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Laboratory note

4-Substituted anilino imidazo[1,2-a] and triazolo[4,3-a]quinoxalines. Synthesis and evaluation of in vitro biological activity

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Abstract

Fifteen imidazo[1,2-a] and [1,2,4]triazolo[4,3-a]quinoxalines were prepared. These compounds bear at position 4 various substituents related to the moieties present in classical and non-classical antifolic agents. And we evaluated in vitro antimicrobial, antiviral and antiproliferative activities. In particular, title compounds were evaluated in vitro against representative strains of Gram-positive and Gram-negative bacteria (*S. aureus*, *Salmonella* spp.), mycobacteria (*M. fortuitum*, *M. smegmatis* ATCC 19420 and *M. tuberculosis* ATCC 27294), yeast and moulds (*C. albicans* ATCC 10231 and *A. fumigatus*). Furthermore, their antiretroviral activity against HIV-1 was determined in MT-4 cells together with cytotoxicity. In these assays title compounds were tested for their capability to prevent MT-4 cell growth. Among the examined series, the compounds **5**, **7** and **10** showed cytotoxicity against mock-infected MT-4 cells.

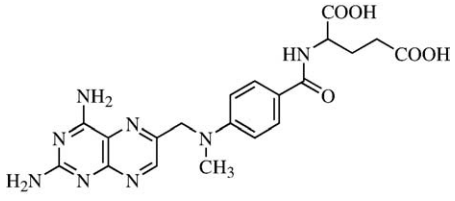
Keywords: Imidazo[1,2-a]quinoxalines; Triazolo[4,3-a]quinoxalines; Biological activity

1. Introduction

Structural analogy with the substrate has been successfully employed for developing an antimetabolite strategy in the case of antifolate drugs. Methotrexate (MTX), the closest drug to folic acid, inhibits dihydrofolate reductase (DHFR) and remains an unvaluable antifolic agent even though accompanied with drug resistance (Fig. 1).

Research efforts have concentrated on the discovery of safer or more potent compounds and a survey of these results has recently appeared [1]. In the past decade only two new agents have been introduced endowed with strong thymidilate synthase (TS) inhibitory activity (Tomudex and pemetrexed) (Fig. 2).

Our research group has been for long involved in a wide program aimed to discover anticancer activity in quinoxaline antifolate analogues on the basis that quinoxaline ring may act as bioisoster of both pteridine and quinazoline rings present in the most representative drugs as MTX, trimetrexate and tomudex. The results on the anticancer activity recorded at NCI (Bethesda) of our compounds showed that most of them were endowed with both anticancer and antifolic activity in the range of 10–0.1 μM concentration [2–17]. In this context we have also designed heterocyclic-fused quinoxalines of Fig. 3



Methotrexate

Fig. 1.

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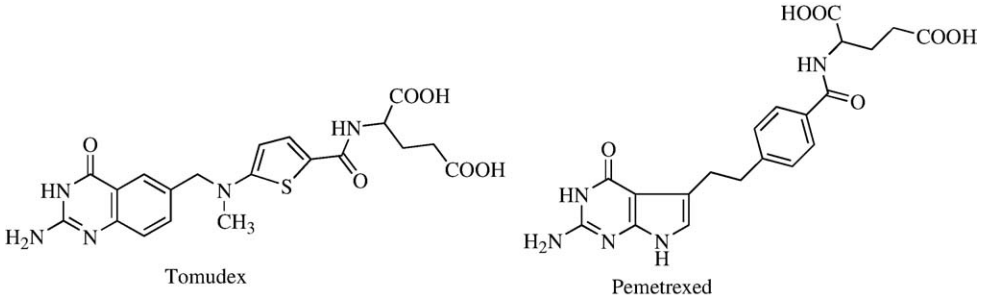


Fig. 2.

