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Synthesis and anti-viral activity of azolo-adamantanes against influenza A virus

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ABSTRACT

Chemotherapy and chemoprophylaxis of influenza is one of the most important directions of health protection activity. Due to the high rate of drug-resistant strains of influenza virus, there is a need for the search and further development of new potent antivirals against influenza with a broad spectrum of activity. In the present study, a set of di-, tri- and tetrazole derivatives of adamantane was efficiently prepared and their anti-influenza activities evaluated against rimantadine-resistant strain A/Puerto Rico/8/34. In general, derivatives of tetrazole possessed the highest virus-inhibiting activity. We demonstrated that several compounds of this set exhibited much higher activity than the currently used antiviral rimantadine, a compound of related structure. Moreover, we showed that these azolo-adamantanes were significantly less toxic. This study demonstrates that influenza viruses can be inhibited by adamantylazoles and thus have potential for developing antiviral agents with an alternate mechanism of action.

1. Introduction

Influenza is a highly contagious acute respiratory infection of humans. According to WHO report, annual epidemics result in 25,000–50,000 deaths all over the world.[1] Beginning in 1997, human influenza cases caused by the highly pathogenic avian influenza virus A(H5N1) were reported. Their number reached 420 by April 2009, 257 of them having resulted in fatalities (http://www.who.int/csr/disease/avian_influenza/country/cases_table_2009_04_21/en/index.html). These facts make influenza a very dangerous pathogen able to cause pandemics with a high rate of mortality. Moreover, in 2009 a new pandemic influenza virus appeared, and on November 2009 more than 206 countries have reported laboratory confirmed cases of pandemic influenza H1N1 2009, including over 6250 deaths (http://www.who.int/csr/don/2009_11_13/en/index.html). In this regard, chemotherapy and chemoprophylaxis of influenza is one of the most important directions for health protection activity.

The influenza virions are enveloped, mostly spherical particles containing an outer lipid membrane with integrated surface proteins of three types (hemagglutinin, neuraminidase and M2 protein). The genome of influenza virus is represented by eight separate segments of single-strand negative RNA associated with nucleoprotein and three enzymes of the polymerase complex. Unlike eukaryotic RNA polymerase, viral polymerase complex lacks error-prone activity. For this reason, similar to other RNA viruses, influenza virus has a very high rate of mutations in its genome leading to the fast selection of drug-resistant strains. The life cycle of influenza virus consists of absorption, penetration into cytoplasm, decapsidation with release of nucleoprotein and its transport into a nucleus where transcription and replication of the viral genome occur. Nascent virus-specific RNAs are transported into the cytoplasm with subsequent translation of viral mRNA and assembly of progeny virions followed by budding from the plasma membrane.

Despite numerous steps in the viral life cycle that are potential targets for drug intervention, only two of them are now available for clinical administration. Currently, two main classes of chemical compounds are used for the treatment of influenza. They differ in their viral targets and mechanism of action.[2] Derivatives of adamantane (amantadine and rimantadine) block viral M2 protein, which is necessary for the release of viral RNA into infected cells.[3] Neuraminidase inhibitors zanamivir and oseltamivir interfere with the activity of viral neuraminidase, which plays an essential role in the release of progeny virus particles from the host cell. It should be noted that derivatives of adamantane inhibit influenza viruses of type A but not type B. Inhibitors of neuraminidase are expensive, and drug-resistant strains have already been discovered, particularly for the H5N1 subtype.[4] There is thus a need for the search and further development of new potent antivirals against influenza, which have a broad spectrum of activity and alternative mechanisms of action.

Several studies describe the virus-inhibiting properties of synthetic compounds targeting viral RNA polymerase,[5–11] fusion protein,[12,13] or the pool of intracellular GTP.[14] Nevertheless, none of

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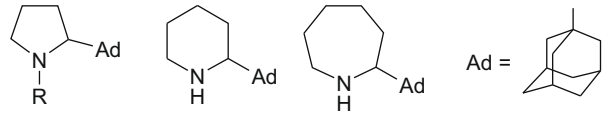
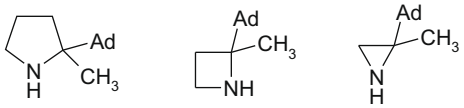
these compounds have been developed far enough to become a clinically available drug.

Rimantadine was used worldwide for several decades for prophylaxis and treatment of influenza A infection. Due to lack of activity against several strains of influenza A and all influenza B viruses, numerous attempts were undertaken to modify the molecule for broadening the range of its anti-viral activity. In particular, many investigations aimed at obtaining high-basic C-adamantyl azacyclanes are carried out at present. 1-Methyl-2-(1-adamantyl)pyrrolidine[15–17] was found to be as effective as rimantadine against H3N2 virus, whereas 2-(1-adamantyl)pyrrolidine and 2-(1-adamantyl)piperidine[15] are particularly effective against H2N2 virus. 2-(1-adamantyl)hexahydroazepine, the product of further ring expansion, does not show anti-viral activity.[15]



R = H, CH_3

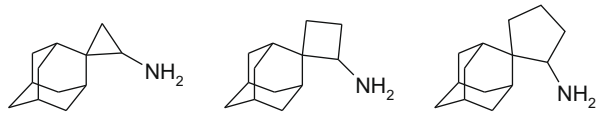
Like rimantadine, C-(1-adamantyl)azacyclanes containing an Ad–CH(CH_3)–N fragment, such as 2-(1-adamantyl)-2-methylpyrrolidine, -2-methylazetidine, and -2-methylaziridine, were also investigated. These compounds are more active than rimantadine against H2N2 and H3N2 viruses while showing, however, higher cytotoxicity.[18]



Rimantadine analogues bearing an ethylamino group at the 2-position of the adamantane framework are also of significant interest. 1-(2-Adamantyl)-1-aminoethane and 1-(2-methyl-2-adamantyl)-1-aminoethane were found less active than rimantadine against H3N2 virus, but significantly more active against H2N2 virus.[19]

2-Adamantyl derivatives were also obtained for azacyclanes. 2-(2-Adamantyl)piperidine was found to be more effective than rimantadine and amantadine against H2N2 virus, but less effective against H3N2 virus.[20] A similar case occurs for 3-(2-adamantyl)pyrrolidines.[21]

Anti-viral activity of 1-(2-adamantyl)-1-aminoethane compelled some investigators to perform the synthesis of spiro-analogues of rimantadine. Spiro[adamantane-2,1′-cyclopropane]-2′-amine,[22] spiro[adamantane-2,1′-cyclobutane]-2′-amine and spiro[adamantane-2,1′-cyclopentane]-2′-amine were obtained. However, anti-viral activity of these compounds was found quite moderate.[19]



Synthesis of a series of spiro-adamantylazacyclanes has also been described in literature. Hydrogenation of (2-nitro-2-adamantyl)propionic acid ethyl ester led to 3-methyl-spiro[pyrrolidine-2,2′-adamantan]-5-one and then to 3-methyl-spiro[pyrrolidine-2,2′-adamantane].[23] Spiro adamantylazacyclanes and 1,2-annulated adamantine azacyclanes were found quite effective against H1N1, H2N2 and H3N2 viruses.[17,23–26]

In the framework of this research, we would like to report the synthesis of *N*-azolo-derivatives of adamantane based on the acid-catalyzed alkylation of the heterocycle in 1-adamantanol. The main goal of the present study was the evaluation of this class of compounds as influenza A virus-inhibiting agents. In order to study the role of azole and adamantly moieties, as well as contribution of side chains in anti-influenza properties of these compounds, we synthesized a set of substances bearing different functional groups in a framework of azolo-adamantanes, and evaluated their toxicities and anti-viral potencies.

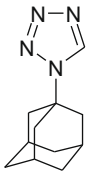
2. Results and discussion

2.1. Chemistry

Since electrophilic reactions of adamantane do not proceed at position-1, a recently studied method of alkylation of azoles with 1-adamantanol in strong acids[27–33] was applied to the synthesis of 1-(1-adamantyl)azoles. It was shown that, in strong acids, 1-adamantanol forms adamantyl-carbocation that reacts readily with tetrazoles,[27,28] 1,2,4-triazoles,[26,29] pyrazoles,[30–32] and other azoles.[33–36,38]

According to the developed method, a variety of *N*-adamantylazoles have been synthesized (Scheme 1), in particular 1-(1-adamantyl)-3,5-dimethylpyrazol-4-amine (**1a**), 1-(1-adamantyl)-4-bromo-3-carboxypyrazole[31] (**1b**), 1-(1-adamantyl)-1,2,4-triazole[29] (**2a**), 1-(1-adamantyl)-3-chloro-1,2,4-triazole[29] (**2b**), as well as a series of 2-(1-adamantyl)-tetrazoles: 2-(1-adamantyl)-tetrazole[27] (**3a**), 2-(1-adamantyl)-5-methyltetrazole[27] (**3b**), 2-(1-adamantyl)-5-carboxymethyltetrazole (**3c**), 2-(1-adamantyl)-5-ethoxycarbonylmethyltetrazole (**3d**), 2-(1-adamantyl)-5-butoxycarbonylmethyltetrazole (**3e**), 2[2-(1-adamantyl)-tetrazol-5-yl]acetohydrazide (**3f**), and 2[2-(1-adamantyl)-tetrazol-5-yl]-N-(2-hydroxyethyl)acetamide (**3g**).

