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Disoxaril-related 3-(diethylamino)-5-phenylisoxazoles

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Abstract

Previous research has shown that 3-(dialkylamino)-5-phenylisoxazoles possessing a compact structure were active against HRV-2 and, consequently, presented a type B activity. In this paper, 3-(diethylamino)-5-phenylisoxazoles, which are structurally more elongated and related to Disoxaril, were synthesized in view to attempt type A activity against HRV-14. Unfortunately, all tested compounds were devoid of activity against HRV-14 (and HIV-1) or exhibited great toxicity.

Keywords: Isoxazole derivatives; Antiviral agents; Antirhinovirus activity

1. Introduction

In previous works we showed that the 3-(dialkylamino)-5-phenylisoxazoles **A** (see Fig. 1) were endowed with significant antirhinovirus activity [1,2]. Because the structure of many **A** derivatives was short and compact, in accordance with the observations of Andries et al. [3], these compounds exerted type B activity. More generally, the isoxazole moiety is known to possess potential antirhinoviral properties as it is widely represented in the class of the WIN compounds (see Figs. 1 and 2). The leader of this class (WIN 51711 or Disoxaril) inhibits picornaviral uncoating by binding to the hydrophobic pocket on the virion surface and, as it possesses an elongated structure, shows type A activity [3]. Although the Disoxaril was a very active antirhinovirus agent, it was withdrawn from clinical studies due to the appearance of crystallurea at high dosages in healthy volunteers [4].

Following our interest in the class of 3-(dialkylamino)-5-phenylisoxazoles, our attention was aimed at amplifying the pattern of activity of **A** compounds and, consequently, some new isoxazole derivatives with an elongated structure were synthesized. All new derivatives carry a similar design in the 4′-position of the phenyl ring concerning a five carbon atom linker between oxygen and nitrogen, this latter having different basic properties. In particular, when R′ was a butoxy chain ending with an oxazoline ring, as in compounds **9** and **10**, the substances obtained were related to WIN compounds with the etherocyclic rings mutually exchanged (see Fig. 2).

2. Chemistry

With the goal of obtaining a suitable chain in the 4′-position of phenylisoxazole **A**, resorcin was treated with 5-chlorovaleronitrile in the presence of dimethylformamide (DMF) and anhydrous potassium carbonate to yield 3-(4-cyanobutoxy)phenol (**1**).

In turn, 2-(diethylamino)chromone (**2**) was prepared with a cyclocondensation reaction between **1** and 3-(diethylamino)-3-oxo-propanoic acid ethyl ester in the presence of phosphorus oxychloride using 1,2-dichloroethane (DCE) as solvent, as previously reported for similar compounds [5] (see Scheme 1).

As depicted in Scheme 1, the treatment of the 2-(diethylamino)chromone (**2**) with hydroxylamine hydrochloride in ethanol in the presence of pyridine gave

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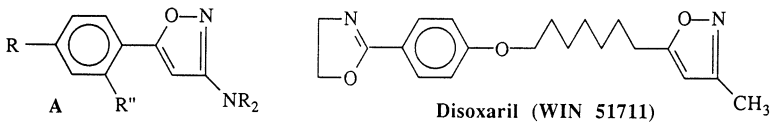


Fig. 1.

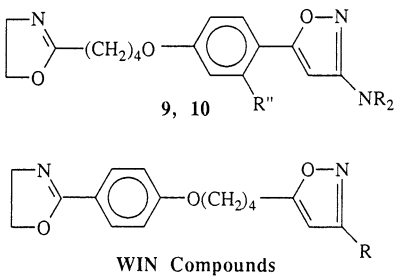


Fig. 2.

a good yield of the 3-(diethylamino)-5-phenylisoxazole derivative (**3**). The reaction mechanism, based on the nucleophilic attack of the hydroxylamine at the 2-position of the chromone ring, was already described [1].

The isoxazole **3** was transformed into the ester **4** by reaction with *p*-nitrobenzoyl chloride and into the ether **5** by reaction with dimethylsulfate (see Scheme 2). Comments on these reactions are given in Section 4.

The cyanodervative **3** was then hydrogenated into the corresponding aminoderivative **6** using platinum oxide as catalyst in chloroform–methanol [6]. The latter compound was in turn acetylated yielding **7** (see Scheme 2).

