Synthesis and antiviral activity of 1-hydroxy-2-(2-hydroxyphenyl)imidazoles against vaccinia virus

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2-(2-Hydroxyphenyl)imidazole derivatives were synthesized and tested for antiviral activity against vaccinia virus in Vero cell culture. 1-Methylimidazole 3-oxides, 1-methoxyimidazoles, and 1*H*-imidazoles showed no activity, whereas some 1-hydroxyimidazole derivatives hold promise, exhibiting antiviral activity and weak cytotoxicity.

Key words: 1-hydroxyimidazoles, vaccinia virus, antiviral activity, cytotoxicity.

In 1966—1980, the World Health Organization (WHO) undertook the Smallpox Eradication Programme, and this disease was officially declared eradicated in 1980. Nevertheless, smallpox virus is considered a potential threat as a biological weapon.[1,2] Hence, there is still a need for new antiviral agents, including small molecule representatives,[3] with activity against smallpox virus and other orthopoxviruses pathogenic for humans.

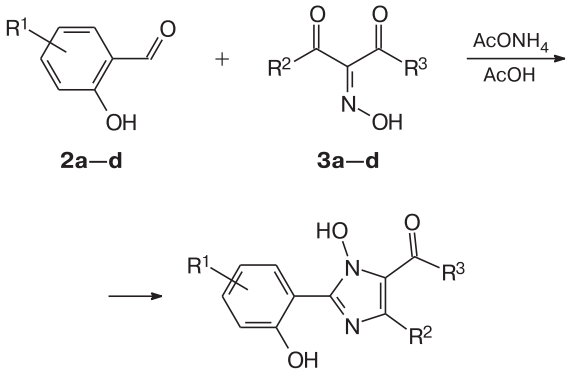
1-Hydroxyimidazole derivatives were found to exhibit different biological activities. This class of compounds includes potential pesticides,[4] herbicides,[5] molecules having hypotensive,[6] antiprotozoal,[7] antibacterial,[8] and antiviral[9] activities, potential selective inhibitors of kinases with different functions,[10] and xanthine oxidase inhibitors.[11,12] The goal of the present work is to synthesize 1-hydroxy-2-(2-hydroxyphenyl)imidazoles, evaluate their antiviral activity against vaccinia virus, and compare their activity with that of other imidazole derivatives.

1-Hydroxyimidazoles **1a—h** were prepared by the condensation of salicylaldehyde derivatives **2a—d** with oximes **3a—d** and ammonium acetate in glacial acetic acid (Scheme 1).

The reaction was performed at room temperature (synthesis of compounds **1a—c,e,f**) or under reflux (when using oxime **3d** in the synthesis of derivatives **1d,g,h**).

To compare antiviral activity of 1-hydroxy-2-(2-hydroxyphenyl)imidazoles with that of 2-(2-hydroxyphenyl) imidazole derivatives, we synthesized 1-methylimidazole 3-oxides **4a—c** and 1-methoxyimidazoles **5a—c** (Scheme 2) according to procedures described previously.[13]

Scheme 1



1	R^1	R^2	R^3
a	H	Me	Me
b	H	Me	OEt
c	H	$CH_2C(Me)_2CH_2$	
d	H	Ph	NHPh
e	5-Cl	Me	Me
f	4-OCH_2Ph	Me	OEt
g	5-Cl	Ph	NHPh
h	5-Br	Ph	NHPh

1*H*-Imidazole **6a** unsubstituted at nitrogen atoms was formed as a by-product in the synthesis of 1-methoxy

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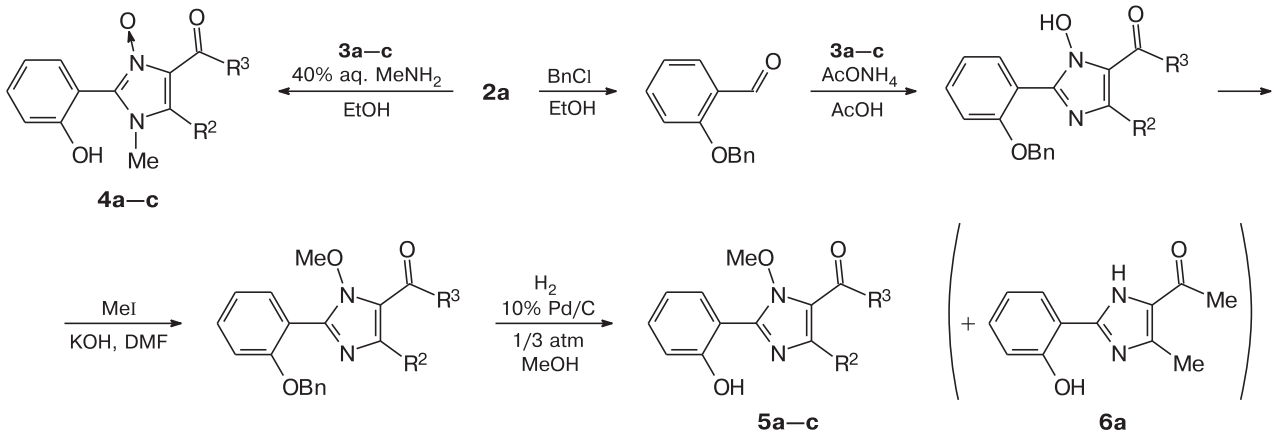
Scheme 2