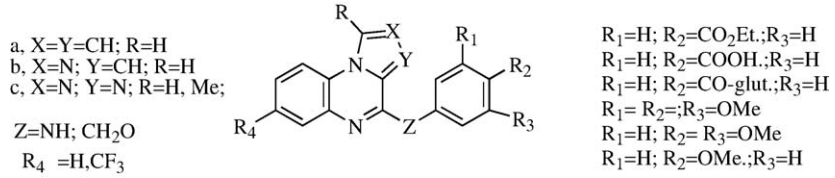


Fig. 3.

which bear the usual side chains of classical and non-classical antifolate drugs.

This modification originates from the observations reported in the literature that this type of annelation was successful in causing selectivity and stronger affinity of a pharmacophore element towards different receptor binding sites. As examples we can cite many current benzodiazepine drugs (alprazolam, triazolam, midazolam) [18]; pyrrolo[1,2-*a*]quinoxalines as 5-HT_3 receptor agonists [19]; non-peptide glucagons antagonists [20]; [1,2,4]triazolo[4,3-*a*]quinoxalines [21] and [1,2,4]triazolo[1,5-*a*]quinoxalylamines [22] and imidazo[1,2-*a*]quinoxalylamines [23] as adenosin antagonists as well as 1,2-dihydro-imidazo[1,2-*a*]quinoxaline-N-oxides as anticancer agents [24].

Thus, annelating the 1,2-bond of the pyrazine ring with an additional "electron-rich" ring might extend on one hand the planarity of the heteroring and on the other hand modulate either the lipophilicity or hydrogen bond accepting property. In other words two requirements for a better alleged affinity for the folate receptor.

With this in mind we previously described a series of pyrroloquinoxalines (a) of Fig. 3 and we found that some of these compounds were endowed with in vitro antiproliferative activities as low as 3.4 μM against a panel of cell lines derived from hematological and solid tumors [25]. In this note we have taken into account the preparation of compounds of b and c type **2**–**10** and **11**–**16** (Fig. 4) where pyrrole[1,2-a]quinoxaline ring has been bioisosterically replaced by both imidazo[1,2-a]- and triazolo[4,3-a]quinoxaline. In addition, they display a substituent in the azole ring that, in our opinion, was supposed to modulate both the lipophilicity and its possible interaction with the target.

Furthermore, the title molecules were also considered for an investigation of whatsoever antimicrobial and antiviral activity since both imidazole and triazole rings are good representatives of different classes of antiinfectives [26–28] and their embodiment in the quinoxaline structure might exhibit this type of activity.

2. Chemistry

The preparation of the compounds was accomplished according to the reactions described in Scheme 1. The starting material chloroimidazo[1,2-a] and chlorotriazolo[4,3-a]quinoxalines (**1a**–**c**) were obtained according to previously reported procedure [23,29]. Nucleophilic attack by the corresponding anilines **17**–**20**, in refluxing ethanol or 1-propanol gave the anilinoimidazo[1,2-a] and anilinotriazolo[4,3-a]quinoxalines **2**–**10** and the esters **11**, **13**, **15** in good yields. The desired acids **12**, **14**, **16** were obtained on alkaline hydrolysis in hydro-alcoholic medium of the esters **11**, **13** and **15**.

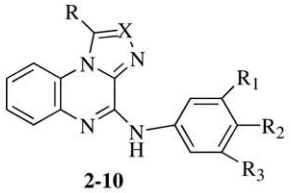
3. Experimental protocols

Melting points are uncorrected and were determined by a Kofler hot stage or Digital Electrothermal apparatus. UV spectra are qualitative and were recorded in nm for solutions in ethanol with Perkin–Elmer Lambda 5 spectrophotometer. IR spectra (Nujol mulls) were recorded with Perkin–Elmer 781 instrument. ^{1}H NMR spectra were recorded with a Varian XL-200 (200 MHz) instrument, using TMS as internal standard. Elemental analyses were performed by the Laboratorio di Microanalisi, Dipartimento di Chimica, University of Sassari, Italy. The analytical results for C, H, N were within ±0.4% of the theoretical values.