From the chromone **2**, the oxazoline **8** was obtained by means of ethanolamine in the presence of anhydrous zinc chloride [7]. Then the benzopyran ring was opened as above with hydroxylamine yielding the isoxazole **9**; this latter was easily methylated with dimethylsulfate to give the oxazolinylisoxazole **10** (see Scheme 3).

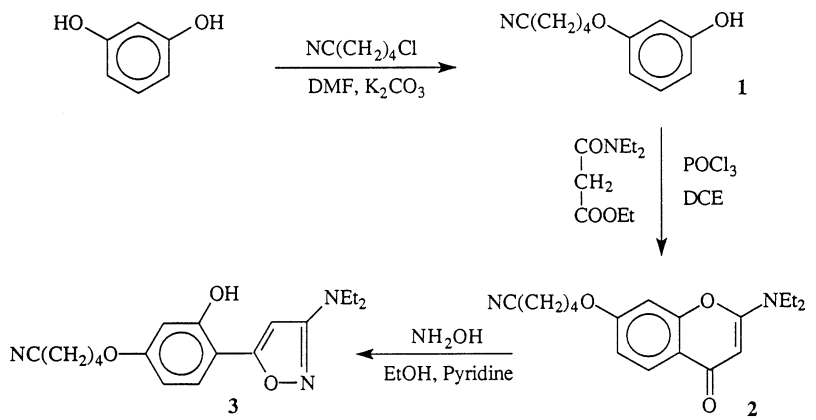
Compounds **1**–**10** are white crystals whose structures are in agreement with elemental analyses and spectral data. In particular, the H-5 of chromones **2** and **8** shows a signal at about 8.10 ppm in the ^{1}H NMR spectra because of the deshielding effect of the carbonyl in the 4-position as described for similar structures [5].

3. Experimental

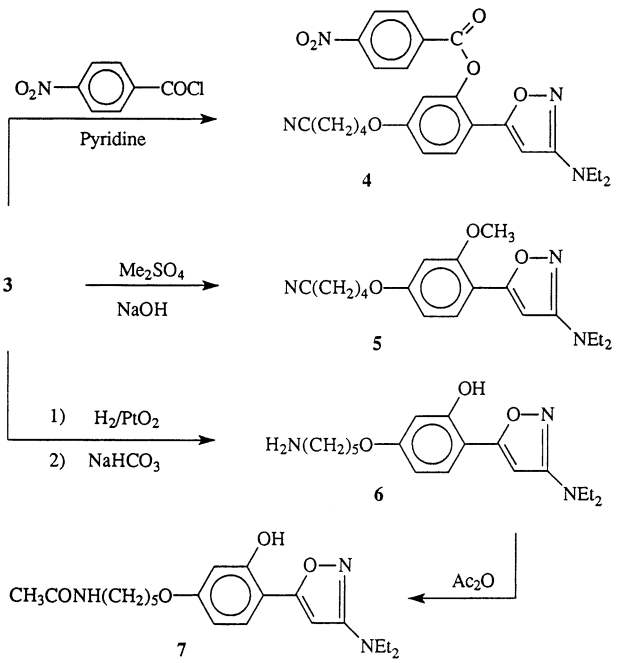
Melting points were determined using an Electrothermal apparatus and are uncorrected. Microanalyses were carried out on a Carlo Erba 1106 elemental analyzer. The results of elemental analysis were within $\pm 0.3\%$ for C and ± 0.1 for H and N of the theoretical value. ^{1}H NMR spectra were performed on a Hitachi Perkin–Elmer R 600 (60 MHz) spectrometer using TMS as internal standard ($\delta = 0$). IR spectra were recorded on a Perkin–Elmer 398 spectrophotometer.

*3.1. 3-(4-Cyanobutoxy)phenol (**1**)*

To 4.0 g (0.036 mol) of resorcinol dissolved in 10 ml of DMF, 8.0 g of anhydrous potassium carbonate and 4.3 g (0.036 mol) of 5-chlorovaleronitrile were added. The mixture was heated at 110–115°C for 5 h under a stream of nitrogen. The final mixture was cooled, poured onto crushed ice (adjusting the pH to 7) and stirred for 30 min. The aqueous solution was extracted several times with chloroform. The pooled organic extracts were counter-extracted with 2 N NaOH. The alkaline solution was acidified with 6 M HCl and the white solid obtained was filtered off. The crude product was crystallized from ethyl ether obtaining **1**; m.p. 69–70°C; 68% yield.



