DOI: 10.1002/cmdc.201100073

Structural Investigation of the Naphthyridone Scaffold: Identification of a 1,6-Naphthyridone Derivative with Potent and Selective Anti-HIV Activity

Oriana Tabarrini,*[a] Serena Massari,[a] Luca Sancineto,[a] Dirk Daelemans,[b] Stefano Sabatini,[a] Giuseppe Manfroni,[a] Violetta Cecchetti,[a] and Christophe Pannecouque[b]

Building upon a large, previously reported series of anti-HIV 6-desfluoroquinolones endowed with a peculiar mechanism of action, the inhibition of Tat-mediated transcription, replacement of the quinolone nucleus with a naphthyridone core was shown to be very productive. In this work, the naphthyridone scaffold was investigated in depth by synthesizing various analogues. This led to the identification of NM13 as the most selective derivative obtained in MT-4 cells. It is the result of the successful combination of the 1,6-naphthyridone nucleus and the C7 benzothiazolpiperazine group, which, for the first time, not only grants potent anti-HIV activity but displays very high selectivity. Further studies aimed at a more thorough investigation of the anti-HIV profile of this new derivative are in progress.

Introduction

Remarkable progress has been made in the treatment of HIV-1 infection/AIDS. However, the emergence of multidrug-resistant HIV strains and the inability of the current anti-HIV treatment to completely eradicate the virus in HIV-infected individuals demands new highly potent drugs capable of interfering with targets other those exploited by the anti-HIV drugs currently on the market. The 6-desfluoroquinolones (6-DFQs), developed by our group,[1–9] are innovative compounds that owe their potent anti-HIV activity to their ability to inhibit the HIV-1 Tat-mediated transcription. This crucial step in HIV replication has not been clinically exploited in anti-HIV therapy. The molecular target of 6-DFQs has not yet been fully identified. Indeed, although the lead compound acts by interfering selectively with the Tat–TAR complex formation,[1,10] this is not strictly correlated to the antiviral activity and moreover, the same activity was not shown by other potent quinolone analogues.[9] Some evidence[1–9] (for example, different activities in various cell lines and the inability to select for resistance mutations), suggests that a host cellular factor or host cellular factor–viral component complex, involved in the Tat-mediated transcription, could be the target. Interfering with host factors essential for viral replication is an attractive way of combating HIV-1 and avoiding the emergence of resistance.[11] Indeed, viral protein–cofactor interfaces are not only much less tolerant to mutations than the catalytic site of the highly variable viral enzyme, but they are particularly attractive targets for developing selective drugs without compromising the normal process of transcriptional control at the cellular level of gene expression.[12–14]

Thus, the 6-DFQs possess beneficial properties that make them particularly attractive for further studies. Whereas we are attempting to identify the molecular target(s) that play a major role in their mechanism of action, our synthetic efforts have led to the identification of some very interesting compounds. The most exciting result was recently achieved by replacing the quinolone ring with the 1,8-naphthyridone core, a classic bioisosteric replacement also applied in the antibacterial quinolone field.[15,16] Naphthyridone derivative HM13N (Figure 1)

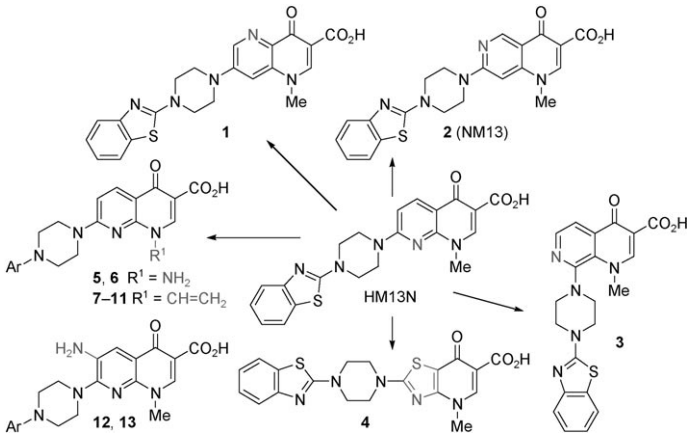


Figure 1. Structures of the compounds synthesized in this study starting from HM13N; for Ar, see Schemes 2 and 3.

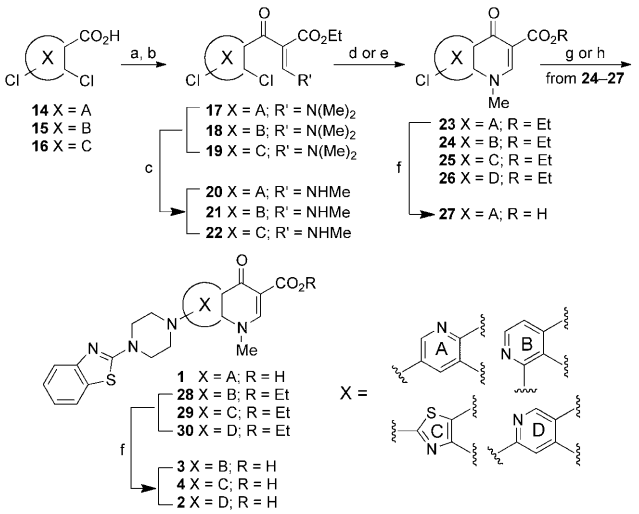
[a] *Prof. O. Tabarrini, Dr. S. Massari, Dr. L. Sancineto, Dr. S. Sabatini, Dr. G. Manfroni, Prof. V. Cecchetti*
Dipartimento di Chimica e Tecnologia del Farmaco
Università di Perugia, Via del Liceo 1, 06123 Perugia (Italy)
Fax: (+39) 075-585-5115
E-mail: oriana.tabarrini@unipg.it

[b] *Dr. D. Daelemans, Prof. C. Pannecouque*
Rega Institute for Medical Research
Katholieke Universiteit Leuven, 3000 Leuven (Belgium)

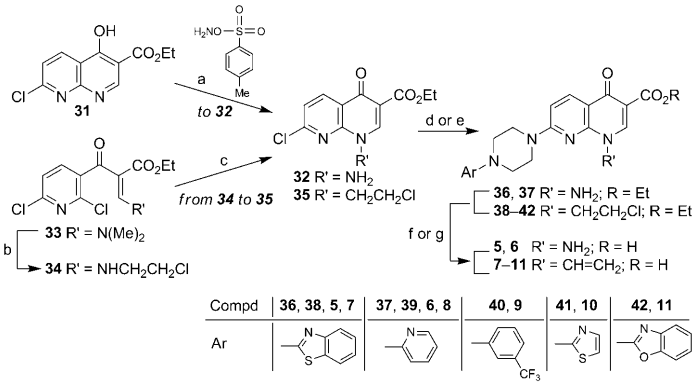
displayed very potent and selective anti-HIV activity with IC_{50} values in the low micromolar range in acutely, chronically, and latently infected cells.[8] The excellent antiviral profile coupled with the absence of any tendency to select for resistance mutations in vitro[8] increased the value of this type of compounds, driving the design and synthesis of the new analogues reported herein.