4, 5: $R^2 = R^3 = Me$ (**a**); $R^2 = Me$, $R^3 = OEt$ (**b**); $R^2 + R^3 = CH_2C(Me)_2CH_2$ (**c**)

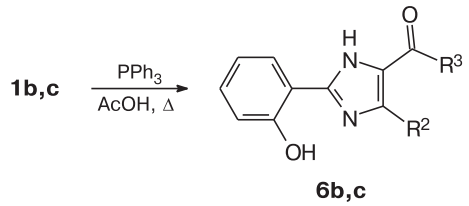
derivative **5a**.[13] Imidazoles **6b,c** were prepared by the reduction of 1-hydroxyimidazoles **1b,c** with triphenylphosphine in glacial acetic acid under reflux (Scheme 3).

1-Hydroxyimidazoles **1a—h** were tested for activity against vaccinia virus in Vero cell culture. The results are given in Table 1.

1-Hydroxy-2-(2-hydroxyphenyl)imidazole derivatives **1a—c**, which were synthesized starting from unsubstituted salicylaldehyde **2a** and which contain an acetyl (**1a**), ethoxycarbonyl (**1b**), or fixed carbonyl group (**1c**) in position 5 of the heterocycle, exhibited potential antiviral activity and low cytotoxicity. Structure **1c** showed most promise. The characteristics of antiviral activity of compounds **1b**,**c** were reliably different from those of compound **1a** (see Table 1).

The presence of substituents (Hal, OCH_2Ph) in the 2-hydroxyphenyl moiety of 1-hydroxyimidazoles **1e**,**f**,**g**,**h** led to an increase in cytotoxicity. 1-Hydroxyimidazoles **1d**,**g**,**h** containing the *N*-phenylcarbamoyl substituent in position 5 appeared to be cytotoxic and showed no inhibitory activity.

Scheme 3



6: $R^2 = Me$, $R^3 = OEt$ (**b**); $R^2 + R^3 = CH_2C(Me)_2CH_2$ (**c**)

We also tested imidazole 3-oxides **4a—c**, 1-methoxyimidazoles **5a—c**, and 1*H*-imidazoles **6a—c** containing a set of substituents in positions 2, 4, and 5 of the heterocycle, which are similar to those in promising 1-hydroxyimidazoles **1a—c** (Table 2).

Table 1. Antiviral activity of 1-hydroxyimidazoles **1a—h** against vaccinia virus (Copenhagen strain) in Vero cell culture

Compound	TC_{50}/μg mL^{-1}	IC_{50}/ μg mL^{-1}	SI (TC_{50}/IC_{50})
1a	64.80±9.36[a]	14.58±0.63[a]	4.39±0.45[a]
1b	478.33±27.15[a,b]	4.07±0.13[a,b]	118.53±10.38[a,b]
1c	203.00±2.04[a,b]	1.29±0.09[a,b]	159.73±11.58[a,b]
1d	0.201	N.a.	—
1e	1.06	N.a.	—
1f	1.434	N.a.	—
1g	<0.16	N.a.	—
1h	<0.16	N.a.	—
Cidofovir (control sample)	275.72±15.54[a,b]	10.03±0.63[a,b]	27.60±1.56[a,b]

Note. TC_{50} is the toxic concentration of the agent causing 50% death of uninfected monolayer cells; IC_{50} is the virus-inhibitory concentration of the agent required to inhibit 50% of infected monolayer cells; SI is the selectivity index of the agent defined as the TC_{50}/IC_{50} ratio; N.a., not active.

[a] The mean values and standard errors are given, the number of repeated measurements of TC_{50}, IC_{50}, and SI is 4.

[b] The difference from **1a** according to Student's t-test at $p \leq 0.001$.