Scheme 1.



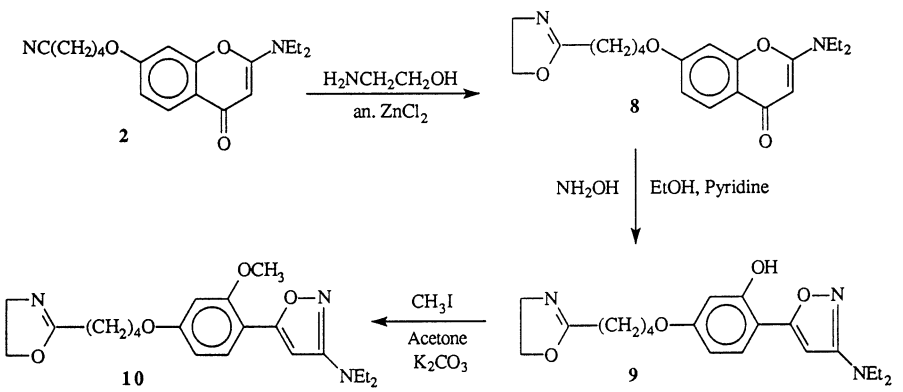
Scheme 2.

^{1}H NMR ($CDCl_3$): δ 1.71–2.18 (m, 4H, OCH_2-(CH_2)$_2$), 2.45 (t, 2H, CH_2CN), 3.99 (t, 2H, OCH_2), 5.83 (s, 1H, OH), 6.32–7.41 (m, 4H, arom. H). IR (KBr) cm^{-1}: 3340, 2260. *Anal.* $C_{11}H_{13}NO_2$: C, H, N.

3.2. 2-(Diethylamino)-7-(4-cyanobutoxy)-4H-1-benzopyran-4-one (2)

In an ice-cooled flask, protected from moisture with a calcium chloride drying tube, 7.0 ml (78.0 mmol) of phosphorus oxychloride were added dropwise with stirring to 10.30 g (55.0 mmol) of 3-(diethylamino)-3-oxopropanoic acid ethyl ester [8]. After the addition, the mixture was removed from the ice bath and maintained at room temperature (r.t.) for 30 min. To the resulting yellow mixture, a solution of 9.56 g (50.0 mmol) of 3-(4-cyanobutoxy)phenol (**1**) in 40 ml of DCE was added slowly with stirring. The reaction mixture was then heated for 5 h at reflux. After cooling, a solution of 68 g of sodium acetate trihydrate in 200 ml of water was added and the mixture was then heated for 1.5 h at 70°C. After cooling, the organic phase was discarded and the aqueous one was extracted three times with chloroform. The pooled organic extracts were washed with water, dried and evaporated under reduced pressure giving a dark-red oil. The oil was stirred at r.t. for 2 h with 200 ml of 2 N NaOH and 50 ml of light petroleum ether. The obtained solid was filtered off and washed with water. The crude product was crystallized from ethyl acetate obtaining **2**; m.p. 85–86°C; 77.8% yield.

^{1}H NMR ($CDCl_3$): δ 1.25 (t, 6H, NCH_2–CH_3), 1.68–2.31 (m, 4H, OCH_2–(CH_2)$_2$–CH_2CN), 2.48 (t, 2H, CH_2CN), 3.47 (q, 4H, NCH_2–CH_3), 4.09 (t, 2H,



Scheme 3.

OCH_2), 5.47 (s, 1H, H-3), 6.70–7.05 (m, 2H, H-6, H-8), 8.10 (d, 1H, H-5). IR (KBr) cm^{-1}: 2240, 1610, 1540. *Anal.* $C_{18}H_{22}N_2O_3$: C, H, N.

3.3. 3-(Diethylamino)-5-[2′-hydroxy-4′-(4-cyanobutoxy)phenyl]isoxazole ***(3)***

To a solution of 1.2 g (3.82 mmol) of **2** in 40 ml of ethanol, 1 g of hydroxylamine hydrochloride and 1.5 ml of pyridine were added. The mixture was refluxed for 24 h. The final solution was evaporated under reduced pressure to leave a pale yellow solid. The solid was dissolved in a small amount of 2 N NaOH, filtering off any impurity of the unreacted starting product. Then the alkaline solution was acidified with 6 M HCl obtaining a white precipitate, which was filtered off and washed with water. The crude product was crystallized from ethanol obtaining **3**; m.p. 188–189°C; 68.2% yield.

