Synthesis of isomeric 4-(*N*-methyltetrazolylamino)-2-phenyl-4*H*-thiopyrano[2,3-*b*]quinoline-3-carbaldehydes and 4-hydroxy-2-phenyl-4*H*-thiopyrano[2,3-*b*]quinoline-3-carbaldehyde based on tandem thiol-Michael and (aza)-Morita–Baylis–Hillman reactions and an *in vitro* study of the activity of the obtained compounds against influenza virus

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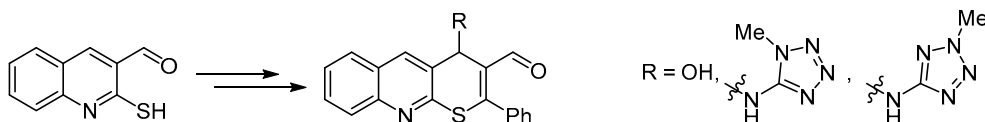
3-{[(1-Methyl-1*H*-tetrazol-5-yl)imino]methyl}quinoline-2-thiol and 3-{[(2-methyl-2*H*-tetrazol-5-yl)imino]methyl}quinoline-2-thiol were synthesized. The sequence of the thiol-Michael reaction and the (aza)-Morita–Baylis–Hillman reaction yielded 4-[(1-methyl-1*H*-tetrazol-5-yl)amino]-2-phenyl-4*H*-thiopyrano[2,3-*b*]quinoline-3-carbaldehyde, 4-[(2-methyl-2*H*-tetrazol-5-yl)amino]-2-phenyl-4*H*-thiopyrano[2,3-*b*]-quinoline-3-carbaldehyde, and 4-hydroxy-2-phenyl-4*H*-thiopyrano[2,3-*b*]quinoline-3-carbaldehyde. Cytotoxicity and antiviral activity against the A/Puerto Rico/8/34 (H1N1) influenza virus strain in MDCK cell culture were determined for the obtained compounds. The study showed that the replacement of the hydroxyl group in 4-hydroxy-2-phenyl-4*H*-thiopyrano[2,3-*b*]quinoline-3-carbaldehyde with a 1-methyl- or 5-amino-2-methyltetrazolyl fragment decreased antiviral activity. At the same time, 3-{[(1-methyl-1*H*-tetrazol-5-yl)imino]-methyl}quinoline-2-thiol has a higher activity than 3-{[(2-methyl-2*H*-tetrazol-5-yl)imino]methyl}quinoline-2-thiol. This fact indicates a possible relationship between the arrangement of substituents in the tetrazole ring and the antiviral activity of the tested heterocyclic system.

Keywords: 1-methyl-1*H*-tetrazol-5-amine, 2-methyl-2*H*-tetrazol-5-amine, thiopyranoquinoline, biological activity, influenza A (H1N1) virus, tandem reaction.

In recent years, interest in nitrogen-containing polynuclear heterocycles exhibiting antiviral activity has been rapidly growing. The attention of researchers is attracted by 1,2,4-triazole and quinoline compounds, among which promising ingredients of drugs were found that are effective against pathogens of various pandemic viral infections: HIV, Ebola virus, Zika virus, as well as the new coronavirus SARS-CoV-2.[1,2] It is also known that

thiopyrano[2,3-*b*]quinolines act as antagonists of metabotropic glutamate receptors and strong antioxidants that prevent oxidative DNA damage caused by free radicals.[3–5] It was recently shown that some nonannulated polynuclear heterocyclic compounds containing the tetrazol-2-yl and pyrimidine fragments in the molecule exhibit pronounced activity against dangerous influenza A varieties.[6] However, the relationship between the chemical structure, including

the regioisomeric nature of the *N*-substituted tetrazole ring, as well as the nature of the substituent at the endocyclic carbon atom, and the antiviral activity is not adequately disclosed. Lack of knowledge in this area reduces the effectiveness of computer prediction and experimental study of biological activity.

The current publication presents the results of the synthesis of 3-{[(1-methyl-1*H*-tetrazol-5-yl)imino]methyl}-quinoline-2-thiol and 3-{[(2-methyl-2*H*-tetrazol-5-yl)imino]methyl}quinoline-2-thiol, in the molecules of which regioisomeric 5-amino-1(2)-methyltetrazolyl fragments containing a bulky polynuclear annulated heterocyclic fragment as a substituent at the nitrogen atom of the amino group are recognized.