1-(1-Adamantyl)-tetrazole (**4**) was obtained according to the previously described procedure.[37]



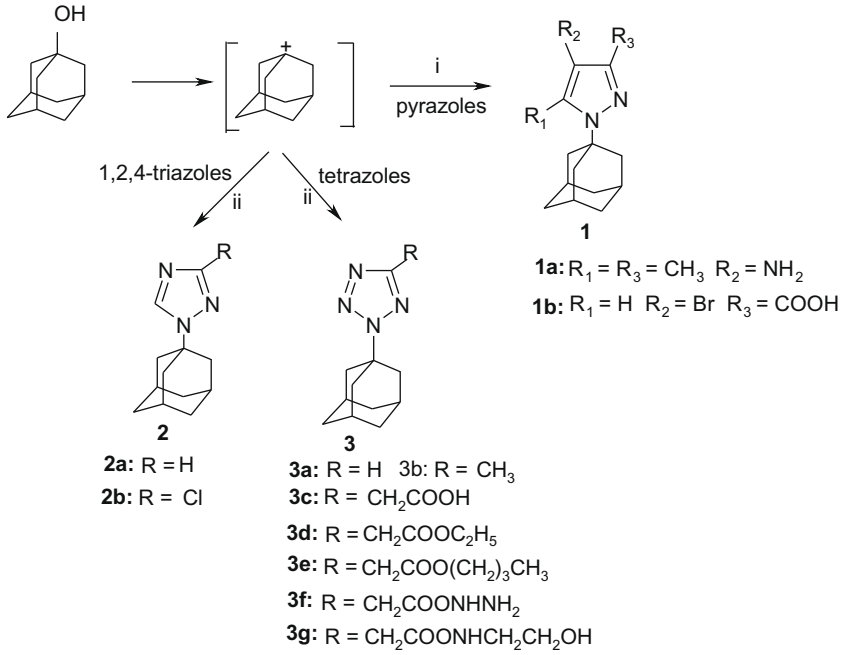
4

A study of the biological activity of 1-(1-adamantyl)pyrazoles and -1,2,4-triazoles showed that their toxicity spans a wide range (minimal toxic dose 5–200 μg/mL), along with very low anti-viral activity (on the biological activity of (1-adamantyl)tetrazoles, see below).

As mentioned above, presence of the highly-basic amino group in the adamantane framework, participating in blocking M2 virus protein, is one of the factors responsible for anti-viral activity of rimantadine and its analogues. We have synthesized a series of *N*-adamantylazoles bearing the amino group in the adamantane framework. Such compounds were obtained by alkylation of azoles with 1-amino- or 1-(1-aminoethyl)-3-hydroxyadamantane in sulfuric acid.

Reaction with ammonium nitrate in sulfuric acid is the most convenient method oto oxidize adamantane derivativto form 1-hydroxy-3-*R*-adamantanes. Occasionally, 1-hydroxy-3-*R*-adamantanes were separated from the reaction mixtures, but in most cases alkylation was carried out while obtaining the corresponding 1-hydroxy-3-*R*-adamantanes in situ. As a result, a broad series of (1-adamantyl)azoles bearing the highly-basic amino group has been obtained.

The following 1,2,4-triazole derivatives were used in the investigation: 1-(3-amino-1-adamantyl)-1,2,4-triazole (**6a**), 1-(3-amino-1-adamantyl)-3-bromo-1,2,4-triazole (**6b**), 1-[3-(1-aminoethyl)-1-



Scheme 1. Reagents and conditions: (i) H_3PO_4–AcOH 4:1 (mass), pyrazoles, 50–60 °C, 3–24 h; (ii) H_2SO_4–AcOH 4:1 (mass), 1,2,4-triazoles or tetrazoles, 20–25 °C, 2–3 h.

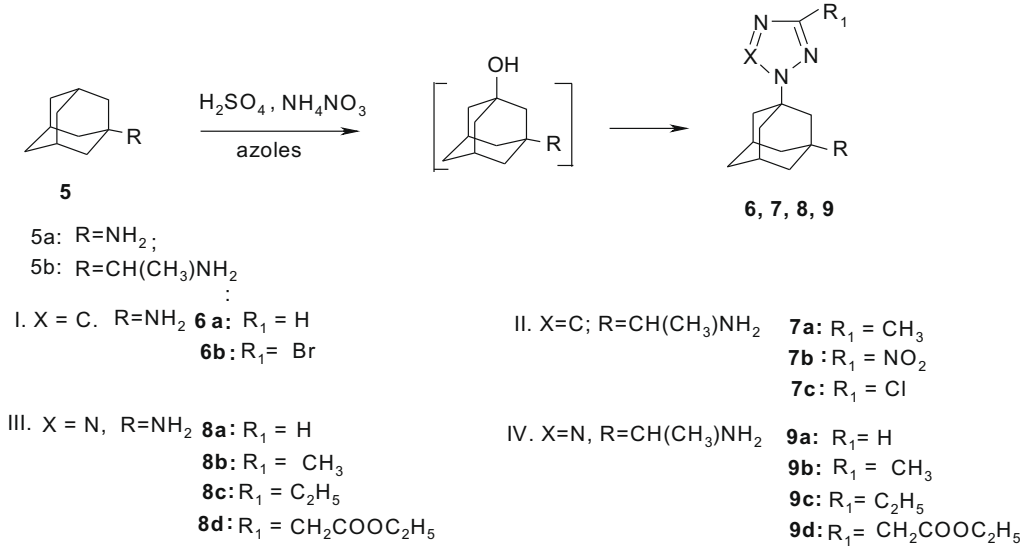
adamantyl]-3-methyl-1,2,4-triazole (**7a**), 1-[3-(1-aminoethyl)-1-adamantyl]-3-nitro-1,2,4-triazole (**7b**), 1-[3-(1-aminoethyl)-1-adamantyl]-3-chloro-1,2,4-triazole (**7c**).

Similarly, a series of (3-amino-1-adamantyl)- and [3-(1-aminoethyl)-1-adamantyl]-tetrazoles has been synthesized: 2-(3-amino-1-adamantyl)tetrazole (**8a**), 2-(3-amino-1-adamantyl)-5-methyltetrazole (**8b**), 2-(3-amino-1-adamantyl)-5-ethyltetrazole (**8c**), 2-(3-amino-1-adamantyl)-5-ethoxycarbonylmethyltetrazole (**8d**), 2-[3-(1-aminoethyl)-1-adamantyl]tetrazole (**9a**), 2-[3-(1-aminoethyl)-1-adamantyl]-5-methyltetrazole (**9b**), 2-[3-(1-aminoethyl)-1-adamantyl]-5-ethyltetrazole (**9c**), and 2-[3-(1-aminoethyl)-1-adamantyl]-5-ethoxycarbonylmethyltetrazole (**9d**) (Scheme 2).

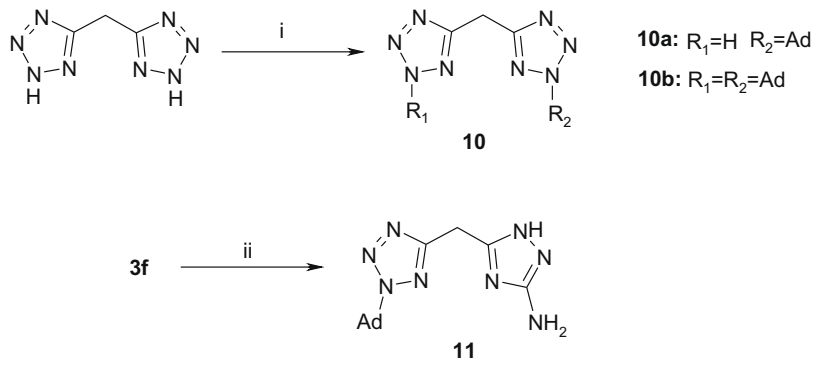
Amino derivatives **6**–**9** were studied in the form of hydrochlorides.

In order to evaluate the biological activity of heterocyclic ring systems as a dimer without and in the presence of the adamantane scaffold, 2-(1-adamantyl) derivatives of bis(5-tetrazolyl)methane (**10a** and **10b**) were synthesized. They exhibited low activity, as well as [2-(1-adamantyl)tetrazolyl-5](3-amino-1,2,4-triazolyl-5)methane (**11**) (Scheme 3).

Among adamantyltetrazoles, a significant number of tetrazol-5-thione and tetrazol-5-one derivatives were studied. 1-(1-Adamantyl)tetrazol-5-thione (**12**) was obtained by cyclocondensation of 1-adamantyl isothiocyanate[39] with sodium azide, instead of direct adamantylation. Oxidation of 1-adamantyl isothiocyanate with



Scheme 2. Reagents and conditions: sulfuric acid, ammonium nitrate, corresponding azole, 20–25 °C, 24 h.