^{1}H NMR ($CDCl_3$): δ 1.10 (t, 6H, NCH_2–CH_3), 1.55–2.12 (m, 4H, $OCH_2(CH_2)_2$), 2.51 (t, 2H, CH_2CN), 3.38 (q, 4H, NCH_2–CH_3), 3.65–4.20 (m, 3H, OCH_2, OH), 6.42 (s, 1H, H-4), 6.52–6.70 (m, 2H, H-3′, H-5′), 7.73 (d, 1H, H-6′). IR (KBr) cm^{-1}: 3000 (broad), 2240, 1620, 1590. *Anal.* $C_{18}H_{23}N_3O_3$: C, H, N.

3.4. 3-(Diethylamino)-5-[2′-p-nitrobenzoyloxy-4′-(4-cyanobutoxy)phenyl]isoxazole ***(4)***

To 1 g (3.04 mmol) of **3** in 5 ml of pyridine, 0.98 g (5.3 mmol) of *p*-nitrobenzoyl chloride was added and the solution was heated at 110°C for 8 min. At the end the mixture was cooled and 15 ml of water was added obtaining a solid. The solid was filtered off and washed with water. The crude product was crystallized from ethyl acetate–cyclohexane obtaining **4**; m.p. 80–81°C; 51% yield.

^{1}H NMR ($CDCl_3$): δ 1.07 (t, 6H, NCH_2–CH_3), 1.72–2.16 (m, 4H, OCH_2–$(CH_2)_2$–CH_2CN), 2.50 (t, 2H, CH_2CN), 3.22 (q, 4H, NCH_2–CH_3), 4.13 (t, 2H, OCH_2), 5.94 (s, 1H, H-4), 6.80–7.13 (m, 2H, H-3′, H-5′), 7.87 (d, 1H, H-6′), 8.46 (near s, 4H, arom. H). IR (KBr) cm^{-1}: 2245, 1745, 1625, 1610. *Anal.* $C_{25}H_{26}N_4O_6$: C, H, N.

3.5. 3-(Diethylamino)-5-[2′-methoxy-4′-(4-cyanobutoxy)phenyl]isoxazole ***(5)***

To 1 g (3.04 mmol) of **3** dissolved in 5 ml of 2 N NaOH, 0.2 ml of dimethylsulfate was added dropwise. The mixture was heated at 70°C for 2 h and then, after cooling, extracted three times with diethyl ether. The pooled organic extracts were washed with water, dried and evaporated under reduced pressure to give a white solid which was crystallized from ethyl acetate–cyclohexane obtaining **5**; m.p. 100–101°C; 46% yield.

^{1}H NMR ($CDCl_3$): δ 1.20 (t, 6H, NCH_2–CH_3), 1.78–2.16 (m, 4H, OCH_2–$(CH_2)_2$–CH_2CN), 2.45 (t, 2H, CH_2CN), 3.38 (q, 4H, NCH_2–CH_3,), 3.88–4.23 (m, 5H, OCH_3, OCH_2), 6.26 (s, 1H, H-4), 6.45–6.73 (m, 2H, H-3′, H-5′), 7.88 (d, 1H, H-6′). IR (KBr) cm^{-1}: 1615, 1565, 1545. *Anal.* $C_{19}H_{25}N_3O_3$: C, H, N.

3.6. 3-(Diethylamino)-5-[2′-hydroxy-4′-(5-aminopentoxy)phenyl]isoxazole ***(6)***

A solution of 1 g (3.04 mmol) of **3** in 20 ml of chloroform and 20 ml of methanol was hydrogenated at 60 psi in a Parr apparatus with 0.25 g of platinum oxide until gas uptake ceased. The catalyst was filtered off and the solvent was distilled under reduced pressure. The residue was treated with a solution of sodium hydrogencarbonate and the free base was crystallized from ethyl acetate–cyclohexane obtaining **6**; m.p. 128–129°C; 60.5% yield.

