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Antiviral activity of indole derivatives

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ABSTRACT

Unsymmetrical methylene derivatives **5** were prepared following a known method, by reaction of the Mannich bases of 2-naphthols **4** with indoles. All synthesized compounds were tested against a wide panel of viruses, since previous work showed that Mannich bases on 7-hydroxycoumarin **1** and unsymmetrical methylene derivatives **2** were endowed with some antiviral activities. The symmetrical Mannich bases **4** were completely inactive, whereas the unsymmetrical methylene derivatives **5**, although possessing a certain degree of toxicity, showed a significant activity against RSV. Some of compounds **5** showed a moderate antiviral activity against HIV-1, BVDV, YFV and CVB-2. The lack of activity of Mannich bases **4** demonstrates the crucial importance for antiviral activity of coumarin moiety present in Mannich bases **1**.

1. Introduction

Many of the emerging infections in humans and animals are caused by RNA viruses. As part of our antiviral research program, we have recently synthesized and tested *in vitro* a series of coumarin derivatives, such as the Mannich bases **1** and the compounds **2** (Fig. 1) characterized by the presence of two heterocycle moieties, namely the indole and the coumarin, linked by a methylene bridge (Giampieri et al., 2007; Mazzei et al., 2008). Compounds **1** and **2** were tested, for cytotoxicity and antiviral activity, against viruses representative of two of the three genera of the Flaviviridae family, i.e. Flaviviruses (yellow fever virus, YFV) and Pestiviruses (bovine viral diarrhea virus, BVDV), as hepaciviruses can hardly be used in routine cell-based assays. Compounds **1** and **2** were also tested against representatives of other virus families, particularly respiratory syncytial virus (RSV). Briefly, when the amino substituent in compounds **1** was piperidine, morpholine, N-methylpiperazine, tetrahydroisoquinoline, etc., the antiviral activity was very poor, but when the amino substituent was piperazine, 2,5-dimethylpiperazine, N,N′-dimethylethylenediamine, etc., forming a double Mannich base, activity against BVDV was noted. In this case, the best activity was found for the piperazine derivative 1,4-bis[(7-hydroxycoumarin-8-yl)methyl]piperazine (NM14), whose antiviral data against BVDV are reported in Table 1A. The indole derivatives **2** were in general quite toxic and endowed with minor activity against BVDV, YFV and RSV [see, for instance, 3-(4′-methyl-7′-propionyloxycoumarin-8′-yl)methyl-2-phenylindole (**7l**) in Tables 1A and 1B].

In this paper we take the opportunity to produce the unsymmetrical methylene derivatives **5** (similar to compounds **2**) changing the portion derived from coumarin into a naphthalene ring, with the hope of reducing toxicity and increasing the antiviral activity. In the meantime, we have the opportunity of testing some symmetrical Mannich bases **4**, strictly related to compound NM14. The synthesized compounds were tested against a panel of several viruses, as specified in Section 5. The resulting data revealed poor anti-Flaviviridae activity of Mannich bases **4**, but the unsymmetrical methylene derivatives **5** showed a slightly minor toxicity in comparison with indoles **2**, a significant activity against BVDV and YFV, a moderate activity against HIV-1 and CVB-2, but, importantly, an interesting activity against RSV emerged.

2. Chemistry

The reaction pathway to synthesize unsymmetrical methylene derivatives (for instance A–CH_2–B) was already described in previous work (Mazzei et al., 2001a,b,c). Briefly, the A moiety of the above formula (in our case the 2-naphthol derivative, see Fig. 1) must be subjected to Mannich reaction and the corresponding Mannich base is then treated, in acetic anhydride, with a second molecule (the B

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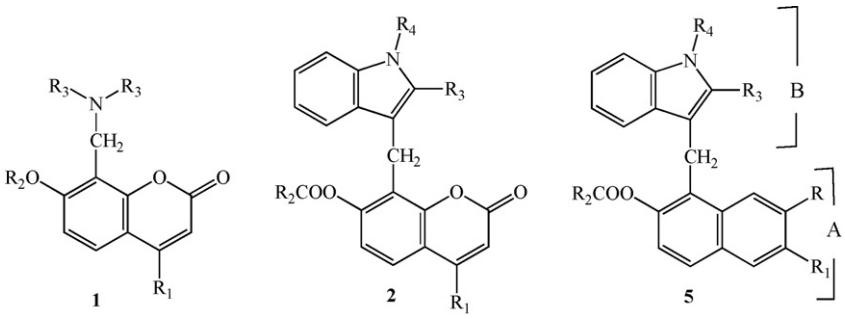


Fig. 1. Major substituents for compounds **1** (from Ref. Mazzei et al., 2008): R_1 = H, methyl; R_2 = benzoyl, *n*-propyl; $N(R_3)_2$ = piperidinyl, morpholinyl, N-methylpiperazinyl, 4-(ethoxycarbonyl)piperazinyl. Major substituents for compounds **2** (from Ref. Mazzei et al., 2008): R_1 = H, methyl; R_2 = methyl, ethyl; R_3 = H, methyl, phenyl; R_4 = H, methyl. *Substituents for reference compounds*: NM14 (from Ref. Giampieri et al., 2007): **1**, R_1 = H; R_2 = H; $N(R_3)_2$ = 4-[(7-hydroxycoumarin-8-yl)methyl]piperazin-1-yl; **7l** (from Ref. Mazzei et al., 2008): **2**, R_1 = methyl, R_2 = ethyl, R_3 = phenyl, R_4 = H.

moiety in Fig. 1, in our case an indole derivative) itself capable of forming a Mannich base.

Sometimes, the aminomethyl substituent can be inserted, with no difference for our synthetic purpose, on one of the two compounds (A or B): such a case was studied by Mazzei et al. (2001b). On the contrary, in the present case, the only way to carry on the reaction is depicted in Scheme 1. This choice was compelled on the basis of the following motivation: if we suppose to operate in an opposite manner, the first step, namely the Mannich base on indole, is easily obtained. But when this Mannich base reacts with the 2-naphthol

Table 1A
Antiviral activity of compounds **4** and **5**.