Scheme 3. Reagents and conditions: (i) H_2SO_4–AcOH 4:1, 1-adamantanol, 20–25 °C, 1–24 h; (ii) ethanole, *S*-methylthiourea, NaOH, reflux.

ammonium nitrate in sulfuric acid led to 3-isothiocyanatoadamantan-1-ol (**13**) which was then transformed into 1-(3-hydroxy-1-adamantyl)tetrazol-5-thione (**14**) (Scheme 4).

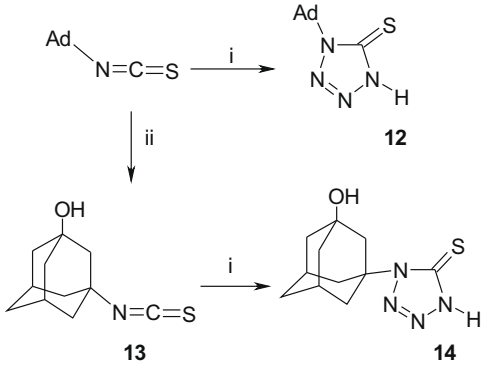
1-*R*-Tetrazol-5-thiones and 1-*R*-tetrazol-5-ones, which are tetrazoles bearing substituents with electron lone pairs at the position-5 in the tetrazole ring, provide other synthetic opportunities for novel antivirals. Instead of alkylation in neutral and basic media, adamantylation in acids mainly leads to *meso*-ionic compounds—1-*R*-3-(1-adamantyl)tetrazolium-5-thiolates and -olates.[40] Such compounds are known to contain a conjugated system that involves exocyclic bonds and interlacing non-integer-valued charges of the cycle atoms.

3-(1-Adamantyl)-1-phenyl-3*H*-tetrazolium-5-thiolate (**16a**) and 3-(1-adamantyl)-1-ethyl-3*H*-tetrazolium-5-thiolate (**16b**) were obtained by adamantylation of the corresponding tetrazolthiones **15**. *S*-substituted derivatives **18a** and **18b** were obtained by hydrolysis of the intermediate tetrazolium salts **17** (Scheme 5).

The reaction of 1-*R*-tetrazol-5-ones **19** and 1-adamantanol in sulfuric acid proceeds selectively at position-3 of the ring to form 1-phenyl-, 1-ethyl-, and 1-(4-methoxycarbonylphenyl)-3-(1-adamantyl)-3*H*-tetrazolium-5-olates **20a**, **20b** and **20c**, respectively (Scheme 6).[40]

2.2. Evaluation of cytotoxicities and antiviral inhibitory effects of compounds

Based on the methods used, a set of compounds have been synthesized and compose of derivatives of di-, tri- and tetrazoles. They were further tested for toxicity and anti-viral activity. The results are summarized in the Table 1.



Scheme 4. Reagents and conditions: (i) NaN_3, dioxane–water, reflux; 8 h, (ii) H_2SO_4, NH_4NO_3, 0–5 °C, 20 h.

As shown in the course of screening, (1-adamantyl) diazoles and 1-(1-adamantyl)-1,2,4-triazoles do not possess high anti-viral activity. Introduction of an amino group into the adamantyl moiety led to a slight increasing of activity of 1,2,4-triazoles (**6a**, **7a** and **7c**). Among derivatives of 1,2,4-triazole, only two compounds—1-(3-chloro-1,2,4-triazol-1-yl)-3-(1-aminoethyl-1)adamantane (**7c**) and 1-(3-methyl-1,2,4-triazol-1-yl)-3-(1-aminoethyl-1)adamantane (**7a**) exert notable anti-influenza activity (SI = 9 and 15, correspondingly).

At the same time, adamantyl derivatives of tetrazole appeared to possess virus-inhibiting properties. First of all, this relates to aminoadamantyl tetrazoles (**9b**, **8b**, **8a**, **9a**, **8c** and **9c**), whose SI could reach 50. Alkyl substitutions in the tetrazole moiety can modulate the activity but their effect differs for 3-amino- and 3-(2-aminoethyl)adamantanes (**8a**–**8c** and **9a**–**9c**).

Interestingly, among adamantyltetrazoles, the compounds without an amino group (**2b**, **4**, **12**, **14**, **16b**, **18**, **20b**, **20c**) exerted anti-influenza activity. It should be noted that the position of the adamantyl moiety in the tetrazole ring was found to be critical for the anti-influenza properties of this class of compounds. Indeed, anti-viral properties of 1- and 2-adamantyltetrazoles **3a** and **4**, differing in the position of adamantyl in the heterocycle, had fivefold difference.

For the anti-influenza activity of compounds tested, both tetrazolyl and adamantyl components of the molecule were required. This related to azolo-adamantanes themselves as well as to hydroxylated derivatives of adamantane (compounds **13** and **14**). Introduction of a thiotetrazole moiety instead of an isothiocyanate substituent led to an approximate sixfold increase in anti-viral activity and a 3–4-fold increase in toxicity (Table 1).

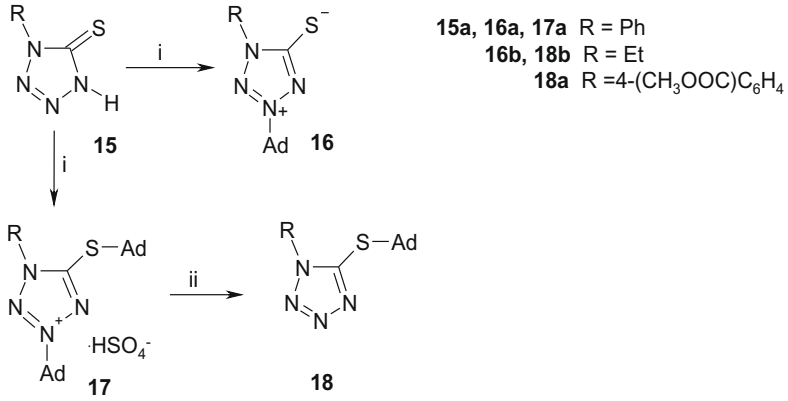
C-methylation of the tetrazole ring in the molecule did not result in changes of its anti-viral activity (compounds **3a** and **3b**). At the same time, its thio-modification at the C-position led to twofold decrease in activity (**4** and **14**). Addition of a phenyl moiety along with or instead of an adamantyl substitutive markedly decreased or completely eliminated the activity of compounds (**15a**, **16a**, **19a**, **20a**).

Addition of a terminal amino group to the C-position of tetrazole through various spacers significantly increased the virus-inhibiting activity of compounds (**3a** and **3f**). Introduction of a hydroxyl group into the adamantyl moiety of the molecule lead to a slight increase in anti-viral activity and an approximate twofold increase in toxicity (**12** and **14**).

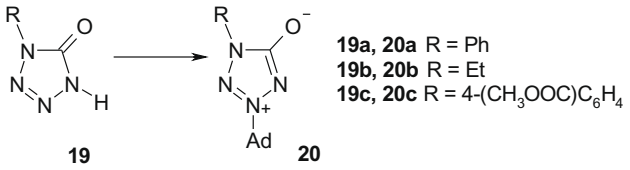
Diadamanthyl and ditetrazole derivatives did not possess anti-viral properties (**10a**, **10b**, **11**, **17a**).

Based on the data obtained we formulated several theories regarding the relationship of structure and anti-viral properties of azolo-adamantanes:

1. The molecule should contain no more than one tetrazole and no more than one adamantlyl moiety.



Scheme 5. Reagents and conditions: (i) 1-adamantanol, H_2SO_4; (ii) H_2O, 100 °C.



Scheme 6. Reagents and conditions: 1-adamantanol, H_2SO_4, 2 h.

Table 1
Activity of *N*-adamantyl azoles against influenza virus A/Puerto Rico/8/34 (H1N1)

Compound	CTD_{50} (microgram/mL)	EC_{50} (microgram/mL)	SI
1a	600	100	6
1b	443	300	1
2a	358	77	5
2b	583	59	10
3a	253	56	5
3b	569	77	7
3c	>500	71	>7
3d	>1000	232	4
3e	<1	>1	<1
3f	383	30	13
3g	884	>1000	<1
4	281	11	25
5b (**Rimantadine**)	60	5	12
6a	1489	123	12
6b	620	111	6
7a	960	62	15
7b	131	120	1
7c	140	15	9
8a	552	22	25
8b	213	13	16
8c	262	25	10
8d	127	18	7
9a	423	23	18
9b	242	8	30
9c	299	6	50
10a	>500	>500	1
10b	120	180	—
11	0.18	0.09	2
12	167	15	11
13	>500	150	>3
14	146	8	18
15a	151	>200	<1
16a	300	130	2
16b	125	3	41
17a	289	57	5
18a	150	2	75
18b	267	61	4
19a	>1000	294	3
20a	52	13	4
20b	604	10	60
20c	300	10	30

2. In most cases, terminal amino groups should be presented as a substitution at the heterocycle and/or adamantyl core, although several exclusions may exist.
3. Anti-viral activity of 1-adamantyltetrazoles is higher than that of their 2-adamantyl isomer.