Interestingly, the 1,8-naphthyridone scaffold was particularly beneficial when it was decorated with the benzothiazolpiperazine moiety at the C7 position; the other 4-arylpiperazines did not confer the same favorable profile.[8] Thus, attempting to investigate this fruitful combination, various naphthyridone derivatives were synthesized. Maintaining the benzothiazolpiperazine at the C7 position, the 8-nitrogen atom was moved to the 5 and 6 positions, synthesizing 1,5-naphthyridone **1** and 1,6-naphthyridone **2**, respectively. Then, 1,7-naphthyridone **3** was prepared by placing the nitrogen at the 7 position while shifting the arylpiperazine to the C8 position. In a more incisive structural modification, a 1,3-thiazole ring replaced the pyridine ring in derivative **4**.

In parallel, the 1,8-naphthyridone scaffold was further investigated by exploiting the knowledge collected for the large series of 6-DFQs. In particular, the recent structure–activity relationship (SAR) acquired for the N1 position indicated that a vinyl group yielded compounds characterized by potent activity coupled with the ability to completely protect the cells from HIV-1 infection, whereas the presence of an amino group characterized low toxic compounds.[7] Thus, 1-amino-derivatives **5** and **6**, and 1-vinyl derivatives **7**–**11**, were synthesized. The 6-aminonaphthyridones **12** and **13**, were also prepared, by placing a classic amino group at the C6 position.[1,2]



Scheme 1. *Reagents and conditions*: a) $SOCl_2$, 80 °C, 3 h; b) ethyl 3-(dimethylamino)-2-propenoate, Et_3N, toluene, 80 °C, 1–3 h; c) $MeNH_2$, Et_2O or/and EtOH, 30 min; d) K_2CO_3, DMF, 60 °C, 24 h; e) NaH, THF, 30 min; f) 4% NaOH, reflux, 30 min; g) 1-(1,3-benzothiazol-2-yl)piperazine, Et_3N, DMF, 50–100 °C, 1–30 h; h) 1-(1,3-benzothiazol-2-yl)piperazine, *N*-methyl-2-pyrrolidone, 105 °C, 24 h.



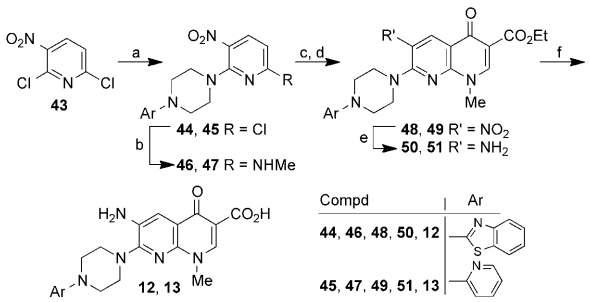
Scheme 2. *Reagents and conditions*: a) NaH, DMF, 0–25 °C, 24 h; b) 2-chloroethylamine hydrochloride, Et_2O/EtOH, RT, 24 h; c) Cs_2CO_3, CH_3CN, reflux, 2 h; d) Ar-piperazine, DMF, 70–80 °C, 4 h; e) Ar-piperazine, Cs_2CO_3, CH_3CN, reflux, 24 h; f) 4% NaOH, reflux, 2–4 h; g) NaOEt, EtOH, reflux, 1 h.

Chemistry

The synthesis of the target compounds **1**–**4** was accomplished following a common pathway as shown in Scheme 1. The reaction of acid chloride of the appropriate dichloroheteroaromatic carboxylic acids with 3-(dimethylamino)-2-propenoate followed by reaction with methylamine and successive base-induced cyclization, gave the key esters **23**–**25**. The nucleophilic reaction of **24**, **25**, and **26**[2] with 1-(1,3-benzothiazol-2-yl)piperazine[17] followed by basic hydrolysis gave target acids **2**–**4**. The hydrolysis step preceded the nucleophilic reaction in the synthesis of 1,5-naphthyridone **1** starting from synthon **23**.

The 1-amino- (**5** and **6**) and 1-vinyl- (**7**–**11**) 1,8-naphthyridones, were synthesized as shown in Scheme 2 by initially preparing the key intermediates **32** and **35**. Compound **32** was prepared by amination of intermediate **31**[18] employing freshly prepared *O*-*p*-tolylsulfonylhydroxylamine (TSH)[19] in the presence of sodium hydride. The 1-vinyl derivative **35**, was instead obtained by a procedure different to that previously reported[20] by reacting acrylate **33**[21] with 2-chloroethylamine to give intermediate **34** followed by cyclization with cesium carbonate. The successive nucleophilic reaction of **32** and **35** with selected 4-arylpiperazines, gave compounds **36**–**42**. The 1-aminoderivatives **36** and **37** were then hydrolyzed to the corresponding target acids **5** and **6** by using 4% sodium hydroxide, whereas employing sodium ethoxide in ethanol at reflux, 1-chloroethyl esters **38**–**42** were directly converted into 1-vinyl derivatives **7**–**11**.

A Gould–Jacob procedure was used to prepare 6-amino-1,8-naphthyridones **12** and **13** (Scheme 3). Starting from 2,6-dichloro-3-nitropyridine **43**, a first nucleophilic reaction with 1-(1,3-benzothiazol-2-yl)piperazine[17] and 1-(2-pyridinyl)piperazine gave intermediates **44** and **45**, respectively. They were then reacted with methylamine under microwave irradiation, followed by reaction with diethyl 2-(ethoxymethylene)malonate (EMME) and thermal cyclization with polyphosphoric acid (PPA), to give naphthyridones **48** and **49**. Catalytic reduction of



Scheme 3. *Reagents and conditions*: a) Ar-piperazine, DMF or toluene, RT, 7–12 h; b) $MeNH_2$, EtOH, MWI, 75 °C, 9 min; c) EMME, 140 °C, 1 week; d) PPA, 90 °C, 2 h; e) Raney Ni, H_2, EtOH/DMF, RT, 1 h; f) 4% NaOH, reflux, 12 h.

the nitro group and basic hydrolysis finally furnished target acids **12** and **13**.

Biological Activity

The newly synthesized compounds were initially evaluated for their anti-HIV-1 (III_B) and anti-HIV-2 (ROD) activity in MT-4 cells and cytotoxicity of the compounds was determined in parallel. The results reported in Table 1 clearly indicate that among the structural modifications made on HM13N, the shifting of the nitrogen atom from the 8 to the 6 position was particularly beneficial. Indeed, the 1,6-naphthyridone **2** (NM13) maintained the same activity of the lead on HIV-1, but it is devoid of any cytotoxic effect ($CC_{50} > 296.58$ μM) which made it very selective with a SI value of ≥ 3707. This is the highest SI value ever obtained in MT-4 cells for the transcription inhibitor quinolone series of compounds. Thus, the benzothiazolpiperazine is confirmed as a very suitable C7 substituent that is able to give potent anti-HIV activity also to the 1,6-naphthyridone scaffold. But, this is the first example in which its presence did not led to toxicity.

In contrast, all the other structural modifications made on the 1,8-naphthyridone scaffold were unproductive. Indeed, the shifting of the nitrogen atom from the 8 to 5 or 7 positions of the naphthyridone nucleus gave inactive compounds **1** and **3**. Whereas the replacement of the pyridine ring with a 1,3-thiazole one, yielded the more cytotoxic derivative **4**.