Compds	MT-4[a] CC_{50} (μM)	HIV-1[b] EC_{50} (μM)	SI	MDBK[c] CC_{50} (μM)	BVDV[d] EC_{50} (μM)	SI	BHK-21[e] CC_{50} (μM)	YFV[f] EC_{50} (μM)	SI	Reo-1[f] EC_{50} (μM)	SI
4a	>100	>100	NA	>100	>100	NA	>100	>100	NA	>100	NA
4b	>100	>100	NA	>100	>100	NA	>100	>100	NA	>100	NA
4c	>100	>100	NA	>100	>100	NA	>100	>100	NA	>100	NA
4d	>100	>100	NA	>100	>100	NA	>100	>100	NA	>100	NA
5a	30	>30	<1	53	3.7	14	23	>23	<1	>23	<1
5b	50	27	2	20	5	4	57	>57	<1	>57	<1
5c	19	>19	<1	12	1.6	8	19	>19	<1	>19	<1
5d	22	11	2	24	7.8	3	19	>19	<1	>19	<1
5e	19	5	4	24	7	3	21	>21	<1	>21	<1
5f	18	>18	<1	22	5	4	19	6.4	3	>19	<1
5g	18	>18	<1	45	4.4	10	19	>19	<1	>19	<1
5h	19	>19	<1	18	1.8	10	6	>6	<1	>6	<1
5i	19	9	2	17	3.6	5	18	2.3	8	>18	<1
5j	34	28	1	48	4.4	11	19	5	4	>19	<1
5k	19	>19	<1	18	4.2	4	19	10	2	>19	<1
5l	16	12	1	11	>11	<1	20	5	4	>20	<1
5m	8	>8	<1	6.6	1.2	6	6.5	≤3.7	2	>6.5	<1
5n	19	7	3	10	1.9	5	19	>19	<1	>19	<1
5o	20	>20	<1	18	4.7	4	19	5	4	>19	<1
5p	55	27	2	22	6	4	47	>47	<1	>47	<1
5q	48	15	3	22	5.6	4	19	7	3	>19	<1
5r	15	>15	<1	11	1.1	10	18	4.2	4	>18	<1
5s	30	8	4	16	2.8	6	20	5	4	>20	<1
5t	47	19	2	26	5	5	19	>19	<1	>19	<1
Reference compds											
NM14	20	>20	<1	>100	1	>100	>100	>100	NA	>100	NA
7l	38	>38	<1	25	7	3	45	>45	<1	>45	<1
3′-Azido-thymidine	50	0.01	5000								
2′-C-Methyl-guanosine	>100	>100	NA	>100	1.8	>56	>100	2.5	>40		
2′-C-Ethynyl-cytidine	≥100	>100	NA	>100	57	>2	>100	>100	NA	>100	NA
Ribavirin	31	>31	<1	100	4	25	>100	50	>2	>100	NA
6-Azauridine	0.2	>0.2	<1	25	>25	<1	>100	26	>4	>100	NA
Mycophenolic acid	0.2	>0.2	<1				>100			>100	NA
Acyclovir				>100	>100	NA	>100	>100	NA	>100	NA

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%. NA: not applicable.

[a] Compd. concn. (μM) required to reduce the viability of mock-infected MT-4 ($CD4^+$ human T-cells containing an integrated HTLV-1 genome) cells by 50%, as determined by the MTT method.

[b] Compound concentration (μM) required to achieve 50% protection of MT-4 cells from the HIV-1-induced cytopathogenicity, as determined by the MTT method.

[c] Compound concentration (μM) required to reduce the viability of mock-infected MDBK (Madin–Darby bovine normal kidney) monolayers by 50%, as determined by the MTT method.

[d] Compound concentration (μM) required to achieve 50% protection of MDBK cells from the BVDV (bovine viral diarrhea virus) induced cytopathogenicity, as determined by the MTT method.

[e] Compound concentration (μM) required to reduce the viability of mock-infected BHK (baby hamster kidney fibroblast) monolayers by 50%, as determined by the MTT method.

[f] Compound concentration (μM) required to achieve 50% protection of BHK from the YFV (yellow fever virus) and Reo-1 (reo virus 1), induced cytopathogenicity, as determined by the MTT method.

Table 1B
Antiviral activity of compounds **4** and **5**.

Compds	VERO-76[a] CC_{50} (μM)	HSV-1[b] EC_{50} (μM)	SI	VV[b] EC_{50} (μM)	SI	CVB-2[b] EC_{50} (μM)	SI	Sb-1[b] EC_{50} (μM)	SI	RSV[b] EC_{50} (μM)	SI	VSV[b] EC_{50} (μM)	SI
4a	>100	>100	NA	>100	NA	>100	NA	>100	NA	>100	NA	>100	NA
4b	>100	>100	NA	>100	NA	>100	NA	>100	NA	>100	NA	>100	NA
4c	>100	>100	NA	>100	NA	>100	NA	>100	NA	>100	NA	>100	NA
4d	>100	>100	NA	>100	NA	>100	NA	>100	NA	>100	NA	>100	NA
5a	65	>65	<1	>65	<1	>65	<1	>65	<1	7	9	>65	<1
5b	40	>40	<1	>40	<1	18	2	>40	<1	5	8	>40	<1
5c	10	>10	<1	>10	<1	>10	<1	>10	<1	>10	<1	>10	<1
5d	20	>20	<1	>20	<1	>20	<1	>20	<1	>20	<1	>20	<1
5e	20	>20	<1	>20	<1	>20	<1	>20	<1	7	3	>20	<1
5f	30	>30	<1	>30	<1	10	3	>30	<1	>30	<1	>30	<1
5g	40	>40	<1	>40	<1	>40	<1	>40	<1	2	20	>40	<1
5h	40	>40	<1	>40	<1	>40	<1	>40	<1	5	8	>40	<1
5i	25	>25	<1	>25	<1	9	3	>25	<1	4	6	>25	<1
5j	60	>60	<1	>60	<1	19	3	>60	<1	3	20	>60	<1
5k	15	>15	<1	>15	<1	>15	<1	>15	<1	4	4	>15	<1
5l	15	>15	<1	>15	<1	>15	<1	>15	<1	2	8	>15	<1
5m	30	>30	<1	>30	<1	>30	<1	>30	<1	1	30	>30	<1
5n	15	>15	<1	>15	<1	>15	<1	>15	<1	1.5	10	>15	<1
5o	15	>15	<1	>15	<1	>15	<1	>15	<1	1	15	>15	<1
5p	25	>25	<1	>25	<1	7	4	>25	<1	4	6	>25	<1
5q	20	>20	<1	>20	<1	6	3	>20	<1	0.5	40	>20	<1
5r	25	>25	<1	>25	<1	10	3	>25	<1	4	6	>25	<1
5s	15	>15	<1	>15	<1	>15	<1	>15	<1	0.7	21	>15	<1
5t	20	>20	<1	>20	<1	9	2	>20	<1	>20	<1	>20	<1
Reference compds													
NM14	>100	>100	NA	>100	NA	>100	NA	>100	NA	>100	NA	>100	NA
7l	>100	>100	NA	>100	NA	>100	NA	>100	NA	>100	NA	>100	NA
3′-Azido-thymidine													
2′-C-Methyl-guanosine	>100	>100	NA	>100	NA	39	>3	58	>2	>100	NA	>100	NA
2′-C-Ethynyl-cytidine	>100	>100	NA	>100	NA	23	>4	18	>6	>100	NA		
Ribavirin	>100					>100	NA	>100	NA			>100	NA
6-Azauridine	40	>40	<1	>40	<1	>40	<1	>40	<1	1.8	22	>40	<1
Mycophenolic acid	13	>13	<1	1.8	7	>13	<1	>13	<1	0.4	33		
Acyclovir	>100	3	>33	>100	NA	>100	NA	>100	NA	>100	NA	>100	NA

