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STRUCTURAL ANALOGS OF UMIFENOVIR. 1. SYNTHESIS AND BIOLOGICAL ACTIVITY OF ETHYL 5-HYDROXY-1-METHYL-2-(*trans*-2-PHENYLCYCLOPROPYL)-1*H*-INDOLE-3-CARBOXYLATE

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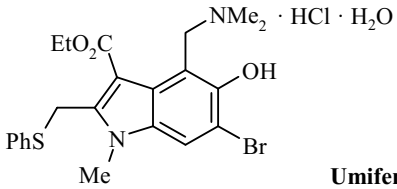
Ethyl 5-hydroxy-1-methyl-2-(trans-2-phenylcyclopropyl)-1H-indole-3-carboxylate is the first prototype of conformationally restricted analogs of umifenovir. It has been prepared using a one-pot method and has undergone an antiviral study.

Keywords: umifenovir, conformationally restricted analogs, antiviral activity.

Umifenovir (as the hydrochloride monohydrate) is the active principle of the antiviral and immunomodulating medication Arbidol, for a long time used in Russia [1].



Umifenovir

At the same time, umifenovir is not authorized for medicinal use in a number of countries due to the absence of clear knowledge regarding its biological target. In addition, existing technology for the preparation of this medicine has two marked drawbacks. In the first the stage of bromination of the ethyl 5-acetoxy-1,2-dimethyl-1*H*-indole-3-carboxylate occurs with low regioselectivity and this results in the appearance of polybrominated admixtures in the commercial product [2]. The second concerns the use of the toxic thiophenol, removal of traces of which from waste water presents a particular problem [3, 4].

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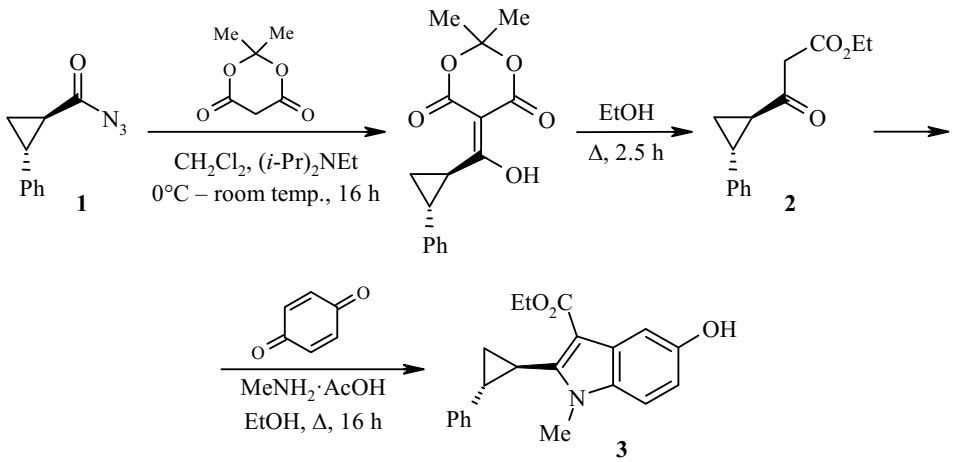
The aim of our work was the creation of debrominated bioisosteric analogs of umifenovir in which the SCH_2 fragment separating the indole and benzene rings is changed to a cyclopropane-1,2-diyl ring. Such a replacement targeted at the introduction of a conformationally rigid link gives the possibility of fixing the mutual positions of the nuclei in the molecule. Based on the comparative activity of the two pairs of diastereomers and the individual enantiomers a possible 3D structure of the pharmacophore can be put forward. In the case of umifenovir, this results in a change in the interaction model with a potential biological target. (For a conformationally mobile thiomethylene link an induced fit model and for the vicinally substituted cyclopropylidene fragment a lock and key model.) Such an approach has been successfully used before in the targeted design of β-secretase inhibitors [5].

Introduction of the cyclopropane-1,2-diyl fragment into the molecular structure avoids the use of thiophenol in the synthesis while preserving the distance between the aromatic and heteroaromatic rings to a significant degree and imitating the electronic structure of the thiomethylene fragment to a known extent.

Within the scope of this work, we have obtained the ethyl 5-hydroxy-1-methyl-2-(*trans*-2-phenylcyclopropyl)-1*H*-indole-3-carboxylate (**3**) by a one-pot reaction of ethyl 3-oxo-3-(*trans*-2-phenylcyclopropyl)propanoate (**2**) with methylammonium acetate and 1,4-benzoquinone in absolute ethanol. The starting 3-oxoester **2** was prepared by the reaction of Meldrum's acid with *trans*-2-phenylcyclopropane-1-carbazide (**1**) [7] and subsequent ethanolysis of the acylation product.