Certain 1,8-naphthyridone analogues were also synthesized by modifying the N1 and C6 positions, while placing different 4-arylpiperazines at the C7 position to enlarge the SAR. The replacement of the N1 methyl group with an amino group gave **5** which maintained the same low cytotoxicity but was markedly less active, especially against HIV-2. The activity further decreased with the 7-pyridinylpiperazine analogue **6**.

The introduction of a vinyl group at the N1 position proved much more valuable; it yielded active and selective anti-HIV compounds (**7**–**11**). The highest activity was obtained with **7**, with an EC_{50} value of 0.02 μM on both HIV-1 and HIV-2, even slightly lower than those of the lead HM13N; however, it was 50-fold more toxic resulting in lower SI values. More selective compounds were obtained by introducing the pyridinylpiperazine at the C7 position, (derivative **8**), and even better when the *m*-trifluoromethylphenylpiperazine was used (derivative **9**). Good activity was also obtained with 1-vinyl derivatives, **10** and **11**. When comparing 1-vinylnaphthyridones with their quinolone counterparts,[7] it became clear that the 1-vinyl moiety confers lower toxicity to the former while maintaining the same high potency.

Finally, the introduction of an amino group at the C6 position of 1,8-naphthyridone nucleus was required as it characterizes many of the potent 6-DFQs.[1,2,5] Benzothiazolyl derivative **12** showed good anti-HIV activity which, however, was not perfectly reproducible, whereas **13**, bearing the pyridinylpiperazine as a C7 substituent, showed moderate activity coupled with low cytotoxicity leading to positive SI values.

Table 1. Anti-HIV-1 and -HIV-2 activity and cytotoxicity of naphthyridone derivatives in MT-4 cells.

Compd	EC_{50} [μM][a,c]		CC_{50} [μM][b,c]	SI[d]	
	HIV-1 (III_B)	HIV-2 (ROD)		III_B	ROD
1	> 118.63	> 118.63	> 118.63	1	1
2 (NM13)	≥ 0.08	> 296.58	> 296.58	≥ 3707	1
3	> 25.57	> 25.57	25.57 ± 3.02	< 1	< 1
4	> 2.05	> 2.05	2.05 ± 1.31	< 1	< 1
5	1.18 ± 0.18	2.57 ± 1.55	23.77 ± 3.07	20	9
6	12.01 ± 3.68	≥ 15.69	172.99 ± 22.90	14	≤ 11
7	0.02 ± 0.00	0.025 ± 0.004	0.44 ± 0.38	22	18
8	0.16 ± 0.06	0.21 ± 0.015	16.66 ± 12.63	104	79
9	0.60 ± 0.27	0.78 ± 0.20	120.27 ± 49.91	200	154
10	1.46 ± 1.14	4.93 ± 0.65	34.61 ± 3.39	24	7
11	0.86 ± 0.47	≥ 1.03	18.30 ± 9.98	21	≤ 18
12	≥ 0.029	≥ 0.077	0.34 ± 0.32	≤ 12	≤ 4
13	1.49 ± 0.65	2.57 ± 0.76	51.86 ± 25.31	35	20
HM13N[e]	0.06 ± 0.00	0.04 ± 0.00	26.33 ± 6.79	439	658

[a] EC_{50}: compound concentration required to achieve 50% protection of MT-4 cells from HIV-induced cytopathogenicity, as determined by the MTT method; '>' indicates that no EC_{50} value (50% inhibition of HIV-induced CPE) was observed at a concentration less than the CC_{50} value obtained for the compound in MT-4 cells; '≥' means that in one separate experiment, performed in triplicate, a reliable EC_{50} value was obtained, whereas in the second experiment, although protection against the HIV-1 induced CPE was observed, the level of 50% protection was not reached (no EC_{50} value). [b] CC_{50}: compound concentration that decreases the viability of mock-infected cells by 50%, as determined by the MTT method. [c] All data represent mean values ± SD for at least two separate experiments. [d] SI: CC_{50}/EC_{50} ratio. [e] Ref. [8].

Conclusions

Herein we report the synthesis of an enlarged series of naphthyridone derivatives starting from 1,8-naphthyridone HM13N. The structural modifications confirmed that the 1,8-naphthyridone nucleus is very appropriate to obtain potent and selective anti-HIV compounds with the best results obtained when a vinyl moiety was placed at the N1 position coupled with a pyridinylpiperazine or a *m*-trifluoro-

methylphenylpiperazine at the C7 position (compounds **8** and **9**).

Although the nitrogen atom must not be moved from the 8 to the 5 or 7 positions, as demonstrated by the inactivity of derivatives **1** and **3**, the shifting to the 6 position is the real novelty of this study. Indeed, 1,6-naphthyridone NM13 is endowed with potent anti-HIV-1 activity, which combined with low toxicity, is the most selective derivative in acutely infected MT-4 cells. It is worth noting that a 1,6-naphthyridone analogue, bearing a pyridinylpiperazine as a C7 substituent, was previously reported by us as a nontoxic compound but devoid of any anti-HIV activity at lower concentrations.[2] Thus, the activity of NM13 is the result of a very successful combination of the 1,6-naphthyridone nucleus and the C7 benzothiazolylpiperazine, which, for the first time not only confers potent anti-HIV activity but also resulted in a highly selective compound.

Further elaborating the anti-HIV profile of this new derivative is clearly warranted. A transcription inhibitor is expected to inhibit viral production in latently and chronically infected cells, in which HIV-1 proviral DNA has already been integrated in the host-cell genome. The evaluation of its antiviral activity on these cells is currently in progress along with studies to confirm which step of the viral replicative cycle is inhibited. Moreover, we are attempting to increase its solubility which is lower than that of HM13N. Improving this parameter is mandatory to enable future in vivo studies.

Experimental Section

Chemistry

All reactions were routinely checked by thin-layer chromatography (TLC) on silica gel 60 F_{254} (Merck) and visualized by UV light or iodine. Flash column chromatography separations were carried out on Merck silica gel 60 (mesh 230–400). Melting points were determined in capillary tubes (Büchi Electrothermal Model 9100) and are uncorrected. Elemental analyses were performed on a Fisons elemental analyzer, Model EA1108CHN, and the data for C, H, and N are within $\pm$0.4% of the theoretical values. ^{1}H NMR and ^{13}C NMR spectra were recorded at 200 MHz (Bruker Avance DPX-200) and 400 MHz (Bruker Avance DRX-400) using residual solvents such as $CHCl_3$ (δ = 7.26) or DMSO (δ = 2.48) as an internal standard. Chemical shifts (δ) are given in ppm, and the spectral data are consistent with the assigned structures. Reagents and solvents were purchased from common commercial suppliers and were used as supplied. After extraction, organic solutions were dried over anhydrous Na_2SO_4, filtered, and concentrated with a Büchi rotary evaporator at reduced pressure. Yields are of purified product and were not optimized. Microwave irradiated reactions were carried out in an Initiator 2.0 (Biotage) using 5, 10, and 20 mL Pyrex vials. All starting materials were commercially available unless otherwise indicated.