To access annulated polynuclear heterocyclic compounds ("hybrid systems"), tandem transformations are conveniently used, which in some cases make it possible to carry out multistep synthesis of the active ingredients of antiviral drugs in a one-pot mode.[7] A method is described in the literature for the preparation of 4-amino-4*H*-chromenes by the tandem oxo-Michael and aza-Baylis–Hillman reactions of *N*-tosylsalicylaldehyde imine with 3-phenyl-2-propinal (Scheme 1).[8] The reaction is carried out in the presence of proline or its derivatives.

Scheme 1
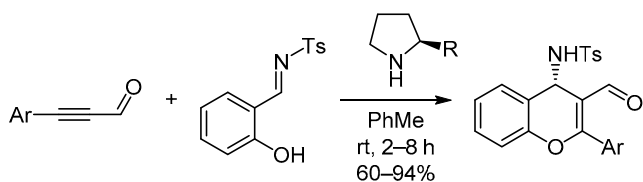
We succeeded in carrying out the thiol-Michael reaction and the aza-Morita–Baylis–Hillman reaction involving azomethines **3**, **6** obtained from 2-sulfanylquinoline-3-carbaldehyde (**1**) and the regioisomeric 1-methyl-1*H*-tetrazol-5-amine (**2**) and 2-methyl-2*H*-tetrazol-5-amine (**5**), and 3-phenylpropyn-2-al (**8**). The more accessible Et$_3$N was used as a catalyst in this case. As a result, annulated heterocyclic compounds **4** and **7** containing regioisomeric *N*(1)- and *N*(2)-methyltetrazolyl fragments at the amino nitrogen atom were obtained in 66 and 67% yields, respectively (Scheme 2).
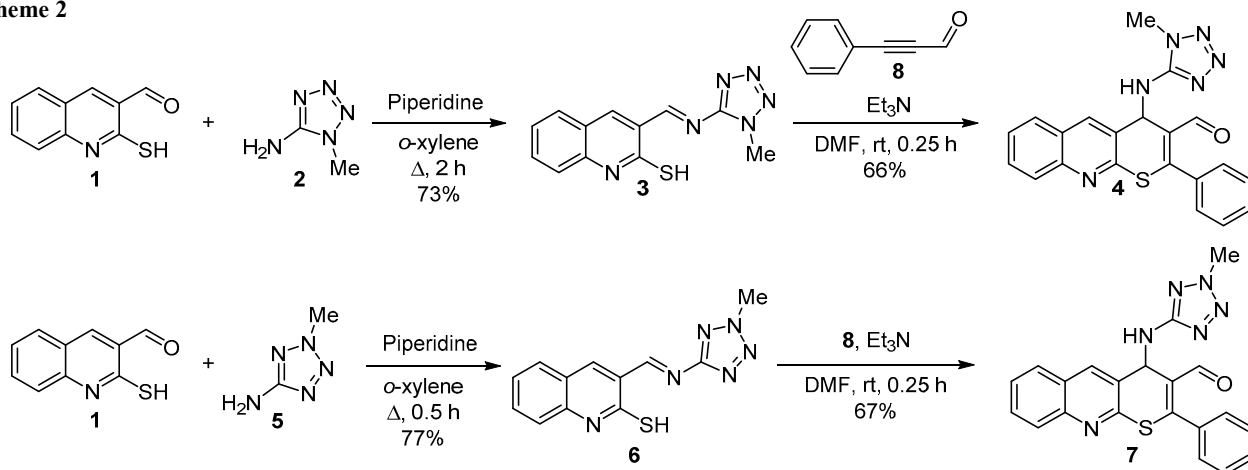
Also, in order to compare the effect of the tetrazole ring on the biological activity of compounds **4** and **7** against influenza A/Puerto Rico/8/34 (H1N1) virus, 4-hydroxy-4*H*-thiopyrano[2,3-*b*]quinoline **9** was synthesized and studied (Scheme 3).