In the present study we investigated the anti-influenza activity of a novel class of chemical compounds composed of adamantyl and heterocyclic azole moieties. This study was considered an attempt to develop a novel, wide range antiviral and, in particular, to overcome the high-level of amantadine/rimantadine resistance among currently circulating influenza viruses.

Adamantane heterocyclic derivatives of similar structure were studied by Kolocouris and co-workers.[17,24,25] The highly-basic amino group of rimantadine was converted to a nitrogen-containing heterocycle. These experiments demonstrated that the presence of five- or six-membered amino heterocycles and adamantane in one frame greatly enhances the anti-viral activity against influenza A virus. In general, our results are in agreement with those obtained previously. Several of our compounds do not contain a free amino group (see above). Despite this, they possess high-level antiviral activity. This suggests that another mechanism, either direct or indirect, might be involved in their virus-inhibiting action.

The target for adamantane-based antivirals against influenza is virus-specific tetrameric transmembrane protein M2. Functionally, it is a proton pump playing an essential role in the virus' life cycle. After formation of virus-containing vacuole, M2 conducts protons into viral particle thus decreasing pH inside the virion which, in turn, facilitates dissociation of nucleoprotein and release into the cytoplasm. Adamantane derivatives block the transmembrane domain of M2 thus preventing normal morphogenesis of the virus.

Nevertheless, drug-resistant variants of the virus can be easily selected in vitro and frequently occur under natural conditions.[41,42] The incidence of resistant strains during the last few years have reached in some countries almost 100% (http://www.cdc.gov/flu/about/qa/antiviralresistance.htm). Mostly, the resistance is due to the specific amino acid substitution S31N in transmembrane domain of M2, although mutations in positions 26, 27, 30 and 34 may also contribute. Several attempts have been undertaken in order to overcome influenza virus resistance to adamantine derivatives. Schoultissek et al.[3] synthesized and tested adamantine-containing compounds with various substitutives differing in size and charge. This study resulted in selecting compounds interfering with both M2 activity and hemagglutinin function that is able to compensate for the inhibition of the proton channel. Nevertheless, none of them appeared effective enough to replace rimantadine and amantadine in clinical practice.

The model virus used in our study was A/Puerto Rico/8/34 whose level of rimantadine resistance is very high due to the S31N mutation in M2. Therefore, one can expect that compounds with high activity against this virus are good prospects in terms of suppression of other rimantadine-resistant viruses. Indeed, some of compounds tested in our study were shown to be more potent inhibitors of virus multiplication than rimantadine. This can be explained in two ways. First, these compounds have higher affinity to M2 protein. Second, the target for these compounds is different. It should be noted that if the target for adamantane-based antivirals is located inside the M2 tetramer,[43] it narrows the set of compounds that fit the channel due to spatial restrictions. If, according to another model,[44] the substances bind with amino acids from outside the M2, one may consider a much wider group of compounds, because spatial requirements in this case are less strict, especially if the target is located in the cytoplasm (not within the channel and not between its outer surface and lipid bilayer of the virion's membrane).

Of particular interest are *meso*-ionic derivatives of adamantyltetrazole (**20b**, **20c**, **16b**, **18a**). First, their anti-influenza activity is relatively high (SI 30–75). Second, these compounds, similarly to adamantane, do not occur in nature. Recently, sulfur-containing azoles have been shown to specifically inhibit the RNA polymerase complex of measles virus.[45] It cannot be ruled out that, although differing in structure, azolo-adamantanes might have the similar target. If so, they would be attractive for further optimization in order to develop new selective and effective anti-influenza drugs.

3. Experimental

3.1. Chemistry

^{1}H and ^{13}C NMR spectra were recorded on a Bruker WM-400 and a Bruker AC-200 spectrometers, respectively. Thin-layer chromatography was performed on Sorbfil and Silufol UV-254 plates, eluent chloroform, chloroform/ethyl acetate (4:1), or chloroform/acetone (1:1). Some products were purified by chromatography on a silica gel (Chemapol L 100/160) column.

3.1.1. 2-(1-Adamantyl)-5-ethoxycarbonylmethyltetrazole (3d)

To a solution of acetic acid (9.2 g) in 25 mL of sulfuric acid cooled to room temperature, 5-ethoxycarbonylmethyltetrazole[46] (1.56 g, 10 mmol) and 1-adamantanol (1.52 g, 10 mmol) were added. The mixture was stirred for 40 min and poured into 150 mL of water with ice. The oily product was extracted with chloroform, the chloroform solution was washed with 5% sodium carbonate solution and water, dried over sodium sulfate, and the solvent was evaporated. The oily residue was dissolved in a minimal quantity of hexane and frozen. The precipitate was filtered and washed with hexane. Yield 2.17 g (75%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.26, 1.28, 1.30 (3H, CH_3), 1.82 (6H, Ad), 2.31 (9H, Ad), 3.89 (2H, CH_2 at 5-position of tetrazole), 4.13, 4.15, 4.18, 4.20 (2H, CH_2 of the ethyl group). Mp 38–40 °C. Anal. Calcd for $C_{15}H_{22}N_4O_2$: C, 62.05; H, 7.64; N, 19.30. Found: C, 62.12; H, 7.88; N, 19.39.

3.1.2. 2-(1-Adamantyl)-5-carboxymethyltetrazole (3c)

2-(1-Adamantyl)-5-ethoxycarbonyl-methyltetrazole (**3d**) (2.9 g, 10 mmol) was dissolved in 30 mL of 1:1 ethanol/water mixture, and potassium hydroxide (2 g) was added. The mixture was refluxed for 1 h. Then 30 mL of water were added and the solution was acidified to pH 2–3 with hydrochloric acid. The precipitate was filtered and recrystallized from aqueous ethanol. Yield 2.1 g (80%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.80 (6H, Ad), 2.37 (9H, Ad), 3.65 (2H, CH_2 at 5-position of tetrazole), 9.26 (1H, COOH). Mp 126–128 °C. Anal. Calcd for $C_{13}H_{18}N_4O_2$: C, 59.53; H, 6.22; N, 21.36. Found: C, 59.66; H, 7.12; N, 21.87.

3.1.3. 2-(1-Adamantyl)-5-butoxycarbonylmethyltetrazole (3e)

2-(1-Adamantyl)-5-carboxymethyltetrazole (**3c**) (2.62 g, 10 mmol) and 1-butanol (5 mL) were dissolved in 50 mL of benzene, and two drops of sulfuric acid were added. The mixture was refluxed for 2 h. Then the solution was washed with 50 ml of 3% aqueous sodium hydroxide and water, dried over sodium sulfate, and the solvent was evaporated. 2.09 g (66%) of the liquid product were obtained. ^{1}H NMR (DMSO-d_6) (δ, ppm): 0.89t (3H, CH_3), 1.31q (2H, CH_2), 1.56 m (2H, CH_2), 1.78 (6H, Ad), 2.26 (9H, Ad), 3.94 (2H, CH_2 at 5-position of tetrazole), 4.07t (2H, CH_2).

3.1.4. 2-[2(1-Adamantyl)-tetrazol-5-yl]-acetohydrazide (3f)

2-(1-Adamantyl)-5-ethoxycarbonylmethyltetrazole (**3d**) (2.9 g, 10 mmol) was dissolved in 20 mL of the 1:1 ethanol/water mixture, and hydrazine hydrate (2 mL) was added. The mixture was refluxed for 1 h and cooled, and the precipitate was filtered. Yield 1.58 g (55%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.77 (6H, Ad), 2.25 (9H, Ad), 3.45 (2H, CH_2 at 5-position of tetrazole), 9.26 (1H, NH), 4.2 (2H, NH_2). Mp 132–134 °C. Anal. Calcd for $C_{13}H_{20}N_6O$: C, 56.50; H, 7.30; N, 30.41. Found: C, 56.98; H, 8.12; N, 31.09.

3.1.5. 2-[2(1-Adamantyl)-tetrazol-5-yl]-N-(2-hydroxyethyl)acetamide (3g)

2-(1-Adamantyl)-5-ethoxycarbonylmethyltetrazole (**3d**) (2.9 g, 10 mmol) were dissolved in 20 mL of ethanol, and 2-aminoethanol (2 mL) was added. The mixture was refluxed for 4 h, diluted with 20 mL of water, cooled, and the precipitate was filtered. Yield 1.98 g (65%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.77 (6H, Ad), 2.25 (9H, Ad), 3.13, 3.15, 3.41, 3.43 (4H, CH_2–CH_2), 3.7 (2H, CH_2 at 5-position of tetrazole), 4.57, 4.58, 4.59 (1H, OH), 8.11 (1H, NH). Mp 178–180 °C. Anal. Calcd for $C_{15}H_{23}N_5O_2$): C, 59.00; H, 7.59; N, 22.93. Found: C, 59.59; H, 8.44; N, 23.15.