Preparation of Ethyl 2-[(3,5-dichloro-2-pyridinyl)carbonyl]-3-(dimethylamino)-2-propenoate (17, Method A). A mixture of 3,5-dichloro-2-pyridinecarboxylic acid **14** (1.3 g, 6.77 mmol) and $SOCl_2$ (5 mL) was heated at 80 °C for ~3 h. The excess $SOCl_2$ was removed by distillation under reduced pressure, to give a residue which was solubilized in toluene and added to a mixture of ethyl 3-(dimethylamino)-2-propenoate (1.16 g, 8.12 mmol) and Et_3N (0.82 g, 8.12 mmol) in toluene (15 mL). The mixture was heated at 80 °C for 1 h. After cooling, the precipitate obtained was filtered and the filtrate was evaporated to dryness to give a residue which was solubilized in EtOAc and washed with H_2O acidified with 2 N HCl. The organic layers were evaporated to dryness to give a residue which was purified by flash chromatography eluting with MeOH/CH_2Cl_2 (3%), to give **17** as an oil (1.0 g, 46%); ^{1}H NMR ($CDCl_3$): δ = 0.90 (t, J = 7.1 Hz, 3H, CH_2CH_3), 3.00 and 3.40 (s, each 3H, CH_3), 3.90 (q, J = 7.1 Hz, 2H, CH_2CH_3), 7.75 (d, J = 1.8 Hz, 1H, H-4), 7.95 (s, 1H, CH), 8.35 ppm (d, J = 1.8 Hz, 1H, H-6).

Ethyl 2-(2,3-dichloroisonicotinoyl)-3-(dimethylamino)-2-propenoate (18). Compound **18** was prepared from 2,3-dichloroisonicotinic acid **15**[22] using Method A (1 h), in 60% yield as an oil after purification by flash chromatography eluting with CH_2Cl_2; ^{1}H NMR ([D$_6$]DMSO): δ = 0.85 (t, 3H, J = 7.1 Hz, CH_2CH_3), 2.95 and 3.40 (s, each 3H, CH_3), 3.85 (q, J = 7.1 Hz, 2H, CH_2CH_3), 7.30 (d, J = 4.0 Hz, 1H, H-5), 7.95 (s, 1H, CH), 8.35 ppm (d, J = 4.0 Hz, 1H, H-6).

Ethyl 2-[(2,4-dichloro-1,3-thiazol-5-yl)carbonyl]-3-(dimethylamino)-2-propenoate (19). Compound **19** was prepared from 2,4-dichloro-1,3-thiazole-5-carboxylic acid **16**[23] using Method A (2 h), in 53% yield, after purification by flash chromatography eluting with EtOAc/cyclohexane (50%); mp: 65–66 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 0.75 (t, J = 7.1 Hz, 3H, CH_2CH_3), 2.20 and 3.10 (s, each 3H, CH_3), 3.75 (q, J = 7.1 Hz, 2H, CH_2CH_3), 7.50 ppm (s, 1H, CH).

Preparation of Ethyl 2-[(3,5-dichloro-2-pyridinyl)carbonyl]-3-(methylamino)-2-propenoate (20, Method B). A mixture of compound **17** (1.0 g, 3.15 mmol) and a 33% solution of $MeNH_2$ in EtOH (0.44 mL, 3.78 mmol) in a mixture of EtOH/Et_2O (1:4, 20 mL), was stirred at RT for 30 min. The reaction mixture was then concentrated in vacuo, giving a residue which was purified by treatment with a mixture of Et_2O/petroleum ether, to give **20** (0.65 g, 68%); mp: 138–139 °C; ^{1}H NMR ($CDCl_3$): δ = 1.00 (t, J = 7.1 Hz, 3H, CH_2CH_3), 3.25 (d, J = 5.0 Hz, CH_3), 4.00 (q, J = 7.1 Hz, 2H, CH_2CH_3), 7.75 (d, J = 1.6 Hz, 1H, H-4), 8.20 (d, J = 14.0 Hz, 1H, CH), 8.45 (bs, 1H, H-6), 11.00 ppm (bs, 1H, NH).

Ethyl 2-(2,3-dichloroisonicotinoyl)-3-(methylamino)-2-propenoate (21). Compound **21** was prepared from **18** by Method B in 73% yield, after crystallization by EtOH; mp: 161–162 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 0.80 (t, J = 7.1 Hz, 3H, CH_2CH_3), 3.15 (d, J = 5.0 Hz, 3H, CH_3), 3.80 (q, J = 6.2 Hz, 2H, CH_2CH_3), 7.20 (d, J = 4.8 Hz, 1H, H-5), 8.10 (d, J = 14.0 Hz, 1H, CH), 8.30 (d, J = 4.8 Hz, 1H, H-6), 10.70 ppm (bs, 1H, NH).

Ethyl 2-[(2,4-dichloro-1,3-thiazol-5-yl)carbonyl]-3-(methylamino)-2-propenoate (22). Compound **22** was prepared from **19** by Method B using Et_2O as solvent, in 73% yield, after treatment with cyclohexane; mp: 74–75 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 1.00 (t, J = 7.1 Hz, 3H, CH_2CH_3), 3.20 (d, J = 4.5 Hz, 3H, CH_3), 4.00 (q, J = 7.1 Hz, 2H, CH_2CH_3), 8.00 and 8.15 (d, J = 15.0 Hz, each 0.5H, CH), 9.30–9.45 and 10.40–10.55 ppm (m, each 0.5H, NH).

Ethyl 7-chloro-1-methyl-4-oxo-1,4-dihydro-1,5-naphthyridine-3-carboxylate (23). A mixture of acrylate **20** (0.60 g, 1.97 mmol) and K_2CO_3 (0.82 g, 5.93 mmol) in DMF (8 mL) was heated at 60 °C for 24 h. After cooling the reaction mixture was poured into ice/H_2O and acidified with 2 N HCl (pH 4), to obtain a precipitate which was filtered and washed with H_2O and then with Et_2O, to give **23** (0.38 g, 72%); mp: 255–256 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 1.25 (t, J = 7.1 Hz, 3H, CH_2CH_3), 3.90 (s, 3H, CH_3), 4.25 (q, J = 7.1 Hz, 2H, CH_2CH_3), 8.40, (s, 1H, H-8). 8.70 (s, 1H, H-2), 8.80 ppm (s, 1H, H-6).

Ethyl 8-chloro-1-methyl-4-oxo-1,4-dihydro-1,7-naphthyridine-3-carboxylate (24). Compound **24** was prepared from acrylate **21** following the procedure used for the synthesis of compound **23** in 71% yield; mp: 129–130 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 1.20 (t, J =

7.0 Hz, 3H, CH_2CH_3), 4.15–4.25 (m, 5H, CH_2CH_3 and CH_3), 8.00 (d, J=5.0 Hz, 1H, H-5), 8.30 (d, J=5.0 Hz, 1H, H-6), 8.60 ppm (s, 1H, H-2).