Scheme 3
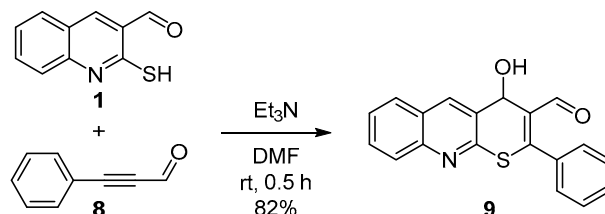
The structure and composition of compounds **3**, **4**, **6**, **7**, and **9** were confirmed by IR, ^{1}H, ^{13}C NMR spectroscopy, and high-resolution mass spectrometry. The individuality of the compounds was confirmed by TLC.

An *in vitro* study of the biological activity of compounds **4**, **7**, and **9** was performed in the Laboratory of Experimental Virology of Saint Petersburg Pasteur Research Institute of Epidemiology and Microbiology using MDCK cell culture against A/Puerto Rico/8/34 (H1N1) influenza virus. Table 1 shows the values of 50% cytotoxic concentration (CC$_{50}$), 50% inhibitory concentration (IC$_{50}$), and the selectivity index SI, the ratio of CC$_{50}$ to IC$_{50}$. Rimantadine was used as the reference drug.

The data in Table 1 demonstrates that compound **4** containing the 1-methyltetrazolyl moiety and compound **9** in which the tetrazolyl moiety is replaced by a hydroxyl group are significantly superior in regards to the selectivity index (SI) to the reference drug rimantadine. The expected advantages of the tetrazole ring in this case could not be revealed: compound **4** turned out to be almost two times less active than compound **9**. To explain this phenomenon, we propose to conduct a detailed study of the structures of both molecules using 3D-QSAR methods. An interesting conclusion can be drawn by comparing the selectivity indices of compounds **4** and **7**. As can be seen, compound **4** containing the *N*(1)-methyltetrazolyl fragment showed a significantly higher activity compared to compound **7**, the

Scheme 2

Table 1. Antiviral properties of compounds **4**, **7**, **9** against influenza A (H1N1) virus in MDCK cell culture

Compound	CC_{50}, µmol	IC_{50}, µmol	SI
4	>749	46	16
7	549	>249	2
9	>939	30	31
Rimantadine	289	58	5

molecule of which contains the regioisomeric *N*(2)-methyl-tetrazolyl fragment.

In conclusion, the studied compounds exhibit activity against the A/Puerto Rico/8/34 (H1N1) influenza virus, which is resistant to rimantadine, an antiviral compound based on adamantane acting as a blocker of the viral M2 ion channel protein. This indicates that tetrazole derivatives have an alternative mechanism of anti-influenza activity. Apparently, their biological target is different from the M2 protein, the traditional target for the adamantane derivatives rimantadine and amantadine.

Experimental

IR spectra were recorded on an IR Affinity-1 Fourier transform spectrometer for studies in the mid-IR range in KBr pellets. ¹H and ¹³C NMR spectra (400 and 101 MHz, respectively) were acquired on a Bruker Avance III HD 400 NanoBay spectrometer in DMSO-*d*₆, using the signals of the deuterated solvent DMSO-*d*₆ (2.50 ppm for ¹H nuclei, 39.5 ppm for ¹³C nuclei) as internal standard. High-resolution mass spectra (electrospray ionization) were obtained on a Shimadzu Nexera X2 LCMS-9030 liquid hybrid quadrupole time-of-flight mass spectrometer. Melting points were determined on a Büchi M-560 apparatus with a heating rate of 1°C/min in the melting range. Monitoring of the reaction progress was done by TLC on Merck Kieselgel 60 F₂₅₄ plates.

Analytical grade solvents were used without additional purification. 1-Methyl-1*H*-tetrazol-5-amine (**2**) and 2-methyl-2*H*-tetrazol-5-amine (**5**) were obtained and purified by known methods. The properties of compounds **2** and **5** correspond to the literature data.[9] 2-Sulfanylquinoline-3-carbaldehyde (**1**) was obtained by a known method.[10]