3.1. Chemistry

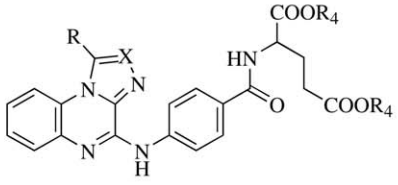
3.1.1. Intermediates

4-Chloroimidazo[1,2-a]quinoxaline (**1a**), 4-chlorotriazolo [4,3-a]quinoxaline (**1b**) and 1-methyl-4-chlorotriazolo[4,3-a] quinoxaline (**1c**) necessary for this work were known and



2-10

Compds	R	R_1	R_2	R_3	X
2	H	OCH_3	OCH_3	OCH_3	CH
3	H	OCH_3	H	OCH_3	CH
4	H	H	OCH_3	H	CH
5	H	OCH_3	OCH_3	OCH_3	N
6	H	OCH_3	H	OCH_3	N
7	H	H	OCH_3	H	N
8	CH_3	OCH_3	OCH_3	OCH_3	N
9	CH_3	OCH_3	H	OCH_3	N
10	CH_3	H	OCH_3	H	N



11-16

Compds	R	R_4	X
11	H	C_2H_5	CH
12	H	H	CH
13	H	C_2H_5	N
14	H	H	N
15	CH_3	C_2H_5	N
16	CH_3	H	N

Fig. 4.

were prepared according to the data of literature: **1a** [23], **1b**, **c** [29].

*3.1.2. General procedure for the preparation of 4-anilino imidazo[1,2-a]quinoxalines (**2–4**) and of 4-anilino triazolo [4,3-a]quinoxalines (**5–10**)*

A mixture of equimolar amounts (1.2 mmol) of **1a–c** and the corresponding substituted anilines **17–19** of Scheme 1 in ethanol (for **3–7**) or 1-propanol (for **2**, **8–10**) was refluxed for 4–5 h (**3–7**) and for 2 h (**2**, **8–10**). After cooling the solvent was removed under reduced pressure and the colored residues **2–10** were further purified as described in Table 1 where yields, melting points, analytical and spectroscopic data were reported.

*3.1.3. Diethyl N-[4-(imidazo[1,2-a]quinoxalin-4-yl)amino] benzoyl-L-glutamate (**11**), diethyl N-[4-(triazolo[4,3-a] quinoxalin-4-yl)amino]benzoyl-L-glutamate (**13**) and diethyl N-[4-(1-methyl-triazolo[4,3-a]quinoxalin-4-yl)amino]benzoyl-L-glutamate (**15**)*

A solution of equimolar amounts (1.5 mmol) of **1a–c** and the *p*-aminobenzoyl-L-glutamate (**20**) in ethanol (90 ml) for **11** and **13** or in 1-propanol (25 ml) for **15** was refluxed for 9 h (**11**, **15**) and 4 h (**13**). After cooling a cream colored solid precipitate (**11** and **15**) was filtered off and washed with ethanol or 1-propanol. In the case of compound **13** the solution was evaporated in vacuo to give a solid residue. Purification methods, yields, melting points, analytical and spectroscopic data are reported in Table 1.

*3.1.4. General procedure for the preparation of the acids **12**, **14**, **16***

A mixture of ester (**11**, **13**, **15**) (0.5 mmol) in ethanol (15 ml) and 1 M NaOH aqueous solution (6 ml) was refluxed for 1 h for **12** and **16**, or 4 h in the case of compound **14**. After cooling the reaction mixture was quenched with an equimolar amount of 1 M HCl aqueous solution. The solids precipitated were filtered off and washed with water.

Compounds **12**, **14** and **16** were purified by recrystallization as reported below. Yields, reaction conditions, melting points, analytical and spectroscopic data are reported in Table 1.