Ethyl 2-chloro-4-methyl-7-oxo-4,7-dihydro[1,3]thiazolo[4,5-*b*]pyridine-6-carboxylate (25). A solution of **22** (0.5 g, 1.61 mmol) in THF (10 mL) was added dropwise to a suspension of NaH (0.04 g, 1.61 mmol) in THF (15 mL). After 30 min the reaction mixture was poured into ice/H_2O, neutralized with 2 N HCl, and extracted with EtOAc. The organic layers were evaporated to dryness to give a residue which was purified by flash chromatography eluting with CH_2Cl_2, to give **25** (0.10 g, 22%); mp: 174–175 °C; ^{1}H NMR ([D$_6$]DMSO): δ=1.20 (t, J=7.0 Hz, 3H, CH_2CH_3), 3.95 (s, 3H, CH_3), 4.30 (q, J=7.1 Hz, 2H, CH_2CH_3), 8.65 ppm (s, 1H, H-2); ^{13}C NMR ([D$_6$]DMSO): δ=14.5, 60.7, 113.9, 126.2, 129.9, 147.5, 151.4, 158.1, 164.4, 169.06 ppm.

Preparation of 7-Chloro-1-methyl-4-oxo-1,4-dihydro-1,5-naphthyridine-3-carboxylic acid (27, method C). A suspension of compound **23** (0.35 g, 1.31 mmol) in 4% NaOH (8 mL) was held at reflux for 30 min. After cooling, the reaction mixture was acidified to pH 4 with 2 N HCl, obtaining a precipitate which was filtered and washed with H_2O, to give **27** (0.16 g, 51%); mp: 309–310 °C; ^{1}H NMR ([D$_6$]DMSO): δ=4.00 (s, 3H, CH_3), 8.60 (s, 1H, H-6) 8.90 (s, 1H, H-8), 9.00 (s, 1H, H-2), 15.00 ppm (s, 1H, CO_2H).

7-[4-(1,3-Benzothiazol-2-yl)-1-piperazinyl]-1-methyl-4-oxo-1,4-dihydro-1,5-naphthyridine-3-carboxylic acid (1). A mixture of the synthon **27** (0.05 g, 0.20 mmol) and 1-(1,3-benzothyazol-2-yl)piperazine[17] (0.26 g, 1.2 mmol) in *N*-methyl-2-pyrrolidone (0.25 mL), was heated at 105 °C for 24 h. After cooling, the reaction mixture was poured into ice/H_2O, obtaining a precipitate which was filtered and crystallized by EtOH/DMF, to give **1** (0.016 g, 18%); mp: 250–251 °C; ^{1}H NMR ([D$_6$]DMSO): δ=4.00 (s, 3H, CH_3), 3.75–3.85 (m, 8H, piperazine CH_2), 7.10 (t, J=7.3 Hz, 1H, benzothiazole CH), 7.25–7.35 (m, 2H, H-8 and benzothiazole CH), 7.50 and 7.80 (d, J=8.1 Hz, each 1H, benzothiazole CH), 8.80 (s, 1H, H-6), 8.90 (s, 1H, H-2), 15.90 ppm (s, 1H, CO_2H); ^{13}C NMR ([D$_6$]DMSO): δ=41.9, 44.4, 47.9, 111.0, 119.1, 121.7, 121.8, 126.4, 127.1, 130.8, 135.1, 138.9, 139.0, 147.9, 151.1, 166.1, 168.4, 177.2; Anal. calcd (%) for $C_{21}H_{19}N_5O_3S$: C 59.85, H 4.54, N 16.62, found: C 60.13, H 4.47, N 16.58.

Preparation of Ethyl 8-[4-(1,3-benzothiazol-2-yl)-1-piperazinyl]-1-methyl-4-oxo-1,4-dihydro-1,7-naphthyridine-3-carboxylate (28, Method D). A mixture of compound **24** (0.07 g, 0.28 mmol), 1-(1,3-benzothyazol-2-yl)piperazine[17] (0.18 g, 0.85 mmol), and Et_3N (0.08 g, 0.85 mmol) in DMF (5 mL) was heated at 80 °C for 30 h. After cooling, the reaction mixture was poured into ice/H_2O giving a precipitate which was washed with H_2O and then with Et_2O, to give **28** (0.036 g, 29%); mp: 229–230 °C; ^{1}H NMR ([D$_6$]DMSO): δ=1.20 (t, J=7.0 Hz, 3H, CH_2CH_3), 2.90–3.00 (m, 2H, piperazine CH_2), 3.45–3.65 (m, 4H, piperazine CH_2), 3.75–3.95 (m, 2H, piperazine CH_2), 4.05 (s, 3H, CH_3), 4.20 (q, J=7.1 Hz, 2H, CH_2CH_3), 7.05 and 7.20 (t, J=7.5 Hz, each 1H, benzothiazole CH), 7.40 (d, J=7.4 Hz, 1H, benzothiazole CH), 7.70 (d, J=5.1 Hz, 1H, H-5), 7.75 (, J=7.4 Hz, 1H, benzothiazole CH), 8.20 (d, J=5.1 Hz, 1H, H-6), 8.50 ppm (s, 1H, H-2).

Ethyl 2-[4-(1,3-benzothiazol-2-yl)-1-piperazinyl]-4-methyl-7-oxo-4,7-dihydro[1,3]thiazolo[4,5-*b*]pyridine-6-carboxylate (29). Compound **29** was prepared from synthon **25**, by Method D (50 °C, 1 h) in 72% yield; mp: 268–269 °C; ^{1}H NMR ([D$_6$]DMSO): δ=1.25 (t, J=7.1 Hz, 3H, CH_2CH_3), 3.75–3.85 (m, 11H, piperazine CH_2 and CH_3), 4.20 (q, J=7.1 Hz, 2H, CH_2CH_3), 7.10 and 7.30 (t, J=7.5 Hz, each 1H, benzothiazole CH), 7.50 and 7.80 (d, J=7.7 Hz, each 1H, benzothiazole CH), 8.35 ppm (s, 1H, H-2).

Ethyl 7-[4-(1,3-benzothiazol-2-yl)-1-piperazinyl]-1-methyl-4-oxo-1,4-dihydro-1,6-naphthyridine-3-carboxylate (30). Compound **30** was prepared from ethyl 7-chloro-1-methyl-4-oxo-1,4-dihydro-1,6-naphthyridine-3-carboxylate **26**[2] by Method D (1 h) in 29% yield, after purification by flash chromatography eluting with MeOH/CH_2Cl_2 (3%); mp: 269–269 °C; ^{1}H NMR ([D$_6$]DMSO): δ=1.25 (t, J=7.0 Hz, 3H, CH_2CH_3), 3.70–3.75 (m, 4H, piperazine CH_2), 3.80 (s, 3H, CH_3), 3.95–4.00 (m, 4H, piperazine CH_2), 4.20 (q, J=7.0 Hz, 2H, CH_2CH_3), 6.68 (s, 1H, H-8), 7.25 and 7.30 (t, J=7.7 Hz, each 1H, benzothiazole CH), 7.50 and 7.80 (d, J=7.5 Hz, each 1H, benzothiazole CH) 8.55 (s, 1H, H-5), 8.95 ppm (s, 1H, H-2).