3-{[(1-Methyl-1*H*-tetrazol-5-yl)imino]methyl}quinoline-2-thiol (3). *o*-Xylene (40 ml), 2-sulfanylquinoline-3-carbaldehyde (**1**) (1.00 g, 5.3 mmol), 1-methyl-1*H*-tetrazol-5-amine (**2**) (0.52 g, 5.3 mmol), and piperidine (3 drops) were added to a flask equipped with a Dean–Stark trap. The reaction mixture was heated under reflux for 2 h with simultaneous collection of H₂O into the trap. Dissolution of the starting compound **1** and precipitation of reaction product **3** were observed in the course of the reaction. The precipitate was filtered off, washed with petroleum ether (bp 40–70°C), and air-dried. No further purification of the product was required. Yield 1.04 g (73%), red powder, mp 287–288°C (decomp.). *R*f 0.29 (EtOAc–hexane, 1:2). ¹H NMR spectrum, δ, ppm (*J*, Hz): 4.11 (3H, s, CH₃); 7.44 (1H, t, *J* = 7.5, H Ar); 7.68 (1H, d, *J* = 8.4, H Ar); 7.73–7.84 (1H, m, H Ar); 8.03 (1H, d,

J = 7.6, H Ar); 8.85 (1H, s, H Ar); 10.03 (1H, s, CH=N); 14.09 (1H, br. s, SH). ¹³C NMR spectrum, δ, ppm: 33.2 (CH₃); 116.8 (C Ar); 122.4 (C Ar); 125.6 (C Ar); 130.7 (C Ar); 132.2 (C Ar); 134.7 (C Ar); 137.3 (C Ar); 141.4; 158.8; 167.9; 181.5. Found, *m/z*: 271.0763 [M+H]⁺. C₁₂H₁₁N₆S. Calculated, *m/z*: 271.0760.

4-[(1-Methyl-1*H*-tetrazol-5-yl)amino]-2-phenyl-4*H*-thiopyrano[2,3-*b*]quinoline-3-carbaldehyde (4). 3-{[(1-Methyl-1*H*-tetrazol-5-yl)imino]methyl}quinoline-2-thiol (**3**) (0.50 g, 1.8 mmol), freshly distilled 3-phenylpropyn-2-al (**8**) (0.24 g, 1.8 mmol), and Et₃N (0.56 g, 5.5 mmol) were dissolved in DMF (30 ml) in a flat-bottom flask. The course of the reaction was monitored by TLC (eluent EtOAc–hexane, 1:2). The reaction product **4** precipitated. The reaction took place in 15 min. The reaction mixture was cooled in the freezer for 10 h. The formed precipitate was filtered off, washed twice with cold MeOH, and air-dried. No further purification of the product was required. Yield 0.49 g (66%), colorless crystals, mp 258–259°C. *R*f 0.2 (EtOAc–hexane, 1:2). ¹H NMR spectrum, δ, ppm (*J*, Hz): 3.67 (3H, s, CH₃); 6.46 (1H, d, *J* = 6.4, C*H*NH); 7.48 (1H, d, *J* = 6.4, CHN*H*); 7.64 (4H, br. s, H Ar); 7.74 (2H, d, *J* = 6.2, H Ar); 7.83 (1H, t, *J* = 7.5, H Ar); 7.99 (1H, d, *J* = 8.3, H Ar); 8.10 (1H, d, *J* = 8.1, H Ar); 8.87 (1H, s, H Ar); 9.35 (1H, s, CH=O). ¹³C NMR spectrum, δ, ppm: 32.5 (CH₃); 49.2 (*C*HNH); 126.6; 127.4 (C Ar); 127.5 (C Ar); 127.7 (C Ar); 128.0 (C Ar); 129.0; 129.5 (C Ar); 130.9 (C Ar); 131.6 (C Ar); 131.7; 133.4; 138.5 (C Ar); 147.0; 153.9; 155.2; 159.7; 186.6 (CH=O). Found, *m/z*: 401.1183 [M+H]⁺. C₂₁H₁₇N₆OS. Calculated, *m/z*: 401.1179.