4. Microbiology

The new compounds were evaluated in vitro against representative strains of Gram-positive and Gram-negative bacteria (*S. aureus*, *Salmonella* spp.), various mycobacterial strains (*M. fortuitum*, *M. smegmatis* ATCC 19420 and *M. tuberculosis* ATCC 27294), and yeast and mould strain (*C. albicans* ATCC 10231 and *A. fumigatus*). Title compounds were also evaluated for anti-HIV-1 activity in MT-4 cells.

4.1. Microbiological assays

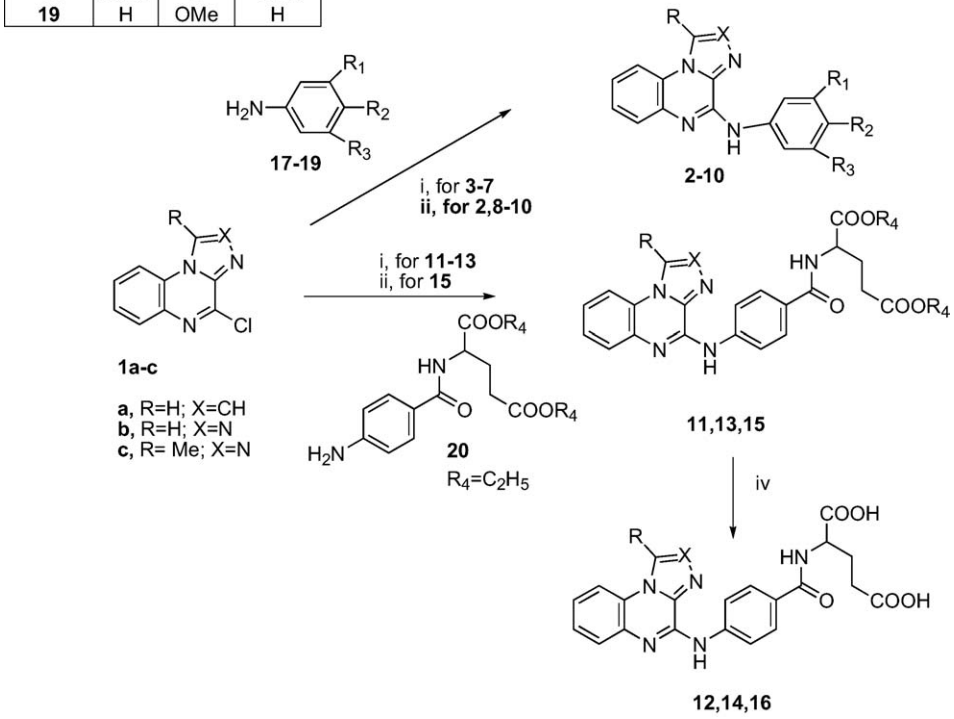
4.1.1. Compounds

Test compounds were solubilized in DMSO at 100 mM and then diluted into culture medium.

4.2. 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).

Anilines	R_1	R_2	R_3
17	OMe	OMe	OMe
18	OMe	H	OMe
19	H	OMe	H



Scheme 1. Reaction conditions: i, EtOH reflux Δ/5.5h; ii, propOH, reflux 2h; iii, propOH, reflux 9h; iv; EtOH, NaOH 1M, reflux 2/13h

4.3. Cytotoxicity and antiproliferative assays

Exponentially growing cells were resuspended in growth medium containing serial dilutions of the drugs. 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 [30].

4.4. Antiviral assays

Activity of compounds against HIV-1 was based on inhibition of virus-induced cytopathogenicity in MT-4 cells acutely infected at a multiplicity of infection of 0.01.

Activity against YFV, DENV-2 and WNV was based on inhibition of virus-induced cytopathogenicity in acutely infected BHK-21 cells. Activity against BVDV was based on inhibition of virus-induced cytopathogenicity in acutely infected MDBK cells. Cells were seeded overnight at a rate of 5×10^4 per well into 96-well plates in growth medium at 37 °C, in a humidified CO_2 (5%) atmosphere. Cell monolayers were infected with 50 μl of a proper virus dilution to give an m.o.i. = 0.01. Then, serial dilutions of test compounds in Dulbecco's modified Eagle's medium, supplemented with 2% inactivated fetal calf serum, were added. After a 3 days incubation at 37 °C, cell viability was determined by the MTT method.