3.1.6. 1-(3-Amino-1-adamantyl)-1,2,4-triazole hydrochloride (6a)

1,2,4-Triazole (0.69 g, 10 mmol), 1-aminoadamantan (1.51 g, 10 mmol), and ammonium nitrate (2.4 g, 30 mmol) were sequentially dissolved in 30 mL of 94% sulfuric acid. The mixture was maintained for 24 h at room temperature, poured onto ice, and the solution was neutralized to pH 8–9 with aqueous ammonia. The product was extracted with chloroform (8 × 50 mL), and the solvent was evaporated. Yield of the base was 1.87 g (86%). The product was dissolved in 50 mL of chloroform–diethyl ether mixture, and dry hydrogen chloride was bubbled through the mixture. The precipitate was filtered. Yield 1.70 g (67%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.70 (2H, Ad), 1.90 (4H, Ad), 2.05 (4H, Ad), 2.25 (4H, Ad), 8.10 (1H, H at 5-position of 1,2,4-triazole), 8.65 (3H, NH_3^+), 8.85 (1H, H at 3-position of 1,2,4-triazole). Mp 284–287 °C (2-propanol). Anal. Calcd for $C_{12}H_{19}ClN_4$: C, 56.56; H, 7.53; N, 21.99. Found: C, 56.32; H, 7.21; N, 21.26.

3.1.7. 1-(3-Amino-1-adamantyl)-3-bromo-1,2,4-triazole hydrochloride (6b)

3-Bromo-1,2,4-triazole (0.99 g, 6.7 mmol), 1-aminoadamantan (1.01 g, 6.7 mmol), and ammonium nitrate (1.6 g, 20 mmol) were sequentially dissolved in 25 mL of 94% sulfuric acid. The mixture was maintained for 24 h at room temperature, poured onto ice, and the solution was neutralized to pH 8–9 with aqueous ammonia. The product was extracted with chloroform (8 × 50 mL), and the solvent was evaporated. Yield of the base was 1.32 g (66%). The product was dissolved in 50 mL of chloroform-diethyl ether mixture, and dry hydrogen chloride was bubbled through the mixture. The precipitate was filtered. Yield 1.46 g (65%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.60 (2H, Ad), 1.90 (4H, Ad), 2.20 (4H, Ad), 2.50 (4H, Ad), 8.05 (1H, H at

5-position of 1,2,4-triazole), 8.60 (3H, NH_3^+). Mp 272–274 °C (2-propanol). Anal. Calcd for $C_{12}H_{18}BrClN_4$: C, 43.06; H, 5.43; N, 16.74. Found: C, 41.35; H, 5.35, N, 15.31.

3.1.8. 1-[3-(1-Aminoethyl)-1-adamantyl]-3-methyl-1,2,4-triazole dihydrochloride (7a)

1,2,4-Triazole (0.83 g, 10 mmol), 1-(1-aminoethyl)adamantan hydrochloride (2.16 g, 10 mmol), and ammonium nitrate (2.4 g, 30 mmol) were sequentially dissolved in 35 mL of 94% sulfuric acid. The mixture was maintained for 24 h at room temperature, poured onto ice, and the solution was neutralized to pH 8–9 with aqueous ammonia. The product was extracted with chloroform (8 × 50 mL), and the solvent was evaporated. Yield of the base was 2.48 g (95%). The product was dissolved in 50 mL of chloroform–diethyl ether mixture, and dry hydrogen chloride was bubbled through the mixture. The precipitate was filtered. Yield 1.58 g (47%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.10 (3H, CH_3 of ethylamino group), 1.60 m (6H, Ad), 2,05 m (6H, Ad), 2.25 (2H, Ad), 2.45 (3H, CH_3 at triazole ring), 2.90 (1H, CH of ethylamino group), 8.25 (3H, NH_3), 9.70 (1H, H at 5-position of 1,2,4-triazole). Mp 238–241 °C (2-propanol). Anal. Calcd for $C_{15}H_{26}Cl_2N_4$: C, 54.04; H, 7.88; N, 16.81. Found: C, 54.09; H, 7.84; N, 17.57.

3.1.9. 1-[3-(1-Aminoethyl)-1-adamantyl]-3-nitro-1,2,4-triazole hydrochloride (7b)

3-Nitro-1,2,4-triazole (1.14 g, 10 mmol), 1-(1-aminoethyl)adamantan hydrochloride (2.16 g, 10 mmol), and ammonium nitrate (2.4 g, 30 mmol) were sequentially dissolved in 40 mL of 94% sulfuric acid. The mixture was maintained for 24 h at room temperature, poured onto ice, and the solution was neutralized to pH 8–9 with aqueous ammonia. The product was extracted with chloroform (9 × 30 mL), and the solvent was evaporated. Yield of the base was 2.05 g (70%). The product was dissolved in 50 mL of chloroform–diethyl ether mixture, and dry hydrogen chloride was bubbled through the mixture. The precipitate was filtered. Yield 1.53 g (47%). 1.19 (3H, CH_3 of ethylamino group), 1.62 m (6H, Ad), 2.10 m (6H, Ad), 2.33 (2H, Ad), 2.94 (1H, CH of ethylamino group), 8.20 (3H, NH_3), 9.00 (1H, H at 5-position of 1,2,4-triazole). Mp 233–235 °C (2-propanol). Anal. Calcd for $C_{14}H_{22}ClN_5O_2$: C, 51.28; H, 6.78; N, 21.37. Found: C, 51.01; H, 6.70; N, 21.39.

3.1.10. 1-[3-(1-Aminoethyl)-1-adamantyl]-3-chloro-1,2,4-triazole hydrochloride (7c)

3-Chloro-1,2,4-triazole (1.04 g, 10 mmol), 1-(1-aminoethyl)adamantan hydrochloride (2.16 g, 10 mmol), and ammonium nitrate (2.4 g, 30 mmol) were sequentially dissolved in 40 mL of 94% sulfuric acid. The mixture was maintained for 24 h at room temperature, poured onto ice, and the solution was neutralized to pH 8–9 with aqueous ammonia. The product was extracted with chloroform (8 × 50 mL), and the solvent was evaporated. Yield of the base was 2.19 g (78%). The product was dissolved in 50 mL of chloroform–diethyl ether mixture, and dry hydrogen chloride was bubbled through the mixture. The precipitate was filtered. Yield 1.38 g (43%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.18 (3H, CH_3 of ethylamino group), 1.63 m (6H, Ad), 2,02 m (6H, Ad), 2.29 (2H, Ad), 2.90 (1H, CH of ethylamino group), 8.17 (3H, NH_3), 8.61 (1H, H at 5-position of 1,2,4-triazole). Mp 268–271 °C (2-propanol). Anal. Calcd for $C_{14}H_{22}Cl_2N_4$: C, 52.99; H, 7.00; N, 17.66. Found: C, 52.24; H, 6.89; N, 17.13.

3.1.11. 2-(3-Amino-1-adamantyl)-tetrazole (8a)

Tetrazole (0.70 g, 10 mmol), 1-amino-adamantan hydrochloride (1.87 g, 10 mmol), and ammonium nitrate (2.4 g, 30 mmol) were sequentially dissolved in 25 mL of 94% sulfuric acid. The mixture was maintained for 24 h at room temperature, poured onto ice, and the solution was neutralized to pH 8–9 with aqueous ammonia. The product was extracted with chloroform (3 × 30 mL), and the solvent was evaporated to the volume of 15–20 mL, and dry hydrogen chloride was bubbled through the mixture. 50 mL of diethyl ether was added, and the precipitate was filtered and recrystallized from methanol–diethyl ether mixture. Yield 1.67 g (65%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.59, 1.62, 1.69, 1.72 (2H, Ad), 1.86, 1.90, 1.92, 1.94 (4H, Ad), 2.11, 2.13, 2.21, 2.24 (4H, Ad), 2.35 (4H, Ad), 8.50 (3H, NH_3^+), 8.82 (1H, CH of tetrazole). Mp 294–297 °C. Anal. Calcd for $C_{11}H_{20}ClN_5$: C, 51.26; H, 7.82, N, 27.17. Found: C, 50.88; H, 8.55; N, 27.69.

3.1.12. 2-(3-Amino-1-adamantyl)-5-methyltetrazole hydrochloride (8b)

5-Methyltetrazole (0.84 g, 10 mmol), 1-aminoadamantan hydrochloride (1.87 g, 10 mmol), and ammonium nitrate (2.4 g, 30 mmol) were sequentially dissolved in 25 mL of 94% sulfuric acid. The mixture was maintained for 24 h at room temperature, poured onto ice, and the solution was neutralized to pH 8–9 with aqueous ammonia. The product was extracted with chloroform (3 × 30 mL), and the solvent was evaporated to the volume of 15–20 mL, and dry hydrogen chloride was bubbled through the mixture. 50 mL of diethyl ether was added, and the precipitate was filtered and recrystallized from methanol–diethyl ether mixture. Yield 2.03 g (75%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.58, 1.61, 1.68, 1.71 (2H, Ad), 1.86, 1.89, 1.91, 1.94 (4H, Ad), 2.01, 2.12, 2.20, 2.23 (4H, Ad), 2.39 (4H, Ad), 2.45 (3H, CH_3), 8.50 (3H, NH_3^+). Mp 285–288 °C. Anal. Calcd for $C_{12}H_{22}ClN_5$: C, 53.03; H, 8.16; N, 25.77. Found: C, 53.53; H, 8.98; N, 24.99.