An initial experiment to convert the 3-oxoester **3** to an enaminoester (for use in the Nenitzescu reaction) by the method reported for ethyl 3-oxo-4-(phenylsulfanyl)butanoate [6] failed. The target compound was obtained in trace amounts while the main products were due to the aminolysis of the ethoxycarbonyl group and the product of a Hunsdiecker cleavage.

Antiviral studies of the compounds obtained on infected cell lines were carried out using umifenovir and other antiviral medications as external standards in accordance with the literature methods [8-10]. The comparative data for the antiviral activity of the studied compounds is given in Tables 1-5. Samples for carrying out the biological investigations were initially dissolved in aqueous DMSO, the umifenovir was used as its hydrochloride monohydrate, and compound **3** as the free base.

It is evident from Table 1 that neither umifenovir nor its conformationally rigid analog shows any marked antiviral activity in these tests. At the same time, compound **3** proved less cytotoxic when compared with umifenovir.

The data in Table 2 shows that neither umifenovir nor its conformationally rigid analog **3** exhibits antiviral activity in the studied concentration range; however umifenovir showed marked cytotoxicity. The data obtained for the activity of umifenovir towards the respiratory syncytial virus does not disagree with the literature data (MIC_{50} 8.7 μg/ml [8]).

TABLE 1. Cytotoxicity and Antiviral Activity of the Studied Compounds in Human Erythroleukemia Cells (HEL).

Compound	MCC*, μM (μg/ml)	MIC_{50}*[2], μM (μg/ml)				
		Simplex herpes 1 KOS virus	Simplex herpes 2 G virus	Cowpox virus	Vesicular stomatitis virus	Simplex herpes 1 TK-KOS ACVr virus
3	>300 (>100)	>300 (>100)	>300 (>100)	>300 (>100)	>300 (>100)	>300 (>100)
Umifenovir	188 (100)	>37 (>20)	>37 (>20)	>37 (>20)	>37 (>20)	>37 (>20)
Brivudine	>250	0.04	183	50	>250	2
Cidofovir	>250	6	2	22	>250	6
Acyclovir	>250	0.2	0.08	250	250	2
Ganciclovir	>100	0.08	0.03	>100	>100	0.2

*Minimum cytotoxic concentration of the compound leading to the appearance of morphological changes in the cells (detected microscopically).

*[2]Minimum inhibitory concentration of the compound needed for a 50% decrease in the cytopathic effects of the virus in the cell colony.

TABLE 2. Cytotoxicity and Antiviral Activity of the Studied Compounds in Cervical Cancer Cells (HeLa)

Compound	MCC, μM (μg/ml)	MIC_{50}, μM (μg/ml)		
		Vesicular stomatitis virus	Coxsackie B4 virus	Respiratory syncytial virus
3	>300 (>100)	>300 (>100)	>300 (>100)	>300 (>100)
Umifenovir	37 (20)	>7.5 (>4)	>7.5 (>4)	>7.5 (>4)
(*S*)-DHPA*	>250	>250	>250	>250
Ribavirin	>250	10	112	10

*9-((2*S*)-2,3-Dihydroxypropyl)adenine.

TABLE 3. Cytotoxicity and Antiviral Activity of the Studied Compounds in African Green Monkey Epithelial Kidney Cells (VERO)

Compound	MCs, μM (μg/ml)	MIC_{50}, μM (μg/ml)				
		Parainfluenza 3 virus	Reovirus 1	Sindbis virus	Coxsackie B4 virus	Punta Toro virus
3	>300 (>100)	>300 (>100)	>300 (>100)	>300 (>100)	>300 (>100)	>300 (>100)
Umifenovir	>190 (>100)	>190 (>100)	>190 (>100)	>190 (>100)	>190 (>100)	>190 (>100)
(*S*)-DHPA	>250	>250	>250	>250	>250	>250
Ribavirin	>250	50	>250	>250	>250	112

TABLE 4. Cytotoxicity and Antiviral Activity of the Studied Compounds in Feline Kidney Cells (CRFK)

Compound	MCC, μM (μg/ml)	MIC_{50}, μM (μg/ml)	
		Feline coronavirus	Feline herpes virus
3	>300 (>100)	>300 (>100)	>300 (>100)
Umifenovir	19.0 (10.1)	>7.5 (>4)	>7.5 (>4)
HHA*	>100	13.0	4.3
UDA*[2]	>100	1.1	0.8
Ganciclovir	>100	>100	1.0

*(2S,3S)-*trans*-3-Hydroxy-2,3-dihydroanthranilic acid.
*[2]3'-(1-Carboxy-1-phosphonoxyethoxy)uridinediphosphate-*N*-acetylglucosamine.