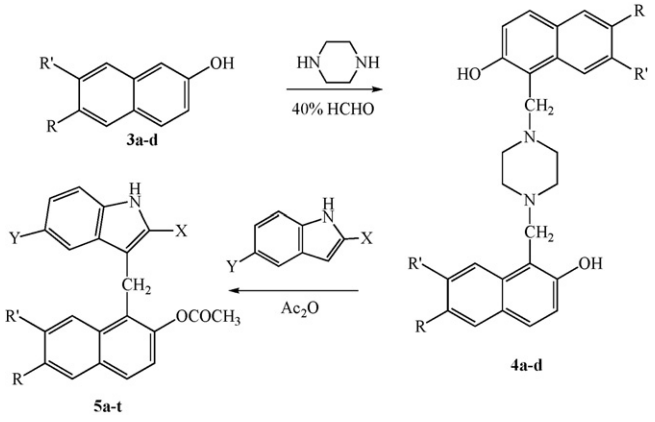
Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%. NA: not applicable.

[a] Compound concentration (μM) required to reduce the viability of mock-infected VERO 76 (monkey normal kidney) monolayers by 50%.

[b] Compound concentration (μM) required to reduce the plaque number of HSV-1 (herpes virus 1), VV (vaccinia virus), CVB-2 (Coxsackie virus B2), Sb-1 (polio virus 1), RSV (respiratory syncytial virus) and VSV (vesicular stomatitis virus) by 50% in VERO-76 monolayers.

in acetic anhydride, the reaction of acetylation occurs very quickly, and 2-acetylnaphthol is incapable of forming a Mannich base. Thus, the final product is the symmetrical methylene derivative of starting indole, obtained via partial deaminomethylation of the Mannich base (Mazzei et al., 2001b).

To obtain the unsymmetrical methylene derivatives **5**, the intermediate Mannich bases **4** can be formed using any secondary amine since the amine is no longer present in the final product. Generally, the choice depends on simple amines (e.g. piperidine, morpholine) possessing a good yield in the aminomethylation reaction. Instead, we utilized piperazine for several reasons. First, it is cheap and gives a high yield in the Mannich reaction. Secondly, piperazine gives Mannich bases which tend to precipitate in the reaction medium, making the separation of the final product easier (note that piperidino- or morpholinomethyl derivatives provide a good yield but the separation from unreacted starting material may be difficult and/or time consuming). Third, the intermediate piperazine derivatives **4** are structurally very similar to other Mannich bases with significant activity against BVDV (see Section 6).



Scheme 1.

All synthesized compounds **4** and **5** are white crystals and their structures are in agreement with elemental analyses and spectral data.

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 ±0.3% for C and ±0.1% for H and N of the theoretical value. ^{1}H NMR and ^{13}C NMR spectra were performed on a Varian Gemini 200 (200 MHz) spectrometer using tetramethylsilane (TMS) as internal standard ($\delta = 0$). IR spectra were recorded on a Perkin-Elmer 398 Spectrophotometer. GC–MS spectra were performed with HP 6890-5973. GC parameters: injector temperature 250 °C; capillary column HP5 poly(methylphenylsiloxane) 30 m, 0.35 mm, 0.25 μm; temperature program: from 100 to 300 °C at 10 °C/min. MS parameters: mode SCAN 40–600 amu; t_r = retention time.

3.1. Mannich bases **4a–d**

To 20.0 mmol of appropriate 2-naphthol (**3a–d**, as below specified) dissolved in 20 ml of ethanol, 20.0 mmol of piperazine and 2.0 ml of 40% formaldehyde were added. The resulting mixture was refluxed for 4–6 h. After cooling, the solvent was evaporated under reduced pressure. The pale yellow oil obtained was treated with cooled acetone, leaving a white solid crystallized from suitable solvent obtaining the following compounds.

3.1.1. 1,4-Bis[(2-hydroxynaphthalen-1-yl)methyl]piperazine (**4a**)

(A) 2-Naphthol **3a**. Crystallized from dichloromethane/DMF, m.p. 246–248 °C, yield 78.4%.

IR (KBr) ν (cm^{-1}): 2880, 2834, 1622, 1586, 4167, 1264.

^1H NMR ($CDCl_3$) δ: 3.80–4.25 (m, 8H, N–CH_2CH_2–N), 4.97 (s, 4H, CH_2-bridge), 7.13–8.05 (m, 12H, H arom.).

3.1.2. 1,4-Bis[(2-hydroxy-6-methoxynaphthalen-1-yl)methyl] piperazine (**4b**)

(A) 6-Methoxy-2-naphthol **3b**. Crystallized from dichloromethane/DMF, m.p. 259–261 °C, yield 68.2%.

IR (KBr) ν (cm^{-1}): 2973, 2944, 2890, 2845, 1602, 1457, 1241.

^1H NMR ($CDCl_3$) δ: 3.90–4.20 (m, 14H, N–CH_2CH_2–N, CH_3O), 5.07 (s, 4H, CH_2-bridge), 7.20–8.05 (m, 10H, H arom.).

3.1.3. 1,4-Bis[(6-bromo-2-hydroxynaphthalen-1-yl)methyl] piperazine (**4c**)

(A) 6-Bromo-2-naphthol **3c**. Crystallized from dichloromethane/DMF, m.p. 256–257 °C, yield 73.8%.

IR (KBr) ν (cm^{-1}): 3050, 2886, 2832, 1619, 1589, 1465, 1241.

^1H NMR ($CDCl_3$) δ: 3.90–4.20 (m, 8H, N–CH_2CH_2–N), 5.05 (s, 4H, CH_2-bridge), 7.22–8.10 (m, 10H, H arom.).

3.1.4. 1,4-Bis[(2-hydroxy-7-methoxynaphthalen-1-yl)methyl] piperazine (**4d**)

(A) 7-Methoxy-2-naphthol **3d**. Crystallized from dichloromethane/DMF, m.p. 246–248 °C, yield 79.5%.

IR (KBr) ν (cm^{-1}): 2965, 2882, 2836, 1623, 1447, 1224.

Insoluble in all deuterated solvents.