3.1.13. 2-(3-Amino-1-adamantyl)-5-ethyltetrazole hydrochloride (8c)

5-Ethyltetrazole (0.98 g, 10 mmol), 1-aminoadamantan hydrochloride (1.87 g, 10 mmol), and ammonium nitrate (2.4 g, 30 mmol) were sequentially dissolved in 25 mL of 94% sulfuric acid. The mixture was maintained for 24 h at room temperature, poured onto ice, and the solution was neutralized to pH 8–9 with aqueous ammonia. The product was extracted with chloroform (3 × 30 mL), and the solvent was evaporated to the volume of 15–20 mL, and dry hydrogen chloride was bubbled through the mixture. 50 mL of diethyl ether was added, and the precipitate was filtered and recrystallized from methanol-diethyl ether mixture. Yield 1.56 g (55%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.26 (3H, CH_3), 1.56, 1.61, 1.68, 1.71 (2H, Ad), 1.90q (4H, Ad), 2.01, 2.12, 2.21, 2.23 (4H, Ad), 2.38 (4H, Ad), 2.84q (2H, CH_2 of ethyl group), 8.47 (3H, NH_3^+). Mp 274–277 °C. Anal. Calcd for $C_{13}H_{24}ClN_5$: C, 54.63; H, 8.46; N, 24.50. Found: C, 54.96; H, 9.09; N, 24.78.

3.1.14. 2-(3-Amino-1-adamantyl)-5-ethoxycarbonylmethyltetrazole hydrochloride (8d)

5-Ethoxycarbonylmethyltetrazole (1.56 g, 10 mmol), 1-aminoadamantan hydrochloride (1.87 g, 10 mmol), and ammonium nitrate (1.6 g, 20 mmol) were sequentially dissolved in 50 mL of 94% sulfuric acid. The mixture was maintained for 100 h at room temperature, poured onto ice, and the solution was neutralized to pH 8–9 with aqueous ammonia. The product was extracted with chloroform (8 × 25 mL), and the solvent was evaporated. Yield of the base was 1.82 g (60%). The product was dissolved in 50 mL of chloroform, and dry hydrogen chloride was bubbled through the solution. The solvent was evaporated, and the residue was crystallized from 2-propanole. Yield 1.45 g (42%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.21 (3H, CH_3), 1.69 (2H, Ad), 1.95 (4H, Ad), 2.21 (4H, Ad), 2.43 (4H, Ad), 3.97 (2H, CH_2 at 5-position of tetrazole), 4.10 (2H, CH_2 of ethyl group), 8.62 (3H, NH_3^+). Mp 172–174 °C. Anal. Calcd for $C_{15}H_{24}ClN_5O_2$: C, 52.69; H, 7.09; N, 20.49. Found: C, 52.43; H, 6.79; N, 20.01.

3.1.15. 2-[3-(1-Aminoethyl)-1-adamantyl]-tetrazole hydrochloride (9a)

Tetrazole (0.70 g, 10 mmol), 1-(1-aminoethyl)adamantan hydrochloride (2.16 g, 10 mmol), and ammonium nitrate 2.4 g, 30 mmol) were sequentially dissolved in 25 mL of 94% sulfuric acid. The mixture was maintained for 24 h at room temperature, poured onto ice, and the solution was neutralized to pH 8–9 with aqueous ammonia. The product was extracted with chloroform (3 × 30 mL), and the solvent was evaporated to the volume of 15–20 mL, and dry hydrogen chloride was bubbled through the mixture. 50 mL of diethyl ether was added, and the precipitate was filtered and recrystallized from methanol–diethyl ether mixture. Yield 1.56 g (55%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.14d (3H, CH_3 of the ethylamino group), 1.62 m (6H, Ad), 2.00, 2.03, 2.06, 2.09 q (2H, Ad), 2.18s (4H, Ad), 2.30s (2H, Ad), 2.94q (1H, CH of the ethylamino group), 8.60 (3H, NH_3^+), 8.86 (1H, CH of tetrazole). Mp 287–290 °C. Anal. Calcd for $C_{13}H_{24}ClN_5$: C, 54.63; H, 8.46; N, 24.50. Found: C, 54.08; H, 7.68; N, 24.99.

3.1.16. 2-[3-(1-Aminoethyl)-1-adamantyl]-5-methyltetrazole hydrochloride (9b)

5-Methyltetrazole (0.84 g, 10 mmol), 1-(1-aminoethyl)adamantan hydrochloride (2.16 g, 10 mmol), and ammonium nitrate (2.4 g, 30 mmol) were sequentially dissolved in 25 mL of 94% sulfuric acid. The mixture was maintained for 24 h at room temperature, poured onto ice, and the solution was neutralized to pH 8–9 with aqueous ammonia. The product was extracted with chloroform (3 × 30 mL), and the solvent was evaporated to the volume of 15–20 mL, and dry hydrogen chloride was bubbled through the mixture. 50 mL of diethyl ether was added, and the precipitate was filtered and recrystallized from methanol–diethyl ether mixture. Yield 1.94 g (65%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.14d (3H, CH_3 of the ethylamino group), 1.61 m (6H, Ad), 2.00, 2.03, 2.06, 2.09 q (2H, Ad), 2.44 (3H, CH_3 at 5-position of tetrazole), 2.18s (4H, Ad), 2.30s (2H, Ad), 2.94q (1H, CH of the ethylamino group), 8.50 (3H, NH_3^+). Mp 279–282 °C. Anal. Calcd for $C_{14}H_{26}ClN_5$: C, 56.08; H, 8.74; N, 23.36. Found: C, 56.23; H, 8.99; N, 23.58.

3.1.17. 2-[3-(1-Aminoethyl)-1-adamantyl]-5-ethyltetrazole hydrochloride (9c)

5-Ethyltetrazole (0.98 g, 10 mmol), 1-(1-aminoethyl)adamantan hydrochloride (2.16 g, 10 mmol), and ammonium nitrate (2.4 g, 30 mmol) were sequentially dissolved in 25 mL of 94% sulfuric acid. The mixture was maintained for 24 h at room temperature, poured onto ice, and the solution was neutralized to pH 8–9 with aqueous ammonia. The product was extracted with chloroform (3 × 30 mL), and the solvent was evaporated to the volume of 15–20 mL, and dry hydrogen chloride was bubbled through the mixture. 50 mL of diethyl ether were added, and the precipitate was filtered and recrystallized from methanol–diethyl ether mixture. Yield 1.72 g (55%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.15d (3H, CH_3 of the ethylamino group), 1.25t (3H, CH_3 of the ethyl group at 5-position of tetrazole), 1.62 m (6H, Ad), 2.01, 2.04, 2.07, 2.10q (2H, Ad), 2.19s (4H, Ad), 2.31s (2H, Ad), 2.83q (2H, CH_2 of the ethyl group at 5-position of tetrazole) 2.95q (1H, CH of the ethylamino group), 8.17 (3H, NH_3^+). Mp 268–271 °C. Anal. Calcd for $C_{15}H_{28}ClN_5$: C, 57.40; H, 8.99; N, 22.31. Found: C, 57.98; H, 9.55; N, 22.69.

3.1.18. 2-[3-(1-Aminoethyl)-1-adamantyl]-5-ethoxycarbonylmethyltetrazole hydrochloride (9d)

5-Ethoxycarbonylmethyltetrazole (1.56 g, 10 mmol), 1-(1-aminoethyl)adamantan hydrochloride (2.16 g, 10 mmol), and ammonium nitrate (1.6 g, 20 mmol) were sequentially dissolved in 50 mL of 94% sulfuric acid. The mixture was maintained for 100 h at room temperature, poured onto ice, and the solution was neutralized to pH 8–9 with aqueous ammonia. The product was extracted with chloroform (8 × 25 mL), and the solvent was evaporated. Yield of the base was 1.37 g (41%). The product was dissolved in 50 mL of chloroform, and dry hydrogen chloride was bubbled through the solution. The solvent was evaporated, and the residue was crystallized from 2-propanole. Yield 1.44 g (39%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.19 (3H, CH_3 of ethyl group), 1.20 (3H, CH_3 of ethylamino group), 1,67 (6H, Ad), 2.09 (2H, Ad), 2.21 (4H, Ad), 2.35 (2H, Ad), 2.91 (1H, CH of ethylamino group), 3.95 (2H, CH_2 at 5-position of tetrazole), 4.11 (2H, CH_2 of ethyl group), 8.22 (3H, NH_3^+). Mp 138–140 °C. Anal. Calcd for $C_{17}H_{28}ClN_5O_2$: C, 55.19; H, 7.64; N, 18.93. Found: C, 55.41; H, 7.74; N, 18.78.

