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SYNTHESIS OF NEW DISOXARIL ANALOGUES WITH POTENT AND SELECTIVE ANTI-HUMAN RHINOVIRUS 14 ACTIVITY[1]

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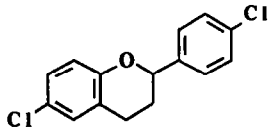
Abstract: New analogues of disoxaril have been synthesized and tested against DNA and RNA viruses. 5-Chloro-2-[4,5-dihydro-2-(oxazolyl)phenoxypropylcarbonyl]thiophene showed the highest potency and selectivity against HRV-14.

In the last decade, numerous compounds, 4',6-dichloroflavan (BW-683c) **1**[2], 1-(5-tetradecyloxy-2-furanyl)ethanone (RMI-15731) **2**[3], 4'-ethoxy-2'-hydroxy-4,6'-dimethoxychalcone (RO 09-0410) **3**[4], 5-[7-[4-(4,5-dihydro-2-oxazolyl)phenoxy]heptyl]-3-methylisoxazole (WIN 51711, disoxaril) **4**[5], have been reported to inhibit a wide range of human rhinoviruses (HRVs) by binding to the viral capsid.[6,7] The more extensive structure-activity studies have been performed on (oxazolinylphenoxy)alkylisoxazoles. Numerous analogues of disoxaril have been prepared, differing in the lenght of the carbon chain bridged between the isoxazole and phenoxy moieties and bearing substituents in different positions of the phenyl and/or oxazoline rings.[8-10] Several compounds in this series exhibit, *in vitro*, a cytotoxicity in the range 3-30 μM, and a broad spectrum of anti-HRV activity at concentrations as low as 0.3 μM.



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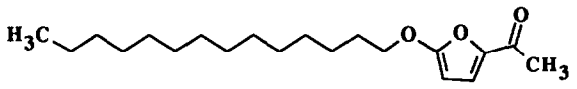

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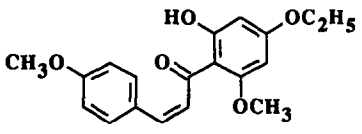

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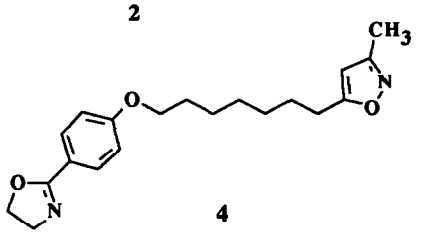

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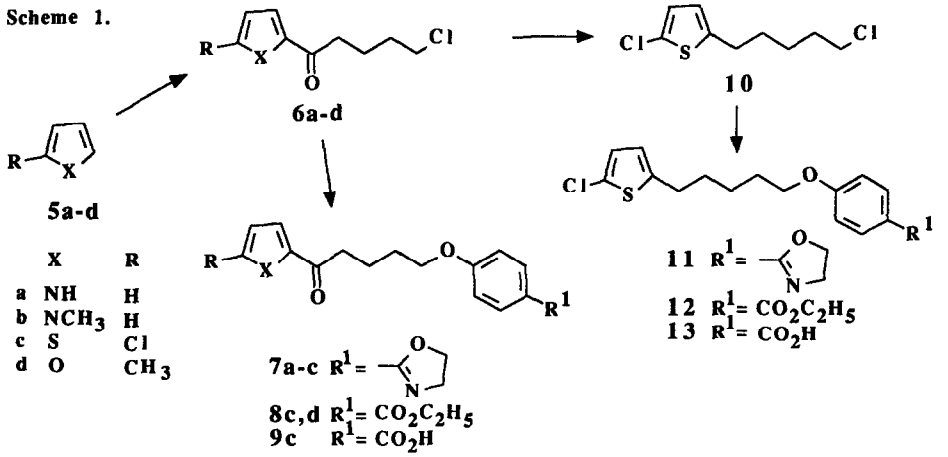
We now report the synthesis of new disoxaril analogues (Table 1), characterized by pentatomic heterocycles different from isoxazole and by the presence of a ketocarbonyl group in the aliphatic chain, and present evidence for their potent and selective anti-HRV-14 activity *in vitro* (Table 2).