3.2. Unsymmetrical methylene derivatives 5a-t

To 5 g of acetic anhydride, 2.0 mmol of suitable indole (as specified below) were added, then 2.0 mmol of Mannich bases **4** (as specified below) were added and the solution was heated at 95 °C for 1.5 h. After cooling, the solution was poured onto crushed ice and the mixture was stirred for 1–2 h. The precipitate was filtered off and the solid was crystallized from the appropriate solvent. The following compounds were obtained.

3.2.1. 3-(2-Acetoxynaphthalen-1-yl)methylindole (**5a**)

From **4a** and indole. Crystallized from ethanol, m.p. 70–72 °C, yield 57.4%.

IR (KBr) ν (cm^{-1}): 3405, 3053, 1709, 1514, 1202.

^1H NMR ($CDCl_3$) δ: 2.07 (s, 3H, CH_3CO), 4.47 (s, 2H, CH_2-bridge), 6.1 (s, 1H, H-2), 6.70–7.97 (m, 10H, H arom.).

m/*z* (t_r = 6.29): 315 (M, 17%)$^+$, 207 (M−108, 15%)$^+$, 117 (M−198, 100%)$^+$, 43 (M−272, 66%)$^+$.

UV: λ_{max} 254 nm, ε 2300 L mol^{-1} cm^{-1}.

3.2.2. 3-(2-Acetoxynaphthalen-1-yl)methyl-2-methylindole (**5b**)

From **4a** and 2-methylindole. Crystallized from ethanol, m.p. 178–180 °C, yield 51.2%.

IR (KBr) ν (cm^{-1}): 3357, 3059, 1734, 1514, 1210.

^1H NMR ($CDCl_3$) δ: 1.92 (s, 3H, 2-CH_3), 2.12 (s, 3H, CH_3CO), 4.42 (s, 2H, CH_2-bridge), 7.07–7.95 (m, 10H, H arom.).

^{13}C NMR ($CDCl_3$) δ: 11.2 (1C, CH_3), 20.5 (1C, CH_2-bridge), 20.9 (1C, CH_3–CO), 108.5 (1C, CH), 109.3 (1C, C), 117.3 (1C, CH), 118.5 (1C, CH), 120.2 (1C, CH), 120.9 (1C, CH), 123.8 (1C, CH), 124.5 (1C, C), 125.6 (1C, CH), 126.3 (1C, CH), 127.2 (1C, CH), 127.8 (1C, CH), 128.0 (1C, C), 130.5 (1C, C), 131.2 (1C, C), 132.5 (1C, C), 134.1 (1C, C), 145.9 (1C, C–O), 169.0 (1C, CO).

UV: λ_{max} 275 nm, ε 2600 L mol^{-1} cm^{-1}.

3.2.3. 3-(2-Acetoxynaphthalen-1-yl) methyl-2-phenylindole (**5c**)

From **4a** and 2-phenylindole. Crystallized from ethanol/ethyl acetate, m.p. 136–137 °C, yield 45.3%.

IR (KBr) ν (cm^{-1}): 3377, 3054, 2907, 1731, 1515, 1210.

^1H NMR ($CDCl_3$) δ: 2.15 (s, 3H, CH_3CO), 4.55 (s, 2H, CH_2-bridge), 6.91–7.85 (m, 15H, H arom.).

^{13}C NMR (DMSO) δ: 20.7 (1C, CH_2-bridge), 21.0 (1C, CH_3–CO), 111.0 (1C, CH), 112.7 (1C, C), 113.3 (1C, CH), 120.7 (1C, CH), 122.1 (1C, CH), 123.3 (1C, CH), 124.5 (1C, C), 124.6 (1C, CH), 125.3 (1C, C), 126.4 (1C, CH), 126.9 (1C, CH), 127.6 (2C, CH), 128.3 (1C, CH), 128.5 (1C, CH), 128.6 (1C, CH), 131.5 (2C, CH), 132.7 (1C, C), 134.3 (1C, C), 134.6 (1C, C), 136.5 (1C, C), 136.9 (1C, C), 146.0 (1C, C–O), 169.5 (1C, CO).

UV: λ_{max} 292 nm, ε 2700 L mol^{-1} cm^{-1}.

3.2.4. 3-(2-Acetoxynaphthalen-1-yl) methyl-5-bromoindole (**5d**)

From **4a** and 5-bromoindole. Crystallized from ethanol, m.p. 219–221 °C, yield 42.6%.

IR (KBr) ν (cm^{-1}): 3385, 3064, 2941, 1739, 1514, 1208.

^1H NMR ($CDCl_3$) δ: 2.17 (s, 3H, CH_3CO), 4.31 (s, 2H, CH_2-bridge), 6.50 (s, 1H, H-2), 7.11–7.96 (m, 9H, H arom.).

^{13}C NMR ($CDCl_3$) δ: 20.1 (1C, CH_3–CO), 21.8 (1C, CH_2-bridge), 109.8 (1C, C), 110.4 (1C, C), 118.9 (1C, C), 119.1 (1C, CH), 120.7 (1C, CH), 121.4 (1C, CH), 124.0 (1C, CH), 124.3 (1C, CH), 125.4 (1C, C), 126.1 (1C, CH), 127.2 (1C, CH), 127.4 (1C, CH), 127.7 (1C, C), 131.3 (1C, C), 132.4 (1C, C), 134.0 (1C, C), 135.1 (1C, C), 145.6 (1C, C–O), 169.1 (1C, CO).

UV: λ_{max} 288 nm, ε 2200 L mol^{-1} cm^{-1}.

3.2.5. 3-(2-Acetoxynaphthalen-1-yl)methyl-5-methoxyindole (**5e**)

From **4a** and 5-methoxyindole. Crystallized from ethanol, m.p. 166–168 °C, yield 47.5%.

IR (KBr) ν (cm^{-1}): 3408, 2940, 2828, 1741, 1213.

^1H NMR ($CDCl_3$) δ: 2.21 (s, 3H, CH_3CO), 3.95 (s, 3H, CH_3O), 4.45 (s, 2H, CH_2-bridge), 6.42 (s, 1H, H-2), 7.1–7.98 (m, 9 H, H arom.).

m/*z* (t_r = 28.59): 345 (M, 58%)$^+$, 302 (M−43, 50%)$^+$, 189 (M−156, 34%)$^+$, 147 (M−198, 100%)$^+$, 132 (M−211, 45%)$^+$, 43 (M−302, 39%)$^+$.

UV: λ_{max} 274 nm, ε 2700 L mol^{-1} cm^{-1}.

3.2.6. 3-(2-Acetoxy-6-methoxynaphthalen-1-yl)methylindole (**5f**)

From **4b** and indole. Crystallized from ethanol, m.p. 168–170 °C, yield 39.6%.

IR (KBr) ν (cm^{-1}): 3370, 2959, 1754, 1512, 1207.