7-[4-(1,3-Benzothiazol-2-yl)-1-piperazinyl]-1-methyl-4-oxo-1,4-dihydro-1,6-naphthyridine-3-carboxylic acid (2). Compound **2** was prepared from intermediate **30** by Method C (24 h). After cooling, the reaction mixture was filtered and the solid obtained was suspended in 2 N HCl and stirred for ~1 h. The precipitate was then filtered, washed with Et_2O, and crystallized by DMF to give **2** in 92% yield; mp 359–360 °C; ^{1}H NMR ([D$_6$]DMSO): δ=3.70–3.80 (m, 4H, piperazine CH_2), 3.95–4.05 (m, 7H, piperazine CH_2 and CH_3), 6.80 (s, 1H, H-8), 7.15 and 7.35 (t, J=7.5 Hz, each 1H, benzothiazole CH), 7.55 and 7.85 (d, J=8.0 Hz, each 1H, benzothiazole CH), 8.90 (s, 1H, H-5), 9.15 ppm (s, 1H, H-2); ^{13}C NMR ([D$_6$]DMSO): δ=36.2, 44.2, 47.8, 89.8, 112.5, 119.1, 121.7, 121.8, 126.4, 130.8, 147.8, 148.7, 151.9, 152.7, 160.7, 166.2, 168.5, 175.3 ppm; Anal. calcd for $C_{21}H_{19}N_5O_3S$: C 59.84, H 4.54, N 16.62, found: C 60.14, H 4.60, N 16.70.

Sodium 8-[4-(1,3-benzothiazol-2-yl)-1-piperazinyl]-1-methyl-4-oxo-1,4-dihydro-1,7-naphthyridine-3-carboxylate (3). Compound **3** was prepared from **28** using Method C (15 min). After cooling, the precipitate obtained was filtered and washed with Et_2O to give **3** in 74% yield; mp: 101–102 °C; ^{1}H NMR ([D$_6$]DMSO): δ=2.90–3.05 (m, 2H, piperazine CH_2), 3.45–3.65 (m, 4H, piperazine CH_2), 3.90–4.05 (m, 2H, piperazine CH_2), 4.10 (s, 3H, CH_3), 7.15 and 7.30 (t, J=8.0 Hz, each 1H, benzothiazole CH), 7.50 (d, J=8.4 Hz, 1H, benzothiazole CH), 7.70–7.80 (m, 2H, benzothiazole CH and H-5), 8.15 (d, J=5.1 Hz, 1H, H-6), ppm 8.50 (s, 1H, H-2); ^{13}C NMR ([D$_6$]DMSO): δ=42.8, 47.8, 49.8, 11.4, 113.6, 119.1, 121.6, 121.8, 126.4, 128.4, 130.8, 136.8, 142.1, 152.7, 153.0, 153.9, 164.5, 168.6, 172.0 ppm; Anal. calcd (%) for $C_{21}H_{18}N_5NaO_3S$: C 56.88, H 4.09, N 15.79, found: C 57.15, H 3.98, N 15.55.

2-[4-(1,3-Benzothiazol-2-yl)-1-piperazinyl]-4-methyl-7-oxo-4,7-dihydro[1,3]thiazolo[4,5-*b*]pyridine-6-carboxylic acid (4). Compound **4** was prepared from **29** using Method C (2 h). After cooling, the precipitate obtained was filtered, suspended in 2 N HCl, and stirred for 30 min. The precipitate was filtered, washed with H_2O, and crystallized by EtOH/DMF, to give **4** in 43% yield; mp: 352–353 °C; ^{1}H NMR ([D$_6$]DMSO): δ=3.80–4.00 (m, 11H, piperazine CH_2 and CH_3), 7.15 and 7.35 (t, J=7.5 Hz, each 1H, benzothiazole CH), 7.55 and 7.85 (d, J=7.5 Hz, each 1H, benzothiazole CH), 8.70 ppm (s, 1H, H-2); ^{13}C NMR ([D$_6$]DMSO): δ=19.0, 56.4, 60.2, 113.5, 119.2, 121.7, 121.9, 126.5, 130.9, 145.2, 152.6, 153.3, 164.9, 168.3, 168.4, 171.2 ppm; Anal. calcd (%) for $C_{19}H_{17}N_5O_3S_2$: C 53.38, H 4.01, N 16.38, found: C 53.60, H 4.00, N 16.19.

Ethyl 1-amino-7-chloro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (32). NaH (0.06 g, 1.58 mmol) was added portionwise to a suspension of ethyl 7-chloro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate **31**[18] (0.4 g, 1.58 mmol) in DMF (5 mL), and the mixture was maintained at RT for 3 h. The reaction mixture was then brought to 0 °C and a solution of freshly prepared *O*-*p*-tolyl-

sulfonylhydroxylamine (TSH)[19] (0.37 g, 1.97 mmol) in CH_2Cl_2 (10 mL) was added. The mixture was maintained at the same temperature for 10 min and then at RT for a further 21 h. The reaction mixture was concentrated in vacuo, diluted with CH_2Cl_2, and washed with H_2O. The organic layers were evaporated to dryness, giving a solid which was washed with Et_2O to give **32** (0.07 g, 17%); mp: 163–165 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta = 1.30$ (t, $J = 7.0$ Hz, 3H, CH_2CH_3), 4.25 (q, $J = 7.0$ Hz, 2H, CH_2CH_3), 6.45 (s, 2H, NH_2), 7.70 (d, $J = 8.2$ Hz, 1H, H-6), 8.55 (d, $J = 8.2$ Hz, 1H, H-5), 8.70 ppm (s, 1H, H-2).

Ethyl 3-[(2-chloroethyl)amino]-2-[(2,6-dichloro-3-pyridinyl)carbonyl]-2-propenoate (34). Compound **34** was prepared from ethyl 2-[(2,6-dichloro-3-pyridinyl)carbonyl]-3-(dimethylamino)-2-propenoate **33**[21] by Method B (24 h), replacing $MeNH_2$ with 2-chloroethylamine hydrochloride, in 86% yield, after treatment with Et_2O; mp: 123–124 °C; ^{1}H NMR ($CDCl_3$): $\delta = 1.00$ (t, $J = 7.1$ Hz, 3H, CH_2CH_3), 3.70–3.80 (m, 4H, CH_2CH_2), 4.00 (q, $J = 7.1$ Hz, 2H, CH_2CH_3), 7.30 (d, $J = 8.0$ Hz, 1H, H-5), 7.55 (d, $J = 8.0$ Hz, 1H, H-4), 8.20–8.25 (m, 1H, CH), 9.45–9.55 and 11.00–11.05 ppm (each m, 1H, NH).

Ethyl 7-chloro-1-(2-chloroethyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (35).[20] Cs_2CO_3 (1.78 g, 5.47 mmol) was added to a solution of **34** (0.77 g, 2.18 mmol) in CH_3CN (10 mL) at reflux. After 2 h, the reaction mixture was cooled and then poured in ice/H_2O, obtaining a precipitate which was filtered and washed with H_2O and then with Et_2O, to give **35** (0.66 g, 96%); mp: 143–145 °C (148–149 °C);[20] ^{1}H NMR ($CDCl_3$): $\delta = 1.40$ (t, $J = 7.1$ Hz, 3H, CH_2CH_3), 4.00 (t, $J = 5.5$ Hz, 2H, CH_2Cl), 4.40 (q, $J = 7.1$ Hz, 2H, CH_2CH_3), 4.70 (t, $J = 5.5$ Hz, 2H, NCH_2), 7.40 (d, $J = 8.2$ Hz, 1H, H-6), 8.60 (s, 1H, H-2), 8.70 ppm (d, $J = 8.2$ Hz, 1H, H-5).