Table 2. Antiviral activity of imidazole 3-oxides **4a—c**, 1-methoxyimidazoles **5a—c**, and 1*H*-imidazoles **6a—c** against vaccinia virus (Copenhagen strain) in Vero cell culture*

Compound	TC_{50}	IC_{50}	SI (TC_{50}/IC_{50})
	μg mL^{-1}		
4a	>100	N.a.	—
4b	>100	N.a.	—
4c	>100	N.a.	—
5a	56.061	N.a.	—
5b	>100	42.926	>2.32
5c	>100	N.a.	—
6a	>100	N.a.	—
6b	5.231	N.a.	—
6c	>100	N.a.	—

* For notations, see Table 1.

It was demonstrated that compounds **4a—c**, **5a—c**, and **6a—c** exhibited no significant activity, that proved the *N*-hydroxy group to be an essential moiety. This factor should be taken into account in the development of chemotherapeutic agents against smallpox based on imidazoles containing a carbonyl group in position 5.

Experimental

Commercially available reagents were used as received. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance^{TM}300 spectrometer (operating at 300 and 75.47 MHz, respectively) in deuterated DMSO using residual signals of the solvent as the internal standard. High-resolution mass spectra (HRMS) were obtained on a BrukerMicrOTOF ESI-TOF mass spectrometer.

Starting diketone monooximes **3a—d** were prepared according to known procedures.[14,15] 1-Hydroxyimidazoles **1a—c**,**f**, imidazole 3-oxides **4a—c**, 1-methoxyimidazoles **5a—c**, and 1*H*-imidazole **6a** were characterized previously.[13,16]

1-[2-(5-Chloro-2-hydroxyphenyl)-1-hydroxy-4-methyl-1*H*-imidazol-5-yl]ethanone (1e). A mixture of aldehyde **2b** (0.78 g, 5.0 mmol), oxime **3a** (0.65 g, 5.0 mmol), and ammonium acetate (0.45 g, 5.8 mmol) in glacial acetic acid (6 mL) was stirred at room temperature and then left for a week. The precipitate was filtered off and purified by refluxing in hexane. Chromatographically pure product **1e** was obtained as beige powder with m.p. 176—178 °C in a yield of 0.61 g (46%). ^{1}H NMR (DMSO-d_6), δ: 13.60 (br.s, 2 H, 2 OH); 7.70 (s, 1 H, H(6′)); 7.43 (dd, 1 H, H(4′), J = 8.9 Hz; J = 2.6 Hz); 6.95 (d, 1 H, H(3′), J = 8.8 Hz); 2.66 (s, 3 H, CH_3); 2.48 (s, 3 H, CH_3). ^{13}C NMR (DMSO-d_6), δ: 181.3; 157.2; 135.7; 131.9; 127.1; 126.7; 122.1; 121.0; 119.5; 113.3; 30.1; 21.2. MS, found: m/z 267.0531 $[M + H]^+$; $C_{12}H_{11}ClN_2O_3$; calculated: $[M + H]^+$ = 267.0536.

***N*-Phenyl-1-hydroxy-2-(2-hydroxyphenyl)-4-phenyl-1*H*-imidazole-5-carboxamide (1d).** A mixture of aldehyde **2a** (0.61 g, 5.0 mmol), oxime **3d** (1.34 g, 5.0 mmol), and ammonium acetate (0.62 g, 8.1 mmol) in glacial acetic acid (10 mL) was stirred at reflux for 5 h and then cooled to room temperature. The precipitate was filtered off, washed on a filter with diethyl ether, and purified by refluxing in acetonitrile. Chromatographically pure product **1d** was obtained as yellowish powder with m.p. 262—264 °C in a yield of 1.06 g (57%). ^{1}H NMR (DMSO-d_6), δ: 13.94 (br.s, 1 H, NH); 12.04 (br.s, 2 H, OH); 7.73—7.85 (m, 4 H, Ar); 7.67 (d, 2 H, Ar, J = 8.1 Hz); 7.42—7.57 (m, 4 H, Ar); 7.36 (m, 2 H, Ar); 7.12 (m, 1 H, Ar); 6.98—7.06 (m, 2 H, Ar). ^{13}C NMR (DMSO-d_6), δ: 156.8; 155.5; 137.3; 136.4; 133.3; 132.4; 132.1; 129.2; 129.0; 128.9; 128.5; 127.4; 123.5; 119.5; 119.3; 118.7; 118.5; 111.2. MS, found: m/z 372.1343 $[M + H]^+$; $C_{22}H_{17}N_3O_3$; calculated: $[M + H]^+$ = 372.1348.