^1H NMR ($CDCl_3$) δ: 2.18 (s, 3H, CH_3CO), 3.98 (s, 3H, CH_3O), 4.44 (s, 2H, CH_2-bridge), 6.45 (s, 1H, H-2), 7.12–7.90 (m, 9H, H arom.).

m/*z* (t_r = 19.17): 345 (M, 55%)$^+$, 303 (M−42, 33%)$^+$, 186 (M−159, 22%)$^+$, 117 (M−228, 100%)$^+$, 43 (M−302, 11%)$^+$.

UV: λ_{max} 268 nm, ε 2400 L mol^{-1} cm^{-1}.

3.2.7. 3-(2-Acetoxy-6-methoxynaphthalen-1-yl)methyl-2-methylindole (**5g**)

From **4b** and 2-methylindole. Crystallized from petroleum ether, m.p. 105–110 °C, yield 56.9%.

IR (KBr) ν (cm^{-1}): 3390, 2935, 1749, 1513, 1203.

^1H NMR ($CDCl_3$) δ: 1.96 (s, 3H, 2-CH_3), 2.10 (s, 3H, CH_3CO), 3.85 (s, 3H, CH_3O), 4.40 (s, 2H, CH_2-bridge) 7.05–7.90 (m, 9H, H arom.).

^{13}C NMR (DMSO) δ: 11.4 (1C, CH_3), 20.6 (1C, CH_3–CO), 21.7 (1C, CH_2-bridge), 55.0 (1C, OCH_3), 106.7 (1C, CH), 107.9 (1C, CH), 110.1 (1C, C), 117.4 (1C, CH), 117.9 (1C, CH), 118.3 (1C, CH), 119.7 (1C, CH), 122.3 (1C, CH), 125.9 (1C, C), 126.3 (1C, CH), 127.0 (1C, CH), 127.7 (1C, C), 128.0 (1C, C), 131.4 (1C, C), 132.8 (1C, C), 134.9 (1C, C), 144.7 (1C, C–O), 156 (1C, C) 169.4 (1C, CO).

UV: λ_{max} 275 nm, ε 2700 L mol^{-1} cm^{-1}.

*3.2.8. 3-(2-Acetoxy-6-methoxynaphthalen-1-yl)methyl-2-phenylindole (**5h**)*

From **4b** and 2-phenylindole. Crystallized from ethanol, m.p. 168–170 °C, yield 45.0%.

IR (KBr) ν (cm^{-1}): 3381, 2904, 1730, 1515, 1209.

^{1}H NMR ($CDCl_3$) δ: 2.13 (s, 3H, CH_3CO), 3.78 (s, 3H, CH_3O), 4.53 (s, 2H, CH_2-bridge), 6.95–7.80 (m, 14H, H arom.).

m/*z* (t_r = 26.98): 421 (M, 17%)$^+$, 378 (M−43, 15%)$^+$, 193 (M−228, 100%)$^+$, 43 (M−378, 35%)$^+$.

UV: λ_{max} 287 nm, ε 2700 L mol^{-1} cm^{-1}.

*3.2.9. 3-(2-Acetoxy-6-methoxynaphthalen-1-yl)methyl-5-bromoindole (**5i**)*

From **4b** and 5-bromoindole. Crystallized from ethanol, m.p. 193–194 °C, yield 67.3%.

IR (KBr) ν (cm^{-1}): 3352, 2957, 1755, 1511, 1204.

1H NMR ($CDCl_3$) δ: 2.20 (s, 3H, CH_3CO), 3.95 (s, 3H, CH_3O), 4.32 (s, 2H, CH_2-bridge), 6.43 (s, 1H, H-2), 7.10–7.98 (m, 8H, H arom.).

m/*z* (t_r = 13,40): 315 (M−109, 14%)$^+$, 272 (M−152,14%)$^+$, 117 (M−307, 100%)$^+$, 43 (M−381, 57%)$^+$.

UV: λ_{max} 246 nm, ε 2500 L mol^{-1} cm^{-1}.

*3.2.10. 3-(2-Acetoxy-6-methoxynaphthalen-1-yl)methyl-5-methoxyindole (**5j**)*

From **4b** and 5-methoxyindole. Crystallized from ethanol, m.p. 85–87 °C, yield 46.3%.

IR (KBr) ν (cm^{-1}): 3386, 2998, 2926, 1755, 1513, 1203.

^{1}H NMR ($CDCl_3$) δ: 2.24 (s, 3H, CH_3CO), 3.95 (m, 6H, CH_3O), 4.40 (s, 2H, CH_2-bridge), 6.40–7.90 (m, 9H, H-2, H arom.).

^{13}C NMR (DMSO) δ: 20.6 (1C, CH_3–CO), 21.4 (1C, CH_2-bridge), 55.0 (2C, OCH_3), 100.2 (1C, CH), 106.7 (1C, CH), 110.9 (1C, CH), 112.0 (1C, C), 118.5 (1C, CH), 122.4 (1C, CH), 123.6 (1C, CH), 126.2 (1C, CH), 126.9 (1C, CH), 127.0 (1C, C), 127.2 (1C, C), 127.4 (1C, C), 131.1 (1C, C), 132.0 (1C, C), 133.1 (1C, C), 144.3 (1C, C–O), 152.9 (1C, C), 156.6 (1C, C), 169.4 (1C, CO).

UV: λ_{max} 239 nm, ε 2500 L mol^{-1} cm^{-1}.

*3.2.11. 3-(2-Acetoxy-6-bromonaphthalen-1-yl)methylindole (**5k**)*

From **4c** and indole. Crystallized from ethanol, m.p. 172–174 °C, yield 60.1%

IR (KBr) ν (cm^{-1}): 3372, 3049, 1753, 1214.

^{1}H NMR ($CDCl_3$) δ: 2.23 (s, 3H, CH_3CO) 4.41 (s, 2H, CH_2-bridge), 6.40 (s, 1H, H-2), 7.30–8.10 (m, 9H, H arom.).

^{13}C NMR (DMSO) δ: 20.6 (1C, CH_3–CO), 21.5 (1C, CH_2-bridge), 111.3 (1C, CH), 112.6 (1C, C), 118.2 (1C, C), 118.3 (1C, CH), 118.6 (1C, CH), 121.0 (1C, CH), 123.0 (1C, CH), 123.5 (1C, CH), 126.7 (1C, CH), 126.8 (1C, CH), 127.0 (1C, CH), 127.6 (1C, CH), 129.2 (1C, C), 130.1 (1C, CH), 131.0 (1C, C), 132.9 (1C, C), 136.0 (1C, C), 146.1 (1C, C–O), 169.4 (1C, CO).