3-{[(2-Methyl-2*H*-tetrazol-5-yl)imino]methyl}quinoline-2-thiol (6) was obtained according to the method of synthesis of compound **3** from 2-sulfanylquinoline-3-carbaldehyde (**1**) and 2-methyl-2*H*-tetrazol-5-amine (**5**). According to the ¹H NMR spectrum, a mixture of stereoisomers in a 1:1.5 ratio was obtained. Separation of the isomers was not carried out, the mixture was used for further transformations. Total yield 1.10 g (77%), orange powder. ¹H NMR spectrum, δ, ppm: 4.08 and 4.40 (3H, both s, CH₃); 5.97 (1H, br. s, H Ar); 7.28–7.52 (1H, m, H Ar); 7.61–7.82 (1H, m, H Ar); 7.97–8.14 (1H, m, H Ar); 8.37 and 8.80 (1H, s, H Ar); 10.03 and 10.72 (1H, s, CH=N); 13.96 (1H, br. s, SH). ¹³C NMR spectrum, δ, ppm: 40.5; 116.7; 122.1; 122.5; 125.5 (2C); 130.6; 131.2; 132.3; 132.5; 134.2; 134.8; 136.2; 137.5; 141.1; 141.5; 165.7; 167.6; 169.6; 181.4; 192.3. Found, *m/z*: 271.0763 [M+H]⁺. C₁₂H₁₁N₆S. Calculated, *m/z*: 271.0760.

4-[(2-Methyl-2*H*-tetrazol-5-yl)amino]-2-phenyl-4*H*-thiopyrano[2,3-*b*]quinoline-3-carbaldehyde (7) was obtained according to the method of synthesis of compound **4** from 3-{[(2-methyl-2*H*-tetrazol-5-yl)imino]methyl}quinoline-2-thiol (**6**) and 3-phenylpropyn-2-al (**8**). Yield 0.50 g (67%), yellow crystals, mp 267–268°C (decomp.). *R*f 0.43 (EtOAc–hexane, 1:2). ¹H NMR spectrum, δ, ppm (*J*, Hz): 4.16 (3H, s, CH₃); 6.24 (1H, d, *J* = 6.0, C*H*NH); 7.51 (1H, d, *J* = 6.0, CHN*H*); 7.57–7.69 (4H, m, H Ar); 7.72–7.78 (2H, m, H Ar); 7.82 (1H, t, *J* = 7.3, H Ar); 7.94–8.01 (1H, m, H Ar); 8.09 (1H, d, *J* = 8.1, H Ar); 8.76 (1H, s, H Ar); 9.32 (1H, s, CH=O).

^{13}C NMR spectrum, δ, ppm: 39.7 (CH$_3$); 48.6 (CHNH); 127.1 (C Ar); 127.3; 127.4 (C Ar); 128.0 (C Ar); 128.3; 129.0 (C Ar); 129.3 (C Ar); 130.9 (C Ar); 131.4 (C Ar); 131.5; 133.6; 138.1 (C Ar); 146.8; 154.2; 159.6; 166.8; 186.5 (CH=O). Found, *m/z*: 401.1184 [M+H]$^+$. C$_{21}$H$_{17}$N$_6$OS. Calculated, *m/z*: 401.1179.

4-Hydroxy-2-phenyl-4*H*-thiopyrano[2,3-*b*]quinoline-3-carbaldehyde (9). 2-Sulfanylquinoline-3-carbaldehyde (**1**) (1.00 g, 5.3 mmol), freshly distilled 3-phenylpropyn-2-al (**8**) (0.69 g, 5.3 mmol), and Et$_3$N (1.60 g, 15.8 mmol) in DMF (50 ml) were charged into a flat-bottom flask. Precipitation of product **9** was observed during the course of the reaction. According to thin-layer chromatography (EtOAc–hexane, 1:2), the reaction was over in 30 min. The reaction mixture was cooled in the freezer for 10 h. The formed precipitate was filtered off, washed twice with cold MeOH, and air-dried. No further purification of the product was required. Yield 1.40 g (82%), colorless crystals, mp 178–180°C. *R*$_f$ 0.41 (EtOAc–hexane, 1:2). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 5.69–5.79 (1H, m, OH); 6.00–6.10 (1H, m, C<u>H</u>OH); 7.56–7.70 (6H, m, H Ar); 7.85 (1H, t, *J* = 7.2, H Ar); 8.02 (1H, d, *J* = 8.2, H Ar); 8.09 (1H, d, *J* = 8.0, H Ar); 8.64 (1H, br. s, H Ar); 9.36 (1H, s, CH=O). ^{13}C NMR spectrum, δ, ppm: 62.5 (COH); 127.3 (C Ar); 127.8; 128.1 (C Ar); 128.8 (C Ar); 128.9 (C Ar); 129.5 (C Ar); 130.7 (C Ar); 131.5 (C Ar); 133.7; 138.5 (C Ar); 147.1; 154.2; 158.6; 187.0 (CH=O). Found, *m/z*: 320.0739 [M+H]$^+$. C$_{19}$H$_{14}$NO$_2$S. Calculated, *m/z*: 320.0740.