***N*-Phenyl-2-(5-chloro-2-hydroxyphenyl)-1-hydroxy-4-phenyl-1*H*-imidazole-5-carboxamide (1g).** A mixture of aldehyde **2b** (0.16 g, 1.0 mmol), oxime **3d** (0.27 g, 1.0 mmol), and ammonium acetate (0.12 g, 1.6 mmol) in glacial acetic acid (3 mL) was stirred at reflux for 3 h and then cooled to room temperature. The precipitate was filtered off, washed on a filter with diethyl ether, and purified by refluxing in acetonitrile. Chromatographically pure product **1g** was obtained as white powder with m.p. 268—269 °C in a yield of 0.24 g (59%). ^{1}H NMR (DMSO-d_6), δ: 13.95 (br.s, 2 H, 2 OH); 11.86 (br.s, 1 H, NH); 7.94 (s, 1 H, Ar); 7.81 (d, 2 H, Ar, J = 7.0 Hz); 7.69 (d, 2 H, Ar, J = 8.0 Hz); 7.45—7.58 (m, 4 H, Ar); 7.38 (t, 2 H, Ar, J = 7.8 Hz); 7.15 (t, 1 H, Ar, J = 7.4 Hz); 7.06 (d, 1 H, J = 8.8 Hz). ^{13}C NMR (DMSO-d_6), δ: 156.3; 155.9; 137.9; 135.7; 133.9; 133.6; 132.2; 132.1; 132.0; 129.4; 129.0; 128.0; 124.1; 122.7; 120.9; 120.8; 119.9; 113.2. MS, found: m/z 406.0953 $[M + H]^+$; $C_{22}H_{16}ClN_3O_3$; calculated: $[M + H]^+$ = 406.0958.

***N*-Phenyl-2-(5-bromo-2-hydroxyphenyl)-1-hydroxy-4-phenyl-1*H*-imidazole-5-carboxamide (1h).** A mixture of aldehyde **2d** (0.08 g, 0.4 mmol), oxime **3d** (0.10 g, 0.4 mmol), and ammonium acetate (0.08 g, 1.0 mmol) in glacial acetic acid (2 mL) was stirred at reflux for 2 h and then cooled to room temperature. The precipitate was filtered off, washed on a filter with diethyl ether, and purified by refluxing in acetonitrile. Chromatographically pure product **1h** was obtained as white powder with m.p. 263—265 °C in a yield of 0.11 g (66%). ^{1}H NMR (DMSO-d_6), δ: 13.99 (br.s, 2 H, OH); 11.88 (br.s, 1 H, NH); 8.06 (s, 1 H, Ar); 7.80 (d, 2 H, Ar, J = 6.7 Hz); 7.69 (d, 2 H, Ar, J = 8.1 Hz); 7.61 (d, 1 H, Ar, J = 8.9 Hz); 7.46—7.57 (m, 3 H, Ar); 7.39 (t, 2 H, Ar, J = 7.8 Hz); 7.16 (d, 1 H, Ar, J = 7.5 Hz); 7.00 (d, 1 H, Ar, J = 8.8 Hz). ^{13}C NMR (DMSO-d_6), δ: 156.7; 156.0; 137.9; 135.7; 135.2; 135.0; 133.9; 129.6; 129.4; 129.0; 128.0; 127.4; 124.1; 121.4; 120.9; 119.9; 113.8; 110.1. MS, found: m/z 450.0448 $[M + H]^+$. $C_{22}H_{16}BrN_3O_3$, calculated: $[M + H]^+$ = 450.0453.

Ethyl 2-(2-hydroxyphenyl)-4-methyl-1*H*-imidazole-5-carboxylate (6b). A mixture of 1-hydroxyimidazole **1b** (1.05 g, 4.0 mmol) and triphenylphosphine (1.69 g, 6.4 mmol) in glacial acetic acid (7 mL) was stirred at reflux for 4 h. Then approximately two-thirds of the solvent was distilled off under reduced pressure. The residue was diluted with diethyl ether (15 mL). The precipitate was filtered off and purified by refluxing twice in diethyl ether. Chromatographically pure product **6b** was obtained as beige powder with m.p. 177—178 °C in a yield of 0.40 g (41%). ^{1}H NMR (DMSO-d_6), δ: 13.13 (br.s, 1 H, NH); 12.58 (br.s, 1 H, OH); 7.87 (br.s, 1 H, H(2′)); 7.29 (t, 1 H, Ar, J = 7.7 Hz); 6.88—7.01 (m, 2 H, Ar); 4.29 (q, 2 H, $OC\underline{H}_2CH_3$, J = 7.1 Hz); 2.52 (s, 3 H, CH_3); 1.32 (t, 3 H, $OCH_2C\underline{H}_3$, J = 7.1 Hz). ^{13}C NMR (DMSO-d_6), δ: 161.9; 156.6; 145.0; 131.5/131.4*; 130.5; 128.8/128.7*; 125.1; 118.9; 116.8; 112.5; 59.7; 14.2; 11.1. MS,