UV: λ_{max} 272 nm, ε 2400 L mol^{-1} cm^{-1}.

*3.2.12. 3-(2-Acetoxy-6-bromonaphthalen-1-yl)methyl-2-methylindole (**5l**)*

From **4c** and 2-methylindole. Crystallized from ethanol, m.p. 198–200 °C, yield 43.5%.

IR (KBr) ν (cm^{-1}): 3382, 3026, 2916, 1728, 1208.

^{1}H NMR ($CDCl_3$) δ: 1.93 (s, 3H, 2-CH_3), 2.10 (s, 3H, CH_3CO), 4.31 (s, 2H, CH_2-bridge), 6.92–7.93 (m, 9H, H arom.).

^{13}C NMR (DMSO) δ: 11.4 (1C, CH), 20.6 (1C, CH_3–CO), 21.0 (1C, CH_2-bridge), 107.6 (1C, CH), 110.2 (1C, C), 117.3 (1C, CH), 118.0 (1C, CH), 118.5 (1C, CH), 119.8 (1C, CH), 123.4 (1C, CH), 126.7 (1C, CH), 126.8 (1C, CH), 127.6 (1C, CH), 127.8 (1C, CH), 129.1 (1C, C), 130.1 (1C, CH), 131.2 (1C, C), 131.5 (1C, C), 132.8 (1C, C), 134.9 (1C, C), 146.7 (1C, C–O), 169.2 (1C, CO).

UV: λ_{max} 286 nm, ε 2600 L mol^{-1} cm^{-1}.

*3.2.13. 3-(2-Acetoxy-6-bromonaphthalen-1-yl)methyl-2-phenylindole (**5m**)*

From **4c** and 2-phenylindole. Crystallized from ethanol, m.p. 237–238 °C, yield 63.4%.

IR (KBr) ν (cm^{-1}): 3359, 3059, 1725, 1210.

^{1}H NMR ($CDCl_3$) δ: 2.20 (s, 3H, CH_3CO), 4.57 (s, 2H, CH_2-bridge), 6.80–8.20 (m, 14H, H arom.).

m/*z* (t_r = 23.06): 423 (M−47, 10%)$^+$, 381 (M−89, 12%)$^+$, 186 (M−284, 90%)$^+$, 116 (M−354, 20%)$^+$, 43 (M−427, 100%)$^+$.

UV: λ_{max} 288 nm, ε 2400 L mol^{-1} cm^{-1}.

*3.2.14. 3-(2-Acetoxy-6-bromonaphthalen-1-yl)methyl-5-bromoindole (**5n**)*

From **4c** and 5-bromoindole. Crystallized from ethanol, m.p. 222–224 °C, yield 67.7%.

IR (KBr) ν (cm^{-1}): 3357, 1758, 1213.

^{1}H NMR ($CDCl_3$) δ: 2.33 (s, 3H, CH_3CO), 4.41 (s, 2H, CH_2-bridge), 6.80 (s, 1H, H-2), 7.25–8.30 (m, 8H, H arom.).

^{13}C NMR (DMSO) δ: 20.7 (1C, CH_3–CO), 21.0 (1C, CH_2-bridge), 111.0 (1C, C), 112.5 (1C, CH), 113.3 (1C, C), 118.6 (1C, C), 120.7 (1C, CH), 123.4 (1C, CH), 123.5 (1C, CH), 124.6 (1C, CH), 127.0 (1C, CH), 127.3 (1C, CH), 128.5 (1C, CH), 129.3 (1C, CH), 130.1 (1C, C), 130.8 (1C, CH), 132.9 (1C, C), 134.6 (1C, C), 135.5 (1C,C) 146.4 (1C, C–O), 169.4 (1C, CO).

UV: λ_{max} 287 nm, ε 2300 L mol^{-1} cm^{-1}.

*3.2.15. 3-(2-Acetoxy-6-bromonaphthalen-1-yl)methyl-5-methoxyindole (**5o**)*

From **4c** and 5-methoxyindole. Crystallized from ethanol, m.p. 162–164 °C, yield 59.0%.

IR (KBr) ν (cm^{-1}): 3384, 3080, 2949, 1744, 1213.

^{1}H NMR ($CDCl_3$) δ: 2.11 (s, 3H, CH_3CO), 3.84 (s, 3H, CH_3O), 4.30 (s, 2H, CH_2-bridge), 6.35 (s, 1H, H-2), 7.08–7.96 (m, 8H, H arom.).

^{13}C NMR (DMSO) δ: 20.7 (1C, CH_3–CO), 21.3 (1C, CH_2-bridge), 55.1 (1C, OCH_3), 100.1 (1C, CH), 111.0 (1C,CH), 111.9 (1C, CH), 112.3 (1C, C), 118.5 (1C, C), 123.6 (1C, CH), 126.7 (1C, CH), 126.9 (1C, CH), 127.1 (1C, CH), 127.7 (1C, C), 129.2 (1C, CH), 130.1 (1C, C), 130.9 (1C, CH), 131.1 (1C, C), 132.9 (1C, C), 146.4 (1C, C–O), 152.9 (1C, C), 169.4 (1C, CO).

UV: λ_{max} 267 nm, ε 2900 L mol^{-1} cm^{-1}.

*3.2.16. 3-(2-Acetoxy-7-methoxynaphthalen-1-yl)methylindole (**5p**)*

From **4d** and indole. Crystallized from ethanol, m.p. 165–167 °C, yield 56.0%.

IR (KBr) ν (cm^{-1}): 3442, 2936, 1752, 1512, 1216.

^{1}H NMR ($CDCl_3$) δ: 2.17 (s, 3H, CH_3CO), 3.57 (s, 3H, CH_3O), 4.34 (s, 2H, CH_2-bridge), 6.38 (s, 1H, H-2), 7.03–7.95 (m, 9H, H arom.).

m/*z* (t_r = 19.69): 345 (M, 15%), 302 (M−43, 16%), 186 (M−159, 11%), 130 (M−215, 11%)$^+$, 117 (M−228, 100%)$^+$, 43 (M−302, 43%)$^+$.

UV: λ_{max} 277 nm, ε 2500 L mol^{-1} cm^{-1}.

*3.2.17. 3-(2-Acetoxy-7-methoxynaphthalen-1-yl)methyl-2-methylindole (**5q**)*

From **4d** and 2-methylindole. Crystallized from ethanol, m.p. 167–168 °C, yield 59.5%.

IR (KBr) ν (cm^{-1}): 3384, 3055, 2947, 1751, 1511, 1201.

^{1}H NMR ($CDCl_3$) δ: 1.98 (s, 3H, 2-CH_3), 2.17 (s, 3H, CH_3CO), 3.48 (s, 3H, CH_3O), 4.3 (s, 2H, CH_2-bridge), 6.90–7.66 (m, 9H, H arom.).

