice with saline (0.9% NaCl), and 100 μL of an MTT [3-(4,5-dimethyl-1,3-thiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution (0.5 μg/mL) in phosphate-buffered saline was added to each well. The microplates were incubated for 1 h at 36°C, the liquid phase was removed from the wells, and 0.1 mL of dimethyl sulfoxide was added to each well. The optical density was measured at a wavelength of λ 535 nm with a Victor 2 1440 spectrophotometer, and the compound concentration resulting in 50% cell death (CC_{50}) was calculated.

***In vitro* antiviral activity assay.** A solution of a compound to be tested in MEM, 100 μL per well, was added to MDCK cell monolayer, and the plates were incubated for 1 h at 36°C in a 5% CO_2 atmosphere. Influenza viruses A/Puerto Rico/8/34 (H1N1), 100 μL (MOI 0.01), were then added to each well, and the plates were incubated for 24 h at 36°C in a 5%

CO_2 atmosphere. The virus titer was determined after 24 h on MDCK cells. Ten-fold dilution series were prepared from the culture liquid, added to the cells, and incubated for 48 h at 36°C in a 5% CO_2 atmosphere. A 100-μL portion of culture liquid from each well was transferred to a round-bottom well and hemagglutination assay was performed. For this purpose, an equal amount of 1% suspension of chicken erythrocytes in saline was added to each well and incubated for 40 min at room temperature. The infectious titer was considered as reciprocal to the maximum dilution that caused complete agglutination of erythrocytes. The antiviral activity was evaluated by the reduction of the infectious titer. Based on the obtained data, the 50% effective concentration or the concentration of a compound at which the virus titer decreased twofold (IC50) was calculated, and the selectivity index (SI) was then calculated as the ratio CC_{50}/IC_{50}.

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