TABLE 5. Cytotoxicity and Antiviral Activity of the Studied Compounds in Cocker Spaniel Hepatocytes (MDCK)

Compound	MCC, μM (μg/ml)	CC_{50}*, μM (μg/ml)	MIC_{50}, μM (μg/ml)					
			Influenza A H1N1 virus		Influenza A H3N2 virus		Influenza B virus	
			VA*[2]	CA*[3]	VA	CA	VA	CA
3	34.3 (11.5)	59.6 (20)	>12 (>4)	>12 (>4)	>12 (>4)	>12 (>4)	>12 (>4)	>12 (>4)
Umifenovir	19.6 (10.4)	37 (20)	>7.5 (>4)	>7.5 (>4)	>7.5 (>4)	>7.5 (>4)	>7.5 (>4)	>7.5 (>4)
Zanamivir	>100	>100	0.8	1.2	4	2	0.4	0.3
Ribavirin	>100	>100	9	6.4	9	10.7	20	5.9
Amantadine	>200	>200	89	83.9	4	1.6	>200	>200
Remantadine	>200	>200	20	10.3	0.3	0.3	>200	>200

*The cytotoxic concentration of the substance for which only 50% of living cells remain.
*[2]Visual assessment (using microscopy).
*[3]Colorimetric assessment (using a photoelectrocolorimeter).

Data in Table 3 does not fully agree with the literature data [8] since umifenovir, under these conditions, showed inhibitory activity relative to the parainfluenza 3 virus with MIC_{50} 4.9 μg/ml.

Data in Table 4 shows that toxicity of umifenovir exceeds the ethyl 5-hydroxy-1-methyl-2-(*trans*-2-phenylcyclopropyl)-1*H*-indole-3-carboxylate (**3**) by more than one order (MCC 10.1 μg/ml versus 100 μg/ml). Antiviral activity was not revealed in either compound in the concentration range studied.

In Table 5 it is apparent that both umifenovir and compound **3** show clear cytotoxic properties relative to cocker spaniel hepatocytes (MCC 10.4 and 11.5 μg/ml, respectively, at a CC_{50} value of 20 μg/ml). It was unexpectedly found that none of these compounds possessed antiviral properties towards H1N1 and H3N2 influenza A viruses and to the influenza B virus at a concentration up to 4 μg/ml since umifenovir under these conditions has previously been reported to suppress the replication of the influenza A H1N1 virus with MIC_{50} 2.7-4.0 μg/ml [9, 10]. At the same time, the obtained data does not contradict the information [8] regarding the ability of umifenovir to suppress the replication of H3N2 influenza A and influenza B viruses with MIC_{50} values of 6.7 and 7.1 μg/ml, respectively. It was noted in our studies relative to influenza A viruses that remantadine shows a markedly greater activity than umifenovir which also contradicts the literature data [10].

Hence we propose the synthesis of ethyl 5-hydroxy-1-methyl-2-(*trans*-2-phenylcyclopropyl)-1*H*-indole-3-carboxylate as a convenient one-pot method for preparing the first prototype of conformationally restricted umifenovir analogs. In view of the absence of antiviral activity in the obtained compounds in the range studied, the separation of individual enantiomers in the pure state was not carried out. The inconsistencies with previous evidence in the literature that were revealed in the course of the biological studies of known compounds again confirmed the need for wider investigation of the relationship of chemical structure and antiviral activity in this series of compounds.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded on Bruker AM-360 and AV-600 instruments with TMS as internal standard. HPLC-MS analysis was carried out using an Agilent 1200 instrument. HPLC of umifenovir was carried out under the following conditions: Reprosil-Pur Basic C18 column 250×4.6 mm, 5 μm, with a precolumn. Eluents: A) CF_3COOH–H_2O (113 μl per liter), B) CF_3COOH–MeCN (113 μl per liter). Gradient 0-20 min, 5 to 100% B. HPLC of compound **3** was performed using the following conditions: Vodac Denali C18 120A column, 250×4.6 mm, 5 μm, with a precolumn. Eluents: A) CF_3COOH–H_2O (113 μl per liter), B) CF_3COOH–MeCN (113 μl per liter). Gradient 0-30 min, 40 to 70% B. The flow rate for all of the HPLC analyses was 1 ml/min. Detectors UV (λ 220 nm) and ELSD. All mass-spectrometric analysis used electrospray ionization. Elemental analysis was carried out on a Vario EL Cube apparatus. The halogen content was determined by combustion of a sample of the material in a flask filled with oxygen and using visual mercurometric titration with alkaline hydrogen peroxide solution as absorbent. A separate determination of chloride anion was carried out by the argentometric method. Melting points were measured by the capillary method using a Buchi M-565 apparatus with a heating rate of 1°C/min (corrected values are given). TLC was carried out on Merck Alufolien Kieselgel 60 F_{254} and visualized by UV light at 254 nm. Column chromatography used Alfa Aesar L14002 silica gel of size 0.06-0.20 mm (70-230 mesh). All of the syntheses used Alfa Aesar and Acros Organics chemical reagents and solvents. Solvents were dried by standard methods [11].