^{13}C NMR (DMSO) δ: 11.4 (1C, CH_3), 20.6 (1C, CH_3–CO), 21.0 (1C, CH_2-bridge), 54.8 (1C, OCH_3), 103.7 (1C, CH), 107.9 (1C, CH), 110.2 (1C, C), 117.2 (1C, CH), 117.4 (1C, CH), 118.0 (1C, CH), 119.3 (1C, CH), 119.8 (1C, CH), 125.9 (1C, CH), 126.7 (1C, C), 127.2 (1C, CH), 128.1 (1C, CH), 129.8 (1C, C), 131.7 (1C, C), 133.8 (1C, C), 134.9 (1C, C), 146.7 (1C, C–O), 157.1 (1C, C), 169.3 (1C, CO).

UV: λ_{max} 285 nm, ε 2500 L mol^{-1} cm^{-1}.

*3.2.18. 3-(2-Acetoxy-7-methoxynaphthalen-1-yl)methyl-2-phenylindole (**5r**)*

From **4d** and 2-phenylindole. Crystallized from ethanol, m.p. 235–237 °C, yield 36.5%.

IR (KBr) ν (cm^{-1}): 3321, 2934, 1739, 1513, 1234.

^{1}H NMR ($CDCl_3$) δ: 2.22 (s, 3H, CH_3CO), 3.30 (s, 3H, CH_3O), 4.55 (s, 2H, CH_2-bridge), 6.90–7.60 (m, 14H, H arom.).

m/z (t_r = 24.89): 421 (M, 12%)$^+$, 378 (M−43, 12%)$^+$, 193 (M−228, 100%)$^+$, 43 (M−378, 42%)$^+$.

UV: λ_{max} 302 nm, ε 2600 L mol^{-1} cm^{-1}.

*3.2.19. 3-(2-Acetoxy-7-methoxynaphthalen-1-yl)methyl-5-bromoindole (**5s**)*

From **4d** and 5-bromoindole. Crystallized from ethanol, m.p. 180–181 °C, yield 42.4%.

IR (KBr) ν (cm^{-1}): 3363, 3077, 2952, 1758, 1511, 1201.

^{1}H NMR ($CDCl_3$) δ: 2.21 (s, 3H, CH_3CO), 3.61 (s, 3H, CH_3O), 4.26 (s, 2H, CH_2-bridge), 6.47 (s, 1H, H-2), 7.00–7.90 (m, 8H, H arom.).

^{13}C NMR (DMSO) δ: 20.7 (1C, CH_3–CO), 20.9 (1C, CH_2-bridge), 55.0 (1C, OCH_3), 103.8 (1C, CH), 111.0 (1C, C), 112.6 (1C, CH), 113.4 (1C, C), 117.3 (1C, CH), 119.5 (1C, CH), 120.7 (1C, CH), 123.3 (1C, CH), 124.8 (1C, CH), 126.3 (1C, CH), 126.8 (1C, C), 127.2 (1C, CH), 128.7 (1C, CH), 129.9 (1C, C), 133.6 (1C, C), 134.5 (1C, C), 146.5 (1C, C–O), 157.4 (1C, C), 169.5 (1C, CO).

UV: λ_{max} 271 nm, ε 2500 L mol^{-1} cm^{-1}.

*3.2.20. 3-(2-Acetoxy-7-methoxynaphthalen-1-yl)methyl-5-methoxyindole (**5t**)*

From **4d** and 5-methoxyindole. Crystallized from ethanol, m.p. 149–150 °C, yield 47.9%.

IR (KBr) ν (cm^{-1}): 3418, 2938, 2834, 1753, 1510, 1214.

^{1}H NMR ($CDCl_3$) δ: 2.20 (s, 3H, CH_3CO), 3.60 (s, 3H, CH_3O), 3.83 (s, 3H, CH_3O), 4.28 (s, 2H, CH_2-bridge), 6.47 (s, 1H, H-2), 7.03–7.70 (m, 8H, H arom.).

m/z (t_r = 20.60): 375 (M, 15%)$^+$, 332 (M−43, 16%)$^+$, 189 (M−186, 15%)$^+$, 147 (M−228, 100%)$^+$, 132 (M−243, 17%)$^+$, 43 (M−332, 90%)$^+$.

UV: λ_{max} 286 nm, ε 2400 L mol^{-1} cm^{-1}.

4. Biology

4.1. Cell-based assays

4.1.1. Compounds

Compounds were dissolved in DMSO at 100 mM and then diluted in culture medium to results, for the microbiological assays, concentrations starting from 100 to 0.05 μM.

4.1.2. Cells and viruses

Cell lines were purchased from American Type Culture Collection (ATCC). The absence of mycoplasma contamination was checked periodically by the Hoechst staining method. Cell lines supporting the multiplication of RNA viruses were the following: CD4$^+$ human T-cells containing an integrated HTLV-1 genome (MT-4); Madin–Darby bovine kidney (MDBK); baby hamster kidney (BHK-21); Monkey kidney (Vero 76) cells.

4.1.3. Cytotoxicity assays

For cytotoxicity tests, run in parallel with antiviral assays, MDBK, BHK and Vero 76 cells were resuspended in 96-multiwell plates at an initial density of 6×10^5, 1×10^6 and 5×10^5 cells/mL, respectively, in maintenance medium, without or with serial dilutions of test compounds. Cell viability was determined after 48–120 h at 37 °C in a humidified CO_2 (5%) atmosphere by the MTT method. The cell number of Vero 76 monolayers was determined by staining with the crystal violet dye.

For cytotoxicity evaluations, exponentially growing cells derived from human hematological tumors [CD4$^+$ human T-cells containing an integrated HTLV-1 genome (MT-4)] were seeded at an initial density of 1×10^5 cells/mL in 96-well plates in RPMI-1640 medium supplemented with 10% foetal calf serum (FCS), 100 units/mL penicillin G and 100 μg/mL streptomycin. Cell cultures were then incubated at 37 °C in a humidified, 5% CO_2 atmosphere in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 96 h at 37 °C by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method (Pauwels et al., 1988).

4.1.4. Antiviral assays

Activity of compounds against human immunodeficiency virus type-1 (HIV-1) was based on inhibition of virus-induced cytopathogenicity in MT-4 cells acutely infected with a multiplicity of infection (m.o.i.) of 0.01. Briefly, 50 μL of RPMI containing 1×10^4 MT-4 were added to each well of flat-bottom microtiter trays containing 50 μL of RPMI, without or with serial dilutions of test compounds. Then, 20 μL of an HIV-1 suspension containing 100 $CCID_{50}$ were added. After a 4-day incubation, cell viability was determined by the MTT method.