The study of the biological activity of compounds 4, 7, 9. The cytotoxic properties of the compounds were investigated in a separate series of experiments. A series of threefold dilutions (300–3.7 µg/ml) were prepared from the test compounds, after which they were added to the wells of plates with a monolayer of MDCK cells (ATCC CCL-34). The plates were incubated for 72 h at 36°C. Cell viability was analyzed using the methyltetrazolium assay: a solution of methyltetrazolium bromide was introduced into the wells, which, by the action of mitochondrial enzymes, was converted into an insoluble violet formazan derivative.[11] The plates with cells were incubated for 2 h. The precipitate was dissolved in DMSO (0.1 ml per well). The optical density in the wells was measured on a Thermo Multiskan FC plate photometer (Thermo Fisher Scientific, USA) at a wavelength of 540 nm. Based on the obtained data, CC$_{50}$ values, that is, the concentration of the compound, leading to a decrease in optical density by half compared to wells without the addition of compounds, were calculated.

The study of the antiviral activity of compounds 4, 7, 9 was carried out by determining the reduction of the degree of cytopathic action. The experiments used the influenza A/Puerto Rico/8/34 (H1N1) virus from the collection of viral strains of Saint Petersburg Pasteur Research Institute of Epidemiology and Microbiology. The studied compounds in the range of concentrations were applied to the cells in the wells of the plate, incubated for 1 h, then the cells were infected with the virus at a dose of 0.01 TCID$_{50}$ per cell. The cells were incubated for 72 h, after which cell survival was analyzed using the methyltetrazolium assay as described above. Based on the obtained data, a 50% inhibitory concentration (IC$_{50}$) was calculated for each compound as the concentration that reduces the degree of viral destruction of cells by 50% and the selectivity index (SI) as the ratio of CC$_{50}$ to IC$_{50}$. Compounds with an SI of 10 or higher were considered active.

Supplementary information file containing ^{1}H and ^{13}C NMR and high-resolution mass spectra of the synthesized compounds is available at the journal website http://link.springer.com/journal/10593.

References

1. De, A.; Sarkar, S.; Majee, A. *Chem. Heterocycl. Compd.* **2021**, *57*, 410.
2. Ostrovskii, V. A.; Popova, E. A.; Trifonov, R. E. In *Comprehensive Heterocyclic Chemistry IV*; Elsevier: Oxford, 2022, Vol. 6, p. 182.
3. Alizadeh, A.; Rezaiyehraad, R.; Roosta, A.; Halvagar, M. R. *ChemistrySelect* **2019**, *4*, 12049.
4. Kumar, S.; Muthusubramanian, S.; Perumal, S. *RSC Adv.* **2015**, *5*, 30826.
5. Hepworth, J. D.; Heron, B. M. In *Comprehensive Heterocyclic Chemistry III*; Katritzky, A.; Ramsden, Ch.; Scriven, E.; Taylor, R., Eds.; Elsevier: Oxford, 2008, Vol. 7, p. 727.
6. Ostrovskii, V. A.; Danagulyan, G. G.; Nesterova, O. M.; Pavlyukova, Yu. N.; Tolstyakov, V. V.; Zarubina, O. S.; Slepukhin, P. A.; Esaulkova, Ya. L.; Muryleva, A. A.; Zarubaev, V. V.; Trifonov R. E. *Chem. Heterocycl. Compd.* **2021**, *57*, 448.
7. Ishikawa, H.; Suzuki, T.; Hayashi, Y. *Angew. Chem., Int. Ed. Engl.* **2009**, *48*, 1304.
8. Alemán, J.; Núñez, A.; Marzo, L.; Marcos, V.; Alvarado, C.; García Ruano, J. L. *Chem.–Eur. J.* **2010**, *16*, 9453.
9. Henry, R. A.; Finnegan, W. G. *J. Am. Chem. Soc.* **1954**, *76*, 923.
10. Mistry, B. M.; Jauhari, S. *Med. Chem. Res.* **2013**, *22*, 635.
11. Mosmann, T. *J. Immunol. Methods* **1983**, *16*, 55.