^{1}H NMR (DMSO-d_6): δ 1.21 (t, 6H, NCH_2–CH_3), 1.35–1.90 (m, 6H, OCH_2–$(CH_2)_3$–CH_2), 2.65–2.98 (m, 2H, CH_2NH_2), 3.05–3.57 (m, 6H, NCH_2–CH_3, NH_2), 3.99 (t, 2H, OCH_2), 6.25–6.46 (m, 2H, H-4, OH), 6.50–6.85 (m, 2H, H-3′, H-5′), 7.62 (d, 1H, H-6′). IR (KBr) cm^{-1}: 3350, 3310, 3100 (broad), 1620, 1555. *Anal.* $C_{18}H_{27}N_3O_3$: C, H, N.

3.7. 3-(Diethylamino)-5-[2′-hydroxy-4′-(5-acetylaminopentoxy)phenyl]isoxazole ***(7)***

A solution of 1 g (3.00 mmol) of **6** was treated with 5 ml of acetic anhydride at 70°C for 1 h. At the end, the solution was poured onto crushed ice and the suspension was stirred for 1 h. The precipitate was filtered off and the solid was crystallized from ethyl acetate–ethanol obtaining **7**; m.p. 169–170°C; 84% yield.

^{1}H NMR (DMSO-d_6): δ 1.12 (t, 6H, NCH_2–CH_3), 1.38–1.74 (m, 6H, OCH_2–$(CH_2)_3$–CH_2), 1.85 (s, 3H, $COCH_3$), 2.85–3.54 (m, 6H, NCH_2–CH_3, CH_2NH), 4.00 (t, 2H, OCH_2), 6.38 (s, 1H, H-4), 6.51–6.73 (m, 2H, H-3′, H-5′), 7.50–8.12 (m, 2H, H-6′, NH), 10.52 (s, 1H, OH). IR (KBr) cm^{-1}: 3295 (sharp), 3100 (broad), 1640, 1610, 1565. *Anal.* $C_{20}H_{29}N_3O_4$: C, H, N.

3.8. 2-(Diethylamino)-7-[4-(2′-oxazolinyl)-butoxy]-4H-1-benzopyran-4-one ***(8)***

To 2 g (6.36 mmol) of **2** dissolved in 10 ml of ethanolamine, 0.9 g of anhydrous zinc chloride was added and the mixture was heated at 120°C for 2 h. At the end the mixture was cooled and poured onto crushed ice (adjusting the pH to 7). The suspension was extracted three times with chloroform; then the pooled organic extracts were dried and evaporated under reduced pressure. To the resulting oil, 30 ml of 2 N NaOH and 5 ml of cyclohexane were added and the

mixture was allowed to stir for 1 h on an ice bath. The solid that separated out was filtered off and washed with water. The crude product was crystallized from ethanol obtaining **8**; m.p. 90–91°C; 52.1% yield.

^{1}H NMR ($CDCl_3$): δ 1.28 (t, 6H, NCH_2–CH_3), 1.65–3.00 (m, 6H, OCH_2–$(CH_2)_3$), 3.21–3.90 (m, 8H, NCH_2–CH_3, CH_2 oxazoline), 4.02 (t, 2H, OCH_2), 5.40 (s, 1H, H-3), 6.79–7.08 (m, 2H, H-6, 8), 8.06 (d, 1H, H-5). IR cm^{-1}: 1630, 1605, 1545. *Anal.* $C_{20}H_{26}N_2O_4$: C, H, N.

*3.9. 3-(Diethylamino)-5-[2′-hydroxy-4′-(4-[2′-oxazolinyl]butoxy)phenyl]isoxazole (**9**)*

To a solution of 1 g (2.79 mmol) of **8** in 20 ml of ethanol, 1 g of hydroxylamine hydrochloride and 1.5 ml of pyridine were added. The mixture was refluxed for 24 h. The final solution was evaporated under reduced pressure to yield a pale yellow solid. The solid was dissolved in a small amount of 2 N NaOH, filtering off any impurity of unreacted starting product. Then the alkaline solution was acidified with 6 M HCl obtaining a white precipitate, which was filtered off and washed with water. The crude product was crystallized from ethanol obtaining **9**; m.p. 151–152°C; 74.8% yield.