* In the ^{13}C NMR spectra of 1*H*-imidazoles **6b**,**c**, the ^{13}C NMR signals of the carbon atoms in positions 4 and 5 of imidazole are doubled due to prototropic tautomerism of the heterocycle.

found: *m/z* 247.1077 $[M + H]^+$; $C_{13}H_{14}N_2O_3$; calculated: $[M + H]^+ = 247.1083$.

2-(2-Hydroxyphenyl)-6,6-dimethyl-3,5,6,7-tetrahydro-4*H*-benzimidazol-4-one (6c). A mixture of 1-hydroxyimidazole **1c** (0.54 g, 2.0 mmol) and triphenylphosphine (0.84 g, 3.2 mmol) in glacial acetic acid (4 mL) was stirred at reflux for 4 h, cooled to room temperature, and diluted with diethyl ether (10 mL). The precipitate was filtered off, washed on a filter with diethyl ether, and purified by refluxing in diethyl ether. Chromatographically pure product **6c** was obtained as pale-yellow powder with m.p. 282—284 °C in a yield of 0.42 g (82%). ^{1}H NMR (DMSO-d_6), δ: 13.56 and 13.26 (both br.s, 1 H, NH); 12.52 (br.s, 1 H, OH); 8.11 and 7.88 (both br.d, 1 H, H(2′)); 7.33 (t, 1 H, Ar, J = 7.8 Hz); 6.90—7.04 (m, 2 H, Ar); 2.84 and 2.78 (both s, 2 H, CH_2); 2.42 (s, 2 H, CH_2); 1.10 (s, 6 H, 2 CH_3). ^{13}C NMR (DMSO-d_6), δ: 188.4; 172.0; 157.1; 131.5/131.4*; 131.2; 128.8/128.7*; 125.6; 119.1; 117.0; 112.3; 51.6; 35.5; 28.0; 21.0. MS, found: *m/z* 257.1285 $[M + H]^+$; $C_{15}H_{16}N_2O_2$; calculated: $[M + H]^+ = 257.1290$.

Evaluation of antiviral activity. Antiviral activity and toxicity of the synthesized compounds were evaluated in Vero cell culture. Vaccinia virus (Copenhagen strain), as a typical representative of orthopoxviruses, was used as model. A Vero cell monolayer was grown in wells of 96-well plates. Solutions of the compounds to be tested were prepared in dimethyl sulfoxide at a concentration of 20 mg mL^{-1}. These solutions were used to prepare a series of dilutions in a culture medium. These dilutions were placed in wells of the plates containing a cell monolayer. In one-half of the wells, the virus was added, and another half was used to evaluate the toxicity of the compounds. The dilutions were made in five- or three-fold steps; the initial concentration in the wells of the plates was 100 μg mL^{-1}. Commercially available Cidofovir (Vistide) purchased from Gilead Sciences Inc. (USA) was employed as the control. Cidofovir proved to be active against orthopoxviruses in *in vitro* and *in vivo* experiments.[3]

After incubation for four days, the vital dye neutral red that is absorbed only by living cells was added to the wells. In the wells, in which the cells are destroyed by the virus or damaged by toxicity of the compound, the dye uptake does not occur. The monolayer was washed with a physiological solution to remove the unabsorbed dye. Then a lysis buffer was added to the wells to dissolve the dye absorbed by the cells. The absorbance of the solutions in the wells was measured with an E-Max microplate reader (Molecular Devices, USA) at a wavelength of 490 nm. The absorbance is proportional to the number of surviving cells and characterizes the antiviral activity and toxicity of the compound compared to the virus control and the cell control, respectively. The data were processed with the Soft Max Pro 4.0 software, which calculated the 50% toxic concentration (TC_{50}, μg mL^{-1}) and the 50% virus-inhibitory concentration (IC_{50}, μg mL^{-1}). The selectivity index (SI) defined as SI = $= TC_{50}/IC_{50}$ was determined based on the values of TC_{50} and IC_{50}. The value of SI smaller than 8 is regarded as unacceptable for compounds that can be considered as promising antiviral agents.[3]

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