1-Amino-7-[4-(1,3-benzothiazol-2-yl)-1-piperazinyl]-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (5). Compound **5** was prepared from synthon **32** by Method D (70 °C, 4 h), to afford intermediate **36** in 59% yield, followed by Method C (2 h) in 89% yield, after crystallization by EtOH/DMF; mp: 320–321 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta = 3.70$–3.80 and 4.05–4.15 (m, each 4H, piperazine CH_2), 6.75 (bs, 2H, NH_2), 7.10 (t, $J = 7.5$ Hz, benzothiazole CH), 7.25–7.35 (m, 2H, benzothiazole CH and H-6), 7.50 and 7.80 (d, $J = 7.5$ Hz, each 1H, benzothiazole CH), 8.35 (d, $J = 7.8$ Hz, 1H, H-5), 8.70 (s, 1H, H-2), 15.75 ppm (s, 1H, COOH); ^{13}C NMR ([D$_6$]DMSO): $\delta = 47.9$, 108.6, 111.3, 119.1, 121.7, 121.8, 126.5, 130.8, 136.3, 146.2, 149.9, 152.7, 159.1, 166.5, 168.4, 175.8 ppm; Anal. calcd (%) for $C_{20}H_{18}N_6O_3S$: C 56.86, H 4.29, N 19.89, found: C 56.98, H 4.31, N 19.93

1-Amino-4-oxo-7-[4-(2-pyridinyl)-1-piperazinyl]-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (6). Compound **6** was prepared from synthon **32** by Method D (70 °C, 4 h), replacing 1-(1,3-benzothyazol-2-yl)piperazine with 1-(pyridin-2-yl)piperazine, to afford intermediate **37** in 68% yield, followed by Method C (4 h) 84% yield, after crystallization by EtOH/DMF; mp: 268–269 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta = 3.85$–3.95 and 4.05–4.15 (m, each 4H, piperazine CH_2), 6.95 (t, $J = 6.2$ Hz, 1H, pyridine CH), 7.25 (d, $J = 9.1$ Hz, H-6), 7.30–7.40 and 7.90–8.00 (m, each 1H, pyridine CH), 8.10 (d, $J = 4.7$ Hz, 1H, pyridine CH), 8.30 (d, $J = 9.1$ Hz, 1H, H-5), 8.65 (s, 1H, H-2), 15.90 ppm (bs, 1H, COOH); ^{13}C NMR ([D$_6$]DMSO): $\delta = 45.7$, 46.5, 107.7, 108.9, 111.7, 113.6, 131.5, 136.9, 138.0, 140.0, 146.7, 151.2, 153.4, 159.4, 159.7, 166.7, 177.2 ppm; Anal. calcd (%) for $C_{18}H_{18}N_6O_3$: C 59.01, H 4.95, N 22.94, found: C 59.32, H 4.79, N 22.78

Preparation of 7-[4-(1,3-Benzothiazol-2-yl)-1-piperazinyl]-4-oxo-1-vinyl-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid Hydrochloride (7, Method E). A mixture of synthon **35** (0.25 g, 0.79 mmol), 1-(1,3-benzothyazol-2-yl)piperazine[17] (0.17 g, 0.79 mmol), and Cs_2CO_3 (0.25 g, 0.79 mmol), in CH_3CN (5 mL) was heated at reflux for 24 h. The reaction mixture was then poured into ice/H_2O and extracted with EtOAc. The organic layers were evaporated to dryness to give a residue which was treated with Et_2O/EtOH, to give **38** (0.22 g, 56%); mp 231–232 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta = 1.25$ (t, $J = 7.0$ Hz, 3H, CH_2CH_3), 3.70–3.80 and 3.85–3.95 (m, each 4H, piperazine CH_2), 4.05–4.10 (m, 2H, CH_2Cl), 4.20 (q, $J = 7.0$ Hz, 2H, CH_2CH_3), 4.65–4.70 (m, 2H, NCH_2), 7.05–7.20 (m, 2H, benzothiazole CH and H-6), 7.30 (t, $J = 8.0$ Hz, 1H, benzothiazole CH), 7.45 and 7.80 (d, $J = 8.0$ Hz, each 1H, benzothiazole CH), 8.25 (d, $J = 8.2$ Hz, 1H, H-5), 8.60 ppm (s, 1H, H-2).

A mixture of **38** (0.18 g, 0.36 mmol) and NaOEt (0.17 g, 2.52 mmol) in EtOH (5 mL) was held at reflux for 1 h. After cooling, the reaction mixture was concentrated in vacuo, poured into H_2O, and 2 N HCl (pH 1) was added to obtain a solid which was filtered, washed with H_2O, then EtOH, and finally crystallized by EtOH/DMF, to give **7** (0.09 g, 57%); mp: 313–314 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta = 3.70$–3.80 and 3.95–4.05 (m, each 4H, piperazine CH_2), 5.35 (d, $J = 8.0$ Hz, 1H, CH=CH_2), 5.80 (d, $J = 15.0$ Hz, 1H, CH=CH_2), 7.10 (t, $J = 7.3$ Hz, 1H, benzothiazole CH), 7.20–7.30 (m, 2H, benzothiazole CH and H-6), 7.45 and 7.80 (d, $J = 7.5$ Hz, each 1H, benzothiazole CH), 7.85 (dd, $J = 8.0$ and 15.0 Hz, 1H, CH=CH_2), 8.30 (d, $J = 8.5$ Hz, 1H, H-5), 8.75 (s, 1H, H-2), 15.25 ppm (bs, 1H, COOH); ^{13}C NMR ([D$_6$]DMSO): $\delta = 44.1$, 47.8, 108.6, 108.9, 109.2, 110.8, 119.1, 121.7, 121.8, 126.4, 130.8, 132.8, 136.3, 143.7, 149.3, 152.7, 159.1, 166.0, 168.4, 177.3 ppm; Anal. calcd (%) for $C_{22}H_{20}ClN_5O_3S$: C 56.23, H 4.29, N 14.90, found: C 56.55, H 4.28, N 14.63

4-Oxo-7-[4-(2-pyridinyl)-1-piperazinyl]-1-vinyl-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (8). Compound **8** was prepared from synthon **35** by Method E through intermediate **39**, replacing 1-(1,3-benzothyazol-2-yl)piperazine with 1-(pyridin-2-yl)piperazine and adjusting the pH to 7 in the work up of the hydrolysis reaction. After crystallization by DMF, **8** was obtained in 57% overall yield; mp: 314–315 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta = 3.65$–3.70 and 3.80–3.90 (m, each 4H, piperazine CH_2), 5.35 (d, $J = 8.0$ Hz, 1H, CH=CH_2), 5.80 (d, $J = 15.0$ Hz, 1H, CH=CH_2), 6.70 (t, $J = 7.0$ Hz, pyridine CH), 6.80 (d, $J = 8.6$ Hz, 1H, H-6), 7.25 (d, $J = 8.6$ Hz, pyridine CH), 7.55 (t, $J = 7.5$ Hz, pyridine CH) 7.90 (dd, $J = 8.0$ and 15.0 Hz, 1H, CH=CH_2), 8.15(d, J=8.0 Hz, 1H, pyridine CH), 8.30 (d, $J = 9$ Hz, 1H, H-5), 8.80 (s, 1H, H-2), 15.25 ppm (bs, 1H, COOH); ^{13}C NMR ([D$_6$]DMSO): $\delta = 44.4$, 47.6, 107.6, 108.3, 108.8, 109.3, 110.6, 113.7, 132.8, 136.2, 138.1, 143.9, 148.0, 149.3, 159.0, 159.1, 166.2, 177.2 ppm; Anal. calcd (%) for $C_{20}H_{19}N_5O_3$: C 63.65, H 5.07, N 18.56, found: C 63.98, H 5.01, N 18.28.