^{1}H NMR ($CDCl_3$): δ 1.19 (t, 6H, NCH_2–CH_3), 1.60–2.65 (m, 6H, OCH_2–$(CH_2)_3$), 3.12–3.80 (m, 8H, NCH_2–CH_3, CH_2 oxazoline), 4.00 (t, 2H, OCH_2), 6.40 (s, 1H, H-4), 6.48–6.70 (m, 2H, H-3′ 5′), 7.70 (d, 1H, H-6′). IR cm^{-1}: 1620, 1545, 1450. *Anal.* $C_{20}H_{27}N_3O_4$: C, H, N.

*3.10. 3-(Diethylamino)-5-{2′-methoxy-4′-[4-(2′-oxazolinyl)butoxy]phenyl}isoxazole (**10**)*

To a solution of 1 g (2.68 mmol) of **9** in 20 ml of acetone, 0.5 ml of methyl iodide and 1.4 g of anhydrous potassium carbonate were added and the resulting mixture was heated at 50°C for 24 h. After 12 h of heating, another 0.5 ml of methyl iodide was added. At the end, the solvents were eliminated under reduced pressure and 30 ml of 2 N NaOH were added to the residue, with stirring. The alkaline suspension was extracted many times with chloroform. Then the pooled organic extracts were dried and evaporated under reduced pressure. The crude product was crystallized from ethyl acetate–cyclohexane obtaining **10**; m.p. 85–86°C; 91.3% yield.

^{1}H NMR ($CDCl_3$): δ 1.12 (t, 6H, NCH_2–CH_3), 1.52–2.56 (m, 6H, OCH_2–$(CH_2)_3$), 3.09–3.70 (m, 8H, NCH_2–CH_3, CH_2 oxazoline), 3.90–4.23 (m, 5H, OCH_3, OCH_2), 6.19 (s, 1H, H-4), 6.42–6.70 (m, 2H, H-3′ 5′), 7.80 (d, 1H, H-6′). IR (KBr) cm^{-1}: 1620, 1575, 1545. *Anal.* $C_{21}H_{29}N_3O_4$: C, H, N.

4. Results and discussion

Regarding the chemistry, it is interesting to note that the methylation of the hydroxy group in 2′ of the isoxazole **3**, probably due to the presence of the cyanobutoxy substituent in 4′, is more difficult with respect to the previous series having a methoxy group in 4′; in the present case, there is the formation of many by-products and, consequently, the yield is low. In this context, the use of methyl iodide, instead of dimethyl sulfate, does not achieve any significant improvement in yield (data not shown). When the cyano group is replaced with the oxazoline ring, as in the isoxazole **9**, the methylation occurs in a more favorable yield: in this case methyl iodide gives a slightly better yield with respect to dimethyl sulfate.

The acylation of hydroxy group in 2′ also gives poor results: thus, the benzoylation reaction to form the derivative **4** occurs under strong conditions and low yield (with respect to those acylations reported in [2]) while in the acetylation of **6**, following the reaction conditions used by us, the hydroxy group does not react.

In Table 1, the effect of the synthesized chromones (**2** and **8**) and isoxazoles (**3**, **4**, **5**, **6**, **7**, **9** and **10**) on the multiplication of HRV-14 is reported, this effect being a marker of a type A antirhinovirus activity [1,3]. The biological tests were performed as described in [2]. With regard to antirhinovirus properties, chromones are not toxic but are void of activity. Isoxazoles are ineffective and also very toxic. In this context, the Disoxaril-related isoxazole **10** is the most toxic. Hence, the aim of finding derivatives capable of covering both type A and type B antirhinovirus activity, utilizing a unique 3-(diethylamino)-5-phenylisoxazole moiety was unsuccessful. Furthermore, the synthesized compounds do not present significant activity against HIV-1 (data not shown).

Table 1
Effect of compounds **2**–**10** on the multiplication of HRV-14

Comp.	CC_{50} [a] (μM)	EC_{50} [b] (μM)
	MT-4	HRV-14
2	>200	>200
3	5.6	>5.6
4	5	>5
5	5.8	>5.8
6	35	>35
7	22	>22
8	>200	>200
9	34	>34
10	4.4	>4.4

[a] CC_{50}, compound concentration required to reduce the viability of mock-infected MT-4 by 50% (at 37°C).

[b] EC_{50}, compound concentration required to achieve 50% protection of MT-4 cells against the cytopathic effect of HRV-14 (at 33°C).

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