The antiviral studies were carried out using methods [8-10]. Cell colonies were made available by the Catholic University at Leuven in Belgium.

Ethyl 6-bromo-4-(dimethylamino)methyl-5-hydroxy-1-methyl-2-(phenylsulfanyl)methyl-1*H*-indole-3-carboxylate hydrochloride monohydrate (umifenovir) was prepared by the previously reported method [12]. The product properties not previously reported in the literature [12, 13, 14] are given below. Mp > 162ºC (decomp.) (mp 134-135°C [13], but in later data from the same authors the mp is not given as characteristic value [14]). The major compound content (HPLC) was 100.00% (ELSD), 99.27% (UV); t_R 13.43 min. ^{1}H NMR spectrum (360 MHz, CD_3OD), δ, ppm (*J*, Hz): 1.35 (3H, t, *J* = 7.1, $OCH_2C\underline{H}_3$); 2.93 (6H, s, $N(CH_3)_2$); 3.60 (3H, s, 1-CH_3); 4.29 (2H, q, *J* = 7.1, $OC\underline{H}_2CH_3$); 4.68 (2H, s, CH_2S); 4.85 (2H, s, $C\underline{H}_2NME_2$); 7.25-7.37 (5H, m, H Ph); 7.84 (1H, s, H-7). ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.26 (3H, t, *J* = 7.0, $OCH_2C\underline{H}_3$); 2.74 (6H, s, $N(CH_3)_2$); 3.71 (3H, s, 1-CH_3); 4.20 (2H, q, *J* = 7.2, $OC\underline{H}_2CH_3$); 4.74 (2H, s, CH_2S); 4.95 (2H, s, $C\underline{H}_2NME_2$); 7.29-7.38 (5H, m, H Ph); 8.02 (1H, s, H-7); 9.28 (1H, br. s, OH); 9.44 (1H, br. s, N^+H). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm: 13.8 ($OCH_2\underline{C}H_3$); 29.7 (1-CH_3); 30.4 (CH_2S); 42.1 ($N(CH_3)_2$); 53.0 ($O\underline{C}H_2CH_3$); 60.3 ($\underline{C}H_2NME_2$); 105.2 (C-3); 110.0 (C-6); 111.6 (C-4); 116.5 (C-7); 125.3 (C-3A); 127.5 (C-4 Ph); 129.1 (C-2,6 Ph); 131.4 (C-3,5 Ph); 132.6 (C-2); 134.2 (C-1 Ph); 144.2 (C-7A); 148.9 (C-5); 165.0 (C=O). Mass spectrum (for isotope ^{79}Br), *m/z* (I_{rel}, %): 477.4 $[M+H]^+$ (100). Found, %: C 50.00; H 5.11; Br 15.10; Cl 6.88; N 4.99; S 5.89. $C_{22}H_{28}BrClN_2O_4S$. Calculated %: C 49.68; H 5.31; Br 15.02; Cl 6.67; N 5.27; S 6.03.

Ethyl 3-Oxo-3-(*trans*-2-phenylcyclopropyl)propanoate (2). A solution of *trans*-2-phenylcyclopropane-1-carbazide (**1**) in anhydrous CH_2Cl_2 was prepared from *trans*-2-phenylcyclopropane-1-carbohydrazide (20.0 g, 113 mmol) by method [7] using CH_2Cl_2 (550 ml) in place of PhMe. It was added dropwise with stirring