4.5. Antibacterial and antimycotic assays

S. aureus, *Salmonella* spp. and *A. fumigatus* were clinical isolates, *C. albicans* 10231 was ATCC strain. Assays were carried out in Tryptose agar for *S. aureus*, *Salmonella* spp. and Sabouraud dextrose broth for *C. albicans* and *A. fumigatus*, with an inoculum of 10^3 bacteria per ml and 5×10^3 yeast per ml. *A. fumigatus* inocula were obtained from cultures grown at 37 °C for 1 day and then diluting to 0.05 OD_{50} per ml. Minimum inhibitory concentrations (MIC) were determined after incubations at 37 °C for 18 h in the presence of serial dilutions of test compounds.

4.6. Anti-mycobacterial assays

M. tuberculosis 27294 and *M. smegmatis* 19420 were ATCC strains, *M. fortuitum* was clinical isolate. MICs were assessed in microtiter plates by adding 20 μl aliquots of a culture suspension to 80 μl of Middlebrook 7H9 medium containing serial dilutions of test compounds. At the end of incubation, the number of viable mycobacteria was determined by the MTT method.

5. Results and discussion

All the compounds were evaluated in biological assays (the most significant results are shown in Table 2) in order to identify their potential antimicrobial and antiviral activities. However, none of the extracts showed activity against representatives strains of Gram-positive and Gram-negative bacteria (*S. aureus, Salmonella* spp.), nor against mycobacteria (*M. fortuitum*, *M. smegmatis* and *M. tuberculosis*). Test extracts were also inactive against representative strains of