Activity of compounds against yellow fever virus (YFV) and reo virus type-1 (Reo-1) was based on inhibition of virus-induced cytopathogenicity in acutely infected BHK-21 cells. Activities against bovine viral diarrhoea virus (BVDV), in infected MDBK cells, were also based on inhibition of virus-induced cytopathogenicity.

BHK and MDBK cells were seeded in 96-well plates at a density of 5×10^4 and 3×10^4 cells/well, respectively, and were allowed to form confluent monolayers by overnight incubation in growth medium at 37 °C in a humidified CO_2 (5%) atmosphere. Cell monolayers were then infected with 50 μL of a proper virus dilution (in serum-free medium) to give an m.o.i. = 0.01. Then, 1 h later, 50 μL of MEM Earle's medium, supplemented with inactivated fetal calf serum (FCS), 1% final concentration, without or with serial dilutions of test compounds, were added. After 3–4 days incubation at 37 °C, cell viability was determined by the MTT method.

Activity of compounds against Coxsackie virus, B-2 strain (CVB-2), polio virus type-1 (polio-1), Sabin strain, vesicular dtomatitis virus (VSV), vaccinia virus (VV), herpes virus 1 (HSV-1) and against respiratory syncytial virus (RSV), A-2 strain, in infected Vero 76 cells, was determined by plaque reduction assays in Vero 76 cell monolayers. To this end, Vero 76 cells were seeded in 24-well plates at a density of 2×10^5 cells/well and were allowed to form confluent monolayers by incubating overnight in growth medium at 37 °C in a humidified CO_2 (5%) atmosphere. Then, monolayers were infected with 250 μL of proper virus dilutions to give 50–100 PFU/well. Following removal of unadsorbed virus, 500 μL of Dulbecco's modified Eagle's medium supplemented with 1% inactivated FCS and 0.75% methyl cellulose, without or with serial dilutions of test compounds, were added. Cultures were incubated at 37 °C for 2 days (Sb-1 and VSV), 3 days (CVB-2, VV and HSV-1) or 5 days (RSV) and then fixed with PBS containing 50% ethanol and 0.8% crystal violet, washed and air-dried. Plaques were then counted, and 50% effective concentrations (EC_{50}) values were calculated by the linear regression technique.

AZT (3′-azido-thymidine), NM 108 (2′-C-methyl-guanosine), NM 176 (2′-C-ethynyl-cytidine), NM 299 (6-azauridine), M 5255 (mycophenolic acid) and ACV (acyclovir) were used as reference inhibitors of ssRNA+, ssRNA− and DNA viruses, respectively.

5. Results

The synthesized indole derivatives **5** as well as the intermediate Mannich bases **4** were evaluated *in vitro* in parallel cell-based assays for cytotoxicity and antiviral activity (Tables 1A and 1B) against viruses representative of two of the three genera of the Flaviviridae family, i.e. flaviviruses (YFV) and pestiviruses (BVDV).

Title compounds were also tested against representative members of other virus families. Among ssRNA+ viruses were a retrovirus (human immunodeficiency virus type-1, HIV-1), two picornaviruses (Coxsackie virus type B2, CVB-2, and polio virus type-1, Sabin strain, Sb-1); among ssRNA− viruses were a paramyxovirus (respiratory syncytial virus, RSV) and a rhabdovirus (vesicular stomatitis virus, VSV). Among double-stranded RNA (dsRNA) viruses was a reo virus representative (respiratory enteric orphan virus type-1, Reo-1). Two representatives of the DNA virus families were also included: herpes symplex type-1, HSV-1 (Herpesviridae) and vaccinia virus, VV (Poxviridae). 3′-Azido-thymidine, 2′-C-methyl-guanosine, 2′-C-ethynyl-cytidine, ribavirin, 6-azauridine, mycophenolic acid and acyclovir were used as reference inhibitors of ssRNA+, ssRNA− and DNA viruses, respectively. Moreover, Tables 1A and 1B show data from the antiviral assays on NM14, given as an example of compounds **1**, and **7l**, given as an example of compounds **2**.

6. Discussion

From our biological data it is possible to draw the following general conclusions:

(1) All symmetrical Mannich bases (**4a**–**d**) do not show any toxicity or antiviral activity.
(2) All unsymmetrical methylene derivatives between indoles and 2-acetylnaphthols (**5a**–**t**), although possessing a certain degree of toxicity, present some interesting antiviral activities.

The unsymmetrical methylene derivatives (**5a**–**b**, **5e**, **5g**–**s**) present a significant activity against RSV. This behaviour was not present in the series previously tested (Mazzei et al., 2008). In particular, compounds **5q** and **5s** show an EC_{50} below 1 μM (0.5 and 0.7 μM, respectively), with an acceptable selectivity index (SI) of 40 and 21, respectively. Other compounds (**5l**–**o**) present significant activity against RSV: in particular, **5m** and **5o** show an EC_{50} of 1 μM, with an acceptable SI of 30 and 15, respectively.

Compounds (**5f**, **5i**–**j**, **5l**–**n**, **5o**, **5q**–**s**) present a modest activity against YFV, a virus generally not very susceptible to previous Mannich bases (Mazzei et al., 2008).

Compounds **4** and **5** were totally inactive against HSV-1, VV, Reo-1 and Sb-1.

Mannich bases of 7-hydroxycoumarin (compounds **1**) have been found active against BVDV (Giampieri et al., 2007; Mazzei et al., 2008). Now, the attempt to improve the antiviral activity by the substitution of 7-hydroxycoumarin with 2-naphthols was unsuccessful. In particular, the good activity (EC_{50} of 1 μM) against BVDV found testing NM14, compound similar to the Mannich bases **4** but bearing a 7-hydroxycoumarin-8-yl substituent instead of a 2-hydroxynaphthalen-1-yl substituent (Giampieri et al., 2007), was completely lost. This result indicates that the presence of a coumarin ring in these Mannich bases is mandatory.

On the other hand, when the 2-naphthols were linked to indoles (compounds **5**) some antiviral activity against HIV-1, BVDV, YFV, CVB-2 and RSV appeared. Although the activity against HIV-1, BVDV, YFV, CVB-2 is modest, from the data in Table 1B, it is evident that the unsymmetrical compounds **5** are intrinsically endowed with significant activity against RSV. This type of activity was completely absent in the indoles **2**. Unfortunately, our set of substances still possesses a certain degree of toxicity, but the novelty of this molecular structure for this activity is noteworthy and, certainly, deserves further detailed investigations to find new compounds with a more favorable ratio between activity and toxicity. Indeed, approved therapeutic agents (actually only one, ribavirin) to treat patients with RSV are still unsatisfactory and the search for selective drugs may be of great interest to fight this widespread virus.

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