and cooling in an ice bath to a solution of the 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) (16.3 g, 113 mmol) and (*i*-Pr)$_2$NEt (60 ml, 46.9 g, 363 mmol) in absolute CH_2Cl_2 (160 ml). The reaction mixture was left overnight at room temperature and poured onto a mixture of crushed ice (250 g) and conc. HCl (40 ml). The organic phase was separated, washed with 1 N HCl (3×120 ml), water (3×100 ml), and dried over $MgSO_4$. After filtration from the desiccant, the solvent was removed under reduced pressure and the residue was treated with activated carbon (2.5 g) in absolute EtOH (250 ml). The mixture was refluxed with protection from moisture until gas evolution ceased (about 2.5 h). The product was filtered, solvent was distilled off, and the residue was dissolved in CH_2Cl_2 (400 ml), washed with 5% aqueous $NaHCO_3$ solution (4×75 ml) and then water (3×75 ml), and dried over $MgSO_4$. The dried solution was filtered, the filtrate was evaporated, and the residue was distilled under reduced pressure. The yield (calculated on the *trans*-2-phenylcyclopropane-1-carbo-hydrazide) was 17.1 g (65%). Colorless oil. Bp 156-158°C (2 mm Hg) (138-140°C (0.2 mm Hg) [15]). The ^{1}H NMR spectroscopic data agrees with the literature [5]. Found, %: C 71.99; H 7.12. $C_{14}H_{16}O_3$. Calculated, %: C 72.39; H 6.94.

Ethyl 5-Hydroxy-1-methyl-2-(*trans*-2-phenylcyclopropyl)-1*H*-indole-3-carboxylate (3). A 33% solution of $MeNH_2$ in anhydrous EtOH (2 ml, 16 mmol of $MeNH_2$) was treated with glacial AcOH (1 ml, 1.04 g, 16 mmol), 1,4-benzoquinone (1.08 g, 10 mmol), and ester **2** (2.32 g, 10 mmol) dissolved in absolute EtOH (17 ml). The mixture obtained was refluxed for 16 h. The solvent was distilled off, and the residue was dissolved in EtOAc (150 ml), washed with water (3×75 ml), saturated aqueous NaCl solution (4×75 ml), and dried over $MgSO_4$. The product was filtered and silica gel added (15 g). The obtained mixture was evaporated to dryness and the product (absorbed on silica gel) was transferred to a chromatographic column. The target product was purified by gravity elution of the silica gel column (50×600 mm) with EtOAc in hexane (gradient from 5 to 30%) as an eluent. The fractions containing the target product were combined, evaporated under reduced pressure, and the residue was triturated with Et_2O, filtered, and dried to constant weight. Yield 0.94 g (28%). Colorless crystals. Mp 205-207°C and R_f 0.32 (EtOAc–petroleum ether (40-70°C fraction), 7:12). The major compound content (HPLC) was 98.95% (UV); t_R 21.56 min). ^{1}H NMR spectrum (600 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.36 (3H, t, *J* = 7.1, $OCH_2C\underline{H}_3$); 1.58-1.61 (1H, m) and 1.67-1.71 (1H, m, CH_2 cyclopropane); 2.24-2.28 (1H, m) and 2.32-2.35 (1H, m, CHCH cyclopropane); 3.78 (3H, s, NCH_3); 4.27-4.33 (1H, m) and 4.42-4.48 (1H, m, $OC\underline{H}_2CH_3$); 5.28 (1H, br. s, OH); 6.89 (1H, dd, *J* = 8.8, *J* = 2.3, H-6); 7.19 (1H, d, *J* = 8.8, H-7); 7.25-7.28 (3H, m, H-2,4,6 Ph); 7.37 (2H, t, *J* = 7.6, H-3,5 Ph); 7.70 (1H, d, *J* = 2.3, H-4). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 14.5 ($OCH_2\underline{C}H_3$); 19.1 (C-1 cyclopropane); 19.5 (C-3 cyclopropane); 26.2 (C-2 cyclopropane); 31.1 (NCH_3); 59.7 ($O\underline{C}H_2CH_3$); 105.0 (C-3); 106.4 (C-4); 110.0 (C-7); 112.1 (C-6); 125.7 (C-2,6 Ph); 126.2 (C-4 Ph); 127.7 (C-3A); 128.6 (C-3,5 Ph); 131.6 (C-2); 141.7 (C-1 Ph); 147.1 (C-7A); 151.7 (C-5); 165.5 (C=O). Mass spectrum, *m*/*z* (I_{rel}, %): 336.1 $[M+H]^+$ (100), 232.2 $[M+H\text{-}PHCHCH_2]^+$ (6). Found, %: C 74.99; H 6.60; N 3.80. $C_{21}H_{21}NO_3$. Calculated, %: C 75.20; H 6.31; N 4.18.

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