Table 1
Physical and analytical data of compounds of Fig. 4

Compounds	M.p. (°C) (*)	Yield (%)	Analysis for	IR (Nujol) ν_{max} (cm^{-1})	UV (EtOH) λ_{max} (nm)	^1H NMR, δ_H (J in Hz) Solvent: [A] = $CDCl_3$ [B] = $CDCl_3$/DMSO-d_6 (3:1) [C] = $CDCl_3$/DMSO-d_6 (1:1) [D] = DMSO-d_6
2	74–78[a]	44	$C_{19}H_{18}N_4O_3$	3524, 1622	351, 335, 297, 225, 201	[A] 8.18 (1H, br s, NH), 8.02 (1H, s, H-1), 7.81 (1H, dd, $J_{9,8}$ = 7.8 Hz and $J_{9,7}$ = 1.6 Hz, H-9), 7.74 (1H, dd, $J_{6,7}$ = 7.8 Hz and $J_{6,8}$ = 1.6 Hz, H-6), 7.63 (1H, s, H-2), 7.56–7.40 (2H, m, H-7,8), 7.39 (2H, s, H-2′, 6′), 3.95 (6H, s, 3′,5′-OCH_3), 3.86 (3H, s, 4′-OCH_3)
3	114–115[a]	69	$C_{18}H_{16}N_4O_2$	3356, 1610	332, 293, 284, 261, 227, 211	[C] 8.54(1H, br s, NH), 8.08 (1H, dd, $J_{9,8}$ = 7.8 Hz and $J_{9,7}$ = 1.6 Hz, H-9 and 1H, s, H-1), 7.78 (1H, dd, $J_{6,7}$ = 7.8 Hz and $J_{6,8}$ = 1.6 Hz, H-6), 7.71 (1H, s, H-2), 7.50 (2H, s, H-2′, 6′), 7.50–7.39 (2H, m, H-7,8), 6.21 (1H, s, H-4′), 3.82 (6H, s, 3′, 5′, –OCH_3)
4	145.5–147.5[a]	89.74	$C_{17}H_{14}N_4O$	3308, 1622	340, 295, 286, 264, 227, 202	[A] 10.58 (1H, br s, NH), 8.02 (1H, s, H-1), 7.93 (2H, d, J = 8.2 Hz, H-2′, 6′), 7.85 (1H, dd, $J_{6,7}$ = 8.2 Hz and $J_{6,8}$ = 1.6 Hz, H-6), 7.74 (1H, dd, $J_{9,8}$ = 8.2 Hz and $J_{9,7}$ = 1.6 Hz, H-9), 7.63 (1H, s, H-2), 7.56–7.30 (2H, m, H-7, 8), 6.97 (2H, d, J = 8.2 Hz, H-3′, 5′), 3.81 (3H, s, 4′-OCH_3)
5	225–226[a]	91	$C_{18}H_{17}N_5O_3$	3574, 1584	391, 324, 296, 196	[B] 10.58 (1H, br s, NH), 9.55 (1H, s, H-1), 7.96 (1H, d, $J_{6,7}$ = 7.8 Hz, H-6), 9.74 (1H, d, $J_{9,8}$ = 7.8 Hz, H-9), 7.55 (2H, s, H-2′, 6′), 7.48–7.35 (2H, m, H-7,8), 3.94 (6H, s, 3′, 5′-OCH_3), 3.83 (3H, s, 4′-OCH_3)
6	229–230[a]	81	$C_{17}H_{15}N_5O_2$	3403, 1630	319, 293, 197	[A] 9.21 (1H, s, H-1), 8.19 (1H, br s, NH), 7.89 (1H, dd, $J_{6,7}$ = 6.8 Hz, and $J_{6,8}$ = 1.4 Hz, H-6), 7.79 (1H, dd, $J_{9,8}$ = 6.8 Hz and $J_{9,7}$ = 1.4 Hz, H-9), 7.61–7.57 (2H, m, H-7,8), 7.27 (2H, d, $J_{2',6'}$ = 2.2 Hz, H-2′, 6′), 6.29 (1H, t, H-4′), 3.87 (6H, s, OCH_3)
7	216–219[b]	79	$C_{16}H_{13}N_5O$	3355, 1659	338, 314, 224, 202	[B] 10.58 (1H, br s, NH), 10.05 (1H, s, H-1), 8.24 (1H, dd, $J_{6,7}$ = 7.8 Hz and $J_{6,8}$ = 1.6 Hz, H-6), 7.92 (2H, d, $J_{2',6'}$ = 9 Hz, H-2′, 6′), 7.78 (1H, dd, $J_{9,8}$ = 7.8 Hz and $J_{9,7}$ = 1.6 Hz, H-9), 7.60-7.40 (2H, m, H-7, 8), 6.98 (2H, d, $J_{3',5'}$ = 9 Hz, H-3′, 5′), 3.83 (3H, s, 4′-OCH_3)