Commercially available pentatomic heterocycles, namely 1H-pyrrole **5a**, 1-methyl-1H-pyrrole **5b**, 2-chlorothiophene **5c**, and 2-methylfuran **5d**, were used as starting materials. Friedel-Crafts acylation of **5a-d** with 5-chlorovaleryl chloride afforded the heteroaryl ketones **6a-d**. These readily underwent nucleophilic displacement by 4-(4,5-dihydro-2-oxazolyl)phenol and ethyl 4-hydroxybenzoate in the presence of K_2CO_3 to give compounds **7a-c** and **8c,d**, respectively. Hydrolysis of **8c** afforded the acid **9c**.

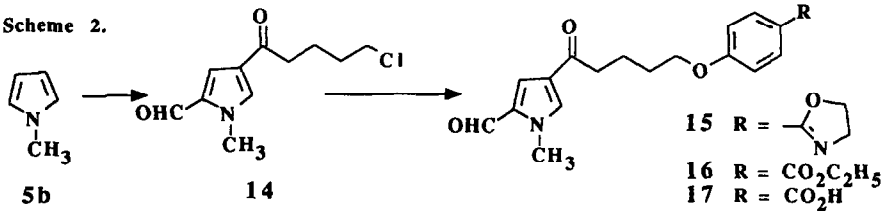
In a similar way, reaction of the haloalkyl derivative **10** (obtained by reduction of **5c** with $LiAlH_4/AlCl_3$) with 4-(4,5-dihydro-2-oxazolyl)phenol and ethyl 4-hydroxybenzoate provided compounds **11** and **12**, respectively. Ester **12** was then hydrolyzed to the corresponding carboxylic acid **13** (Scheme 1).

Scheme 1.



Treatment of 1-methyl-1H-pyrrole **5b** with the Vilsmeier-Haack reagent generated from DMF and $POCl_3$ and with the 5-chlorovaleryl chloride-$AlCl_3$ complex furnished a mixture, whose aqueous work-up gave 4-(5-chlorovaleryl)-1-methyl-1H-pyrrole-2-carboxaldehyde **14**. This compound was reacted with 4-(4,5--dihydro-2-oxazolyl)phenol and ethyl 4-hydroxybenzoate to afford derivatives **15** and **16**, respectively (Scheme 2). Again hydrolysis of the ethyl ester afforded the corresponding acid **17**.

Scheme 2.



Test compounds were screened in Vero cells against Herpes Simplex type 1 (HSV-1), Vaccinia (VV), Vesicular Stomatitis (VSV), Coxsackie B1 (Coxs) and Polio type 1 (Sabin strain, Sb-1) and in HeLa against HRV-14. The latter strain was chosen since its degree of sensitivity to (oxazolinylphenoxy)alkylisoxazoles is fairly predictive of the sensitivity of a broader spectrum of HRV serotypes.[8,9]

Table 1. Chemical and physical data of test compounds

compd	formula	mol. weight	mp (°C)	crystal. from	elemental analyses (%) calcd					found				
					C	H	N	Cl	S	C	H	N	Cl	S
7a	$C_{18}H_{20}N_2O_3$	312.36	155-6	acetonitrile	69.21	6.45	8.97	-	-	68.80	6.57	8.87	-	-
7b	$C_{19}H_{22}N_2O_3$	326.38	133-4	acetonitrile	69.92	6.79	8.58	-	-	69.73	6.85	8.72	-	-
7c	$C_{18}H_{18}NO_3ClS$	363.85	143-4	cyclohexane-benzene	59.41	4.99	3.85	9.75	8.81	59.21	4.97	3.69	9.97	8.66
8c	$C_{18}H_{19}O_4ClS$	366.86	87-9	cyclohexane	58.93	5.22	-	9.67	8.74	58.60	5.25	-	9.55	8.81
8d	$C_{19}H_{22}O_5$	330.37	66-7	cyclohexane	69.07	6.71	-	-	-	69.32	6.60	-	-	-
9c	$C_{16}H_{15}O_4ClS$	338.81	169-71	ethanol	56.72	4.46	-	10.47	9.47	56.57	4.46	-	10.69	9.55
11	$C_{18}H_{20}NO_2ClS$	349.88	99-100	cyclohexane	61.79	5.76	4.00	10.13	9.17	62.13	5.75	3.87	9.80	9.29
13	$C_{16}H_{17}O_3ClS$	324.83	158-9	ethanol	59.16	5.28	-	10.92	9.87	59.32	5.37	-	10.78	10.10
15	$C_{20}H_{22}N_2O_4$	354.39	152-4	ethanol	67.78	6.24	7.91	-	-	67.42	6.14	8.13	-	-
16	$C_{20}H_{23}NO_5$	357.39	117-8	ethanol	67.21	6.49	3.92	-	-	67.29	6.51	3.89	-	-
17	$C_{18}H_{19}NO_5$	329.34	181-3	toluene	65.64	5.82	4.25	-	-	65.40	5.73	4.32	-	-