3.1.19. Di[2-(1-adamantyl)tetrazolyl-5]methane (10a)

Di(tetrazolyl-5)methane[27] (1.52 g, 10 mmol) and 1-adamantanol (3.04 g, 20 mmol) were dissolved in 4:1 (volume) mixture of sulfuric and acetic acids. The mixture was maintained for 24 h at room temperature, poured onto ice, and the precipitate was filtered and recrystallized from ethanol–chloroform (3:1) mixture. Yield 3.36 g (80%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.78 (12H, Ad), 2.26 (18H, Ad), 4.43 (2H, CH_2 between tetrazoles). Mp 230–232 °C. Anal. Calcd for $C_{23}H_{32}N_8$: C, 65.69; H, 7.67; N, 26.64. Found: C, 65.32; H, 7.98; N, 26.52.

3.1.20. [2-(1-Adamantyl)tetrazolyl-5](tetrazolyl-5)methane (10b)

Di(tetrazolyl-5)methane (1.52 g, 10 mmol) and 1-adamantanol (1.52 g, 10 mmol) were dissolved in 4:1 (volume) mixture of sulfuric and acetic acids. The mixture was maintained for 40 min at room temperature, poured onto ice, and the precipitate was filtered and recrystallized from ethanol–water (1:1) mixture. Yield 1.85 g (65%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.73 (6H, Ad), 2.22 (9H, Ad), 4.64 (2H, CH_2 between tetrazoles). Mp 152–154 °C. Anal. Calcd for $C_{13}H_{18}N_8$: C, 54.53; H, 6.34; N, 39.13. Found: C, 54.04, H, 5.65; N, 39.76.

3.1.21. [2-(1-Adamantyl)tetrazolyl-5](3-amino-1,2,4-triazolyl-5)methane (11)

2-(1-Adamantyl)-5-hydrazocarbonylmethyltetrazole (**3f**) (2.76 g, 10 mmol), *S*-methylisothiourea (2.82 g, 15 mmol), and sodium hydroxide (0.6 g, 15 mmol) were sequentially dissolved in 20 mL of ethanol. The mixture was refluxed for 6 h, then the solvent was evaporated. 20 mL of water and sodium hydroxide (0,6 g, 15 mmol) were added to the residue. The solution was refluxed for 40 min and acidified with acetic acid. The precipitate was filtered and recrystallized from ethanol–water (1:1) mixture. Yield 2.28 g (76%). ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.77 (6H, Ad), 2.25 (9H, Ad), 3.96 (2H, CH_2 between heterocycles), 5.63 (2H, NH_2). Mp 230–232 °C. Anal. Calcd for $C_{14}H_{20}N_8$: C, 55.98; H, 6.71; N, 37.31. Found: C, 56.12; H, 6.05; N, 36.98.

3.1.22. 1-(1-Adamantyl)tetrazol-5-thione (12)

Compound **12** was synthesized in 35% yield by reaction of 1-adamantyl isothiocyanate with sodium azide in aqueous dioxane, similar to known procedure.[39] Product was recrystallized from 80% 2-propanol. ^{1}H NMR (DMSO-d_6) (δ, ppm): 2.57 (s, 6H), 2.24 (s, 3H), 1.75 (s, 6H). Mp 179–181 °C. Anal. Calcd for $C_{11}H_{16}N_4S$: C, 55.89; H, 6.84; N, 23.71. Found: C, 56.02; H, 6.83; N, 23.59.

3.1.23. 3-Isothiocyanatoadamantan-1-ol (13)

A solution of 1-adamantyl isothiocyanate (4.2 g, 21 mmol) in 75 mL of 94% sulfuric acid was cooled to 0 °C, and ammonium nitrate (7.2 g, 90 mmol) was added in a rate that allowed to maintain the temperature of 0–5 °C. The reaction mixture was maintained for 20 h at 5 °C, poured onto ice, and the precipitate was filtered. Yield 3.88 g (88%), Mp 159–162 °C (2-propanol). Anal. Calcd for $C_{11}H_{15}NOS$: C, 63.11; H, 7.24; N, 6.69. Found: C, 63.67; H, 6.86; N, 6.89.

3.1.24. 1-(3-Hydroxy-1-adamantyl)tetrazol-5-thione (14)

Compound **14** was synthesized in 39% yield by reaction of **13** with sodium azide in aqueous dioxane, similar to known procedure.[39] ^{1}H NMR ($CDCl_3$) (δ, ppm): 2.55, 2.30, 1.80. Mp 172–175 °C. Anal. Calcd for $C_{11}H_{16}N_4OS$: C, 52.35; H, 6.40; N, 22.20. Found: C, 52.59; H, 6.50; N, 22.53.

1-Phenyltetrazol-5-thione (**15a**)[47] and 1-phenyltetrazol-5-one (**19a**)[48] were synthesized by known methods. 3-(1-Adamantyl)-1-phenyl-3*H*-tetrazolium-5-thiolate (**16a**) and 3-(1-adamantyl)-5-(1-adamantylsulfanyl)-1-phenyltetrazolium hydrosulfate (**17a**) were obtained by adamantylation[40] of **15a**.

3.1.25. 3-(1-Adamantyl)-1-ethyltetrazolium-5-thiolate (16b)

Compound **16b** was obtained in 19% yield by reaction of 1-ethyltetrazol-5-thione[47] with adamantan-1-ol in manner similar to that described for **20a**. Product was recrystallized from hexane/propan-2-ol (10:1). ^{1}H NMR (DMSO-d_6) δ: 4.32 (q, 2H, *J* 6.9 Hz, CH_2), 2.23 (s, 9H, Ad), 1.75 (s, 6H, Ad), 1.42 (t, 3H, *J* 6.9 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ: 172.74, 66.88, 40.73, 34.99, 28.87, 43.00, 12.89. Mp 144–146 °C. Anal. Calcd for $C_{13}H_{20}N_4S$: C, 59.09; H, 7.57; N, 21.21. Found: C, 59.05; H, 6.64; N, 21.35.

3.1.26. 1-Phenyl-3-(1-adamantyl)-3*H*-tetrazolium-5-olate (20a)

A solution of **19a** (0.50 g, 3.09 mmol) and adamantan-1-ol (0.47 g, 3.09 mmol) in 30 mL of 94% sulfuric acid was stirred for 2 h at room temperature. The mixture was then poured onto ice. Precipitate was filtered off, washed with water, and recrystallized from 50% aqueous 2-propanol to give 0.68 g (75%) of **20a**, ^{1}H NMR (DMSO-d_6) δ: 7.93–7.41 (m, 5H, C_6H_5), 2.24 (s, 9H, Ad), 1.72 (s, 6H, Ad); ^{13}C NMR (DMSO-d_6) δ: 158.84, 134.23, 129.46, 128.16, 120.27, 66.61, 40.44, 35.04, 28.76; Mp 179–181 °C. Anal. Calcd for $C_{17}H_{20}N_4O$: C, 68.92; H, 6.76; N, 18.92. Found: C, 69.29; H, 7.27; N, 19.43.

3.1.27. 1-Ethyl-3-(1-adamantyl)-3*H*-tetrazolium-5-olate (20b)

1-Ethyltetrazol-5-thione was alkylated by methyl iodide with phase-transfer catalyst,[49] to give 1-ethyl-5-methylsulfanyl-tetrazole, which was purified by vacuum distillation (bp 92–94 °C, 1 mm). 1-Ethyltetrazol-5-one was obtained by alkaline hydrolysis,[42] of 1-ethyl-5-methylsulfanyl-tetrazole and used without further purification.

A solution of 1-ethyltetrazol-5-one (0.40 g, 3.51 mmol) and adamantan-1-ol (0.54 g, 3.55 mmol) in 94% sulfuric acid (15 ml) was stirred for 2 h at room temperature. The mixture was then poured onto ice and extracted with 100 ml of dichloromethane. The extract was dried over sodium sulfate and the solvent was evaporated. The residue was purified by column chromatography ($CHCl_3$/EtOAc 4:1) and recrystallized from hexane/tetrachloromethane (10:1), to give 0.53 g (60%) of compound **20b**. ^{1}H NMR (DMSO-d_6) δ: 3.97 (q, 2H, *J* 6.9 Hz, CH_2), 2.23 (s, 3H, Ad), 2.17 (s, 6H, Ad), 1.74 (s, 6H, Ad), 1.35 (t, 3H, *J* 6.9 Hz, CH_3); ^{13}C NMR (DMSO-d_6/CCl_4 4:1) δ: 160.30, 65.65, 40.74, 35.35, 28.91, 39.15, 13.63. Mp 110–112 °C. Anal. Calcd for $C_{13}H_{20}N_4O$: C, 62.90; H, 8.06; N, 22.58. Found: C, 63.24; H, 7.99; N, 22.65.