8	242–244[a]	67	$C_{19}H_{19}N_5O_3$	3464, 1611	340, 311, 298, 212	[A] 8.72 (1H, s, NH), 8.01 (1H, d, $J_{6,7}$ = 8.2 Hz, H-6), 7.82 (1H, d, $J_{9,8}$ = 8.2 Hz, H-9), 7.57 (1H, t, H-7), 7.43 (1H, t, H-8), 7.29 (2H, s, H-2′, 6′), 3.88 (6H, s, 3′, 5′-OCH_3), 3.84 (3H, s, 4′-OCH_3), 3.14 (3H, s, CH_3)
9	233–234[a]	65	$C_{18}H_{17}N_5O_2$	3410, 1614	334, 310, 297, 213	[A] 8.30 (1H, s, NH), 7.99 (1H, d, $J_{6,8}$ = 8.4 Hz, H-6), 7.85 (1H, d, $J_{6,7}$ = 8.4 Hz, H-9), 7.52 (1H, t, H-7), 7.30 (1H, t, H-8), 7.24 (2H, d, $J_{2',6'}$ = 1.6 Hz, H-2′, 6′), 6.25 (1H, s, H-4′), 3.84 (6H, s, 3′, 5′-OCH_3), 3.13 (3H, s, CH_3)
10	214–216[a]	60	$C_{17}H_{15}N_5O$	3190, 1611	338, 310, 218	[A] 8.10 (1H, pr s, NH), 8.05 (1H, d, $J_{6,9}$ = 7.9 Hz, H-6), 7.88 (2H, d, $J_{3',5'}$ = 8.8 Hz, H-3′, 5′) 7.85 (1H, d, J = 7.8 Hz, H-9, partially obscured) 7.51 (1H, t, H-7), 7.38 (1H, t, H-8), 6.97 (2H, d, $J_{2',6'}$ = 8.8 Hz, H-2′,6′), 3.84 (3H, s, 4′-OCH_3), 3.13 (3H, s, CH_3)
11	179–181[a]	55	$C_{26}H_{27}N_5O_5$	3321, 1725, 1631	350, 335, 296, 287, 220, 204	[B] 10.50 (1H, br s, NH), 8.47 (1H, s, H-1), 8.37 (1H, d, CONH), 8.27 (2H, d, $J_{3',5'}$ = 7.8 Hz, H-3′, 5′), 8.04 (1H, d, $J_{5,6}$ = 7.8 Hz, H-5), 7.95 (2H, d, $J_{2',6'}$ = 8.6 Hz, H-2′, 6′), 7.85 (1H, d, $J_{8,7}$ = 7.2 Hz, H-8), 7.76 (1H, s, H-2), 7.60–7.41 (2H, m, H-7,6), 4.70–4.56 (1H, m, CH), 4.20 (2H, q, $COOCH_2CH_3$), 4.11 (2H, q, $COOCH_2CH_3$), 2.55–2.00 (4H, m, CH_2CH_3), 1.30 (3H, t, $COOCH_2CH_3$), 1.24 (3H, t, $COOCH_2CH_3$)
12	> 300[c]	42	$C_{22}H_{19}N_5O_5$	3518, 3400(sh) 3306, 1702, 1609	350, 335, 306, 294, 286, 220, 202	[B] 10.50 (1H, br s, NH), 8.63 (1H, s, H-1), 8.46 (1H, s, CONH), 8.33 (2H, d, $J_{3',5'}$ = 8.8 Hz, H-3′, 5′), 8.15 (1H, dd, $J_{6,7}$ = 6.8 Hz and $J_{6,8}$ = 5 Hz, H-6), 7.94 (2H, d, $J_{2',6'}$ = 8.8 Hz, H-2′,6′), 7.81 (1H, dd, $J_{9,8}$ = 7.6 Hz and $J_{9,7}$ = 5 Hz, H-9), 7.72 (1H, s, H-2), 7.53–7.40 (2H, m, H-7,8), 6.00 (2H, br s, COOH), 4.55–4.40 (1H, m, CH), 2.50–1.98 (4H, m, CH_2CH_2)
13	208–210	94	$C_{25}H_{26}N_6O_5$	3296, 1713, 1632, 160	323, 295, 283, 250, 202	[B] 10.50 (1H, br s, NH), 9.88 (1H, s, H-1), 8.39 (1H, d, CONH), 10.27 (2H, d, $J_{3',5'}$ = 8.8 Hz, H-3′, 5′), 8.15 (1H, d, $J_{6,7}$ = 8 Hz, H-6,7), 7.95 (2H, d, $J_{2',6'}$ = 8.Hz, H-2′,6′), 7.8 (1H, d, $J_{9,8}$ = 8. Hz, H-9), 7.58-7.40 (2H, m, H-7,8), 6.00 (2H, br s, COOH), 4.70–4.50 (1H, m, CH), 4.20 (2H, q, CH_2CH_3), 4.11 (2H, q, CH_2CH_3), 2.0-2.00 (4H, m, CH_2CH_2), 1.30 (3H, t, CH_2CH_3), 1.25 (3H, t, CH_2CH_3)