The test compounds were solubilized in DMSO as 200x stock solutions and then were serially diluted in MEM (minimum essential medium) to achieve final concentrations of 600 to 0.01 μM.

Maintenance medium was aspirated from 1-day-old confluent Vero or HeLa (Ohio) cell monolayers, which were infected with 0.2 mL/well of the appropriate virus dilution to give 150 plaque forming units (pfu)/well. Plates were incubated for 1h at 37°C (33°C for HRV-14) in a 5% CO_2 atmosphere (2% for HRV-14). The viral inoculum was removed, and the monolayers were overlaid with MEM containing 2% newborn calf serum, 0.75% carboxymethylcellulose and the test compounds at various concentrations. The overlay medium for HRV-14 also contained 30 mM $MgCl_2$ and 15 μg/mL DEAE-dextrane. Virus-infected controls and uninfected cell controls were run at the same time. Viruses were allowed to replicate (forming plaques) at 37°C (33°C for HRV-14) in a 5% CO_2 atmosphere (2% for HRV-14) for 2-4 days. Cells were fixed with 5% formaldehyde in 2% sodium acetate and stained with 0.25% crystal violet in the fixing solution. Plaques, appearing as clear areas of cell destruction, were counted. The concentration of compound that resulted in a 50% reduction in the number of plaques was determined for each virus by linear regression and recorded as the minimal inhibitory concentration (MIC). The maximum testable level (MTL) was the highest drug concentration that caused no cytotoxic effects on cell monolayers.

Data in Table 2 show that in Vero, as well as in HeLa cells, the majority of the test compounds showed cytotoxicities >15 times lower than those reported for (oxazolinylphenoxy)alkylisoxazoles.

Among 2-chlorothiophene derivatives, compounds bearing a ketone carbonyl group (**7c** and **9c**) showed a lower cytotoxicity than the counterparts with a pentamethylene aliphatic chain (**11** and **13**, respectively). Among compounds with a formyl group, only **16** showed a MTL of 20 μM.

Table 2. Effects of disoxaril analogues on DNA and RNA viruses

compd	MTL[a] (μM) Vero	MIC[b] HSV-1	VV	VSV	Coxs	Sb-1	MTL (μM) HeLa	MIC HRV-14	S.I[c]
7a	>175	>175	>175	>175	>175	60	>640	7.5	-
7b	>300	>300	>300	>300	>300	>300	>610	1.5	>406
7c	>275	>275	>275	>275	>275	200	>550	0.1	>5500
8c	>190	>190	>190	>190	>190	80	>545	0.4	>1362
8d	>120	75	30	>120	60	15	65	2.4	-
9c	>170	>170	>170	>170	>170	30	>590	>50.0	-
11	30	>30	30	>30	>30	25	60	2.8	-
13	60	>60	>60	>60	>60	20	20	>20.0	-
15	>280	>280	>280	>280	>280	>280	>565	20.0	-
16	55	>55	>55	>55	-	5	20	1.4	-
17	380	>380	>380	>380	>380	>380	>610	>50.0	-
Disoxaril[d]							18	0.4	45
WIN 52035[d]							10	0.5	20

[a] MTL (Maximum Testable Level): highest compound concentration that causes no apparent effects on cell monolayers.
[b] MIC (Minimum Inhibitory Concentration): compound concentration required to reduce by 50% the number of plaques. Values represent the mean of three separate experiments.
[c] S.I. (Selectivity Index): ratio MTL/MIC.
[d] Data taken from references 8,9,11.

As far as the antiviral activity is concerned, the test compounds were inactive against HSV-1, VV, VSV, Coxsackie and Poliovirus. On the contrary, many of them selectively inhibited the HRV-14. Derivatives **7c** and **8c** were as potent as disoxaril and its pentamethylene analog WIN 52035, but showed a 30-275 fold superior selectivity index[11] due to their lower citotoxicity.

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