3.1.28. 4-(5-Oxo-tetrazol-1-yl)-benzoic acid methyl ester

A mixture of 4-(5-thioxo-tetrazol-1-yl)-benzoic acid (10 g, 4.5 mol), KOH (3.1 g, 5.54 mol) and methyl iodide (28.4 g, 200 mol) in 200 mL of ethanol was heated for 2 h under reflux. The mixture was diluted with cold water and acidified with hydrochloric acid. The precipitate formed was filtered off and recrystallized from 2-PrOH/DMF (10:1), to give 6.6 g (62%) of 4-(5-methylsulfanyl-tetrazol-1-yl)-benzoic acid.

4-(5-Oxo-tetrazol-1-yl)-benzoic acid was obtained in 57% yield by alkaline hydrolysis[45] of 4-(5-methylsulfanyl-tetrazol-1-yl)-benzoic acid.

A mixture of 4-(5-oxo-tetrazol-1-yl)-benzoic acid (3 g, 14.6 mol), methanol (60 mL), and acetone (30 mL) was heated for 10 h under reflux with stirring and bubbling of dry hydrogen chloride. The mixture was diluted with cold water, the precipitate was filtered off, washed with water, and added into aqueous Na_2CO_3 solution (pH 10). The resulting mixture was filtered and acidified with hydrochloric acid until pH 1. The precipitate formed was filtered off, washed with water, and recrystallized from EtOAc, to give 2.25 g (70%) of the title compound, ^{1}H NMR (DMSO-d_6) δ: 14.82 (s, 1H, NH), 8.02 (d, *J* 8.9 Hz, 2H, C_6H_4), 7.97 (d, *J* 8.9 Hz, 2H, C_6H_4), 3.82 (s, 3H, CH_3). Mp 219 °C (dec). Anal. Calcd for $C_9H_8N_4O_3$: C, 49.09; H, 3.66; N, 25.44. Found: C, 48.89; H, 4.03; N, 25.76.

3.1.29. 1-(4-methoxycarbonylphenyl)-3-(1-Adamantyl)-3*H*-tetrazolium-5-olate (20c)

Similar to the synthesis of **20a**, compound **20c** was synthesized in 81% yield from methyl ester of 4-(5-oxo-tetrazol-1-yl)-benzoic acid and 1-adamantanol. The product was recrystallized from 50% aqueous 2-propanol. ^{1}H NMR (DMSO-d_6) δ: 8.13 (s, 4H, C_6H_4), 3.87 (s, 3H, CH_3), 2.25 (s, 9H, Ad), 1.73 (s, 6H, Ad); ^{13}C NMR (DMSO-d_6) δ: 165.51, 158.82, 138.12, 130.74, 128.72, 119.63, 67.18, 40.53, 35.16, 28.91, 52.49. Mp 159–161 °C. Anal. Calcd for $C_{19}H_{22}N_4O_3$: C, 64.41; H, 6.21; N, 15.82. Found: C, 64.59; H, 7.06; N, 16.20.

3.1.30. 4-(5-Thioxo-tetrazol-1-yl)-benzoic acid methyl ester

A mixture of 4-(5-thioxo-tetrazol-1-yl)-benzoic acid (5 g, 22.5 mol) and methanol (50 mL) was heated for 2 h under reflux with stirring and bubbling of dry hydrogen chloride. The mixture was treated similar to the treatment of methyl ester of 4-(5-oxo-tetrazol-1-yl)-benzoic acid, to give 2.91 g (55%) of desired product, which was recrystallized from 50% aqueous 2-propanol, ^{1}H NMR (DMSO-d_6) δ: 8.11 (s, 4H, C_6H_4), 3.88 (s, 3H, CH_3); Mp 148 °C (dec). Anal. Calcd for $C_9H_8N_4O_2S$: C, 45.76; H, 3.41; N, 23.71. Found: C, 45.72; H, 3.80; N, 23.74.

3.1.31. 4-[5-(1-Adamantylsulfanyl)-tetrazol-1-yl]-benzoic acid methyl ester (18a)

A solution of methyl ester of 4-(5-thioxo-tetrazol-1-yl)-benzoic acid (1.0 g, 4.24 mol) and adamantan-1-ol (1.3 g, 8.55 mol) in 20 mL of 94% sulfuric acid was stirred for 3 h at room temperature. The mixture was poured onto ice and filtered, and the filtrate was extracted with 150 mL of dichloromethane. The extract was dried over sodium sulfate and the solvent was distilled off, to give tetrazolium salt, which was then dissolved in 100 mL of water, and the solution was boiled for 1 h. Products of were extracted with dichloromethane. The extract was dried over sodium sulfate and concentrated. The residue was purified by column chromatography (chloroform/hexane 1:1) and recrystallized from 2-propanol/hexane (1:1), to give 0.9 g (57%) of the title compound, ^{1}H NMR (DMSO-d_6) δ: 8.19 (d, 2H, *J* 8.9 Hz, C_6H_4), 7.75 (d, 2H, *J* 8.9 Hz, C_6H_4), 3.92 (s, 3H, CH_3), 2.07 (s, 9H, Ad), 1.69 (s, 6H, Ad); ^{13}C NMR (DMSO-d_6/CCl_4 4:1) δ: 165.26, 151.19, 137.02, 131.44, 130.71, 125.86, 53.81, 42.66, 35.38, 29.69, 52.68; Mp 150–152 °C. Anal. Calcd for $C_{19}H_{22}N_4O_2S$: C, 61.62; H, 5.94; N, 15.13. Found: C, 61.65; H, 6.59; N, 15.16.

3.1.32. 5-(1-Adamantylsulfanyl)-1-ethyltetrazole (18b)

Compound **18b** was obtained in 34% yield by reaction of 1-ethyltetrazol-5-thione with adamantan-1-ol and further hydrolysis of tetrazolium salt in manner similar to that described for **18a**. Product was recrystallized from hexane, ^{1}H NMR (DMSO-d_6) δ: 4.37 (q, 2H, *J* 6.9 Hz, CH_2), 2.05 (s, 3H, Ad), 2.01 (s, 6H, Ad), 1.66 (s, 6H, Ad), 1.42 (t, 3H, *J* 6.9 Hz, CH_3); ^{13}C NMR (DMSO-d_6/CCl_4 4:1) δ: 149.26, 52.82, 42.99, 35.41, 29.70, 42.66, 14.69; Mp 65–67 °C. Anal. Calcd for $C_{13}H_{20}N_4S$: C, 59.09; H, 7.57; N, 21.21. Found: C, 59.36; H, 7.86; N, 21.48.

3.1.33. Cells and viruses

For in vitro experiments MDCK cells (ATCC CCL 34) were used. Experiments were performed with influenza virus A/Puerto Rico/8/34 (H1N1) from the collection of viral strains from the Influenza Research Institute. Virus was propagated in the allantoic cavity of 10–12 days old chicken embryos for 48 h at 36 °C.

3.1.34. Toxicity assay

For evaluation of anti-viral activity of compounds, MDCK cells were seeded onto 96-wells plates and cultivated in Eagle's minimal essential medium (MEM) with addition of 5% fetal calf serum. After the cell monolayer formed, cells were washed by serum-free MEM. Azolo-adamantanes were dissolved in DMSO to 5 mg/ml, and serial twofold dilutions (1000–1 microgram/ml) were prepared in MEM. The dilutions were applied to the MDCK cells and incubated for 48 h at 37 °C. Cells were washed twice with PBS and the number of survived cells were evaluated by a microtetrazolium test (MTT).[50] Briefly, 3-(4,5-dimethyltiazolyl-2) 2,5-diphenyltetrazolium bromide (ICN Biochemicals Inc., Aurora, Ohio, 0,5 mg/ml, 0,1 ml per well) was added followed by incubation of plates at 37 °C in 5% CO_2 for 60 min. Colored deposit was dissolved in 100 microliters of DMSO. The plates were swirled gently and left in darkness at room temperature for 30 min. Optical density was measured on a spectrophotometer (Victor 1420, Perkin Elmer, Finland) at wavelength 535 nm. Based on these data, the CTD_{50} (compound concentration required to reduce 50% cell viability) value was estimated for each compound.

3.1.35. Antiviral assay

For evaluation of anti-viral activity of azolo-adamantanes, the assay was performed by quantifying virus yield by the end-point dilution method.[51] Briefly, serial twofold dilutions of compounds under investigation were prepared in MEM with addition of arginine (2 mM), glutamine (2 mM) and trypsin (2 microgram/mL). The solutions were applied to MDCK cells and incubated for 1 h at 37 °C followed by infecting with influenza viruses in a dose 1–10^6 50% infecting dose (ID_{50}) in the medium. Control wells did not contain the compounds. Viruses were cultivated at 36 °C for 48 h followed by hemagglutination assay. 100 microliters of supernatant was transferred into round-bottom wells and mixed with 100 microliters of 1% chicken erythrocytes followed by 1 h incubation at room temperature. Virus titer was considered as reciprocal to the final dilution of the inoculum able to cause positive hemagglutination in 50% of cells and expressed in 50% infecting doses.[51] Anti-viral activity of the compounds were evaluated by their ability to decrease the virus titer. Based on the results, 50% effective dose (concentration of compound that decreases the virus production to 0.5 $\log_{10}EID_{50}$) and the selectivity index (relation of CTD_{50} to ED_{50}) were calculated.

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