14	> 300[d]	74	$C_{21}H_{18}N_6O_5$	3500 (sh), 3250, 1704, 1643, 1606	338, 311, 263, 218, 203	[A] 10.55 (1H, br s, NH), 10.11 (1H, s, H-1), 8.55 (1H, d, CONH), 8.34–8.30 (2H, d, $J_{3',5'}$ = 8.2 Hz, H-3′, 5′ and 1H, H-6), 7.95 (2H, d, $J_{2',6'}$ = 8.8 Hz, H-2′, 6′), 7.81 (1H, dd, $J_{9,8}$ = 7.8 Hz and $J_{9,7}$ = 1.8 Hz, H-9), 7.65–7.55 (2H, m, H-7, 8), 6.00 (2H, br s, COOH), 4.50-4.40 (1H, m, CH), 2.55-1.90 (4H, m, CH_2CH_2)
15	226–228[a]	54	$C_{26}H_{28}N_6O_5$	3342, 3309, 1743, 1631, 1605	335, 321, 295, 281, 249, 204	[D] 10.41 (1, s, NH), 6.65 (1H, d, J = 7.2 Hz, CONH), 8.33 (2H, d, $J_{3',5'}$ = 8.6 Hz, H-3′, 5′), 8.21 (1H, d, $J_{6,7}$ = 8.2 Hz, H-6), 7.92 (2H, d, $J_{2',6'}$ = 8.8 Hz, H-2′, 6′), 9.80 (1H, dd, $J_{9,8}$ = 7.8 Hz and $J_{9,7}$ = 1.6 Hz, H-9), 7.62–7.44 (2H, m, H-7, 8), 4.50–4.40 (1H, m, CH), 4.13 (2H, q, $COOCH_2CH_3$), 4.07 (2H, q, $COOCH_2CH_3$), 3.09 (3H, s, CH_3), 2.48 (2H, t, CH_2CO), 2.20-1.9 (2H, m, $COCHCH_2$), 1.21 (3H, t, CH_2CH_3), 1.18 (3H, t, CH_2CH_3)
16	294–296[d]	77	$C_{22}H_{20}N_6O_5$	3315, 1710, 1635, 1610	336, 310, 297, 220, 202	[D] 10.39 (1H, s, NH), 8.58 (1H, d, CONH), 8.32 (2H, d, $J_{3',5'}$ = 8.4 Hz, H-3′, 5′), 8.20 (1H, d, $J_{6,7}$ = 8.4 Hz, H-6), 7.93 (2H, d, $J_{2',6'}$ = 8.4 Hz, H-2′,6′), 7.81 (1H, d, $J_{9,8}$ = 8.4 Hz, H-9), 7.70–7.40 (2H, m, H-7,8), 6.07 (2H, br s, COOH), 4.52–4.35 (1H, m, CH), 3.09 (3H, s, 1-CH_3), 2.60-1.90 (4H, m, CH_2CH_2)

(*) Purification procedure.
[a] Crystallization from EtOH.
[b] Crystallization from EtOH/H_2O.
[c] Crystallization from CH_3COOH.
[d] Crystallization from DMSO; sh, shoulder.

Table 2
Cytotoxicity of compounds **2**–**16**

Compounds	CC50[a] MT-4	Compounds	CC50[a] MT-4
2	= 100	**10**	51
3	> 100	**11**	> 100
4	> 100	**12**	> 100
5	40	**13**	> 100
7	68	**14**	> 100
8	> 100	**15**	> 100
9	93	**16**	> 100

[a] Compound dose (μM) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method.

yeasts and moulds (*C. albicans* ATCC 10231 and *A. fumigatus*).

The compounds were also tested for antiviral activity against HIV, BVDV and YFV (which are used as surrogate models for HCV), other RNA viruses, such as Coxsackie B and Reo, and DNA viruses (HSV-1, HSV-2, Vaccinia and HBV). None of the compounds showed any activities against all viruses we tested. However, the compounds **5**, **7** and **10** showed cytotoxicity against the T-lymphocyte cell line used for the anti-HIV-1 activity.

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