4-Oxo-7-{4-[3-(trifluoromethyl)phenyl]-1-piperazinyl}-1-vinyl-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (9). Compound **9** was prepared from synthon **35** by Method E through intermediate **40**, replacing 1-(1,3-benzothyazol-2-yl)piperazine with 1-[3-(trifluoromethyl)phenyl]piperazine and adjusting the pH to 4 in the work up of the hydrolysis reaction. After crystallization by EtOH/DMF, **9** was obtained in 27% overall yield; mp: 224–225 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta = 3.30$–3.40 and 3.90–4.00 (m, each 4H, piperazine CH_2), 5.35 (d, $J = 8.0$ Hz, 1H, CH=CH_2), 5.80 (d, $J = 15.0$ Hz, 1H, CH=CH_2), 7.05–7.45 (m, 5H, aromatic CH and N6), 7.95 (dd, $J = 8.0$ and 15.0 Hz, 1H, CH=CH_2), 8.30 (d, $J = 8.1$ Hz, 1H, H-5), 8.75 (s, 1H, H-2), 15.25 ppm (bs, 1H, COOH); ^{13}C NMR ([D$_6$]DMSO): $\delta = 44.3$, 47.5, 108.6, 108.9, 109.3, 110.5, 111.4, 115.2, 119.2, 124.8, 130.2, 130.5, 132.8, 136.1, 143.9, 149.4, 151.1, 159.1, 166.0, 177.3 ppm; Anal. calcd (%) for $C_{22}H_{19}F_3N_4O_3$: C 59.46, H 4.31, N 12.61, found: C 59.70, H 4.58, N 12.60.

4-Oxo-7-[4-(1,3-thiazol-2-yl)-1-piperazinyl]-1-vinyl-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (10). Compound **10** was prepared from synthon **35** by Method E through intermediate **41**, replacing 1-(1,3-benzothyazol-2-yl)piperazine with 1-(2-thiazolyl)piperazine.[24] Compound **10** was obtained in 65% overall yield after crystallization by EtOH/DMF; mp: 358–359 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 3.70–3.80 and 4.00–4.10 (m, each 4H, piperazine CH_2), 5.35 (d, J = 8.0 Hz, 1H, CH=CH_2), 5.80 (d, J = 15.0 Hz, 1H, CH=CH_2), 7.10 (d, J = 3.4 Hz, 1H, thiazole CH), 7.30–7.40 (m, 2H, thiazole CH and H-6), 7.95 (dd, J = 8.0 and 15.0 Hz, 1H, CH=CH_2), 8.35 (d, J = 8.9 Hz, 1H, H-5), 8.75 ppm (s, 1H, H-2); ^{13}C NMR ([D$_6$]DMSO): δ = 43.8, 48.3, 108.7, 108.8, 109.1, 109.3, 110.7, 132.8, 136.2, 143.8, 149.3, 157.3, 159.0, 166.0, 171.0, 177.4 ppm; Anal. calcd (%) for $C_{18}H_{17}N_5O_3S$: C 56.38, H 4.47, N 18.27, found: C 56.70, H 4.33 N 18.01.

7-[4-(1,3-Benzoxazol-2-yl)-1-piperazinyl]-4-oxo-1-vinyl-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (11). Compound **11** was prepared from synthon **35** by Method E, through intermediate **42**, replacing 1-(1,3-benzothyazol-2-yl)piperazine with 1-(1,3-benzoxazol-2-yl)piperazine.[24] Compound **11** was obtained in 24% overall yield after crystallization by EtOH/DMF: mp: 302–303 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 3.75–3.85 and 3.95–4.05 (m, each 4H, piperazine CH_2), 5.40 (dd, J = 2.0 and 8.0 Hz, 1H, CH=CH_2), 5.80 (dd, J = 2.0 and 15.0 Hz, 1H, CH=CH_2), 7.00 and 7.20 (t, J = 7.5 Hz, each 1H, benzoxazole CH), 7.30 (d, J = 9.1 Hz, 1H, H-6), 7.35 and 7.45 (d, J = 7.6 Hz, each 1H, benzoxazole CH), 7.90 (dd, J = 8.0 and 15.0 Hz, 1H, CH=CH_2), 8.30 (d, J = 9.1 Hz, 1H, H-5), 8.80 ppm (s, 1H, H-2); ^{13}C NMR ([D$_6$]DMSO): δ = 44.2, 47.5, 108.6, 108.8, 109.3, 109.7, 110.6, 116.4, 121.0, 124.6, 132.8, 136.2, 142.5, 143.8, 148.2, 149.3, 159.1, 161.7, 166.0, 177.3 ppm; Anal. calcd (%) for $C_{22}H_{19}N_5O_4$: C 63.30, H 4.59, N 16.78, found: C 63.10, H 4.36, N 16.72.

2-[4-(6-Chloro-3-nitro-2-pyridinyl)-1-piperazinyl]-1,3-benzothiazole (44). A mixture of 2,6-dichloro-3-nitropyridine **43** (1.0 g, 5.18 mmol), 1-(1,3-benzothyazol-2-yl)piperazine[17] (1.4 g, 6.73 mmol), and Et_3N (0.53 g, 6.73 mmol) in DMF (5 mL) was maintained at RT for 12 h. The precipitate obtained was filtered and washed with EtOAc. The filtrate was evaporated to dryness obtaining a residue which was purified by flash chromatography eluting with Et_2O/petroleum ether (20%), to give **44** (0.75 g, 39%); mp: 144–145 °C; ^{1}H NMR ($CDCl_3$): δ = 3.60–3.70 and 3.75–3.85 (m, each 4H, piperazine CH_2), 6.80 (d, J = 8.0 Hz, 1H, H-5), 7.15 and 7.30 (t, J = 8.0 Hz, each 1H, benzothiazole CH), 7.65 and 7.70 (d, J = 8.0 Hz, each 1H, benzothiazole CH), 8.20 ppm (d, J = 8.0 Hz, 1H, H-4).

1-(6-Chloro-3-nitro-2-pyridinyl)-4-(2-pyridinyl)piperazine (45). Compound **45** was prepared following the procedure used for the synthesis of compound **44** (7 h), replacing 1-(1,3-benzothyazol-2-yl)piperazine with 1-(pyridin-2-yl)piperazine and using toluene as solvent. Compound **45** was obtained in 80% yield; mp: 128–129 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 3.45–3.55 and 3.60–3.70 (m, each 4H, piperazine CH_2), 6.65–6.70 (m,,1H, pyridine CH), 6.80 (d, J = 9.0 Hz, 1H, pyridine CH), 6.95 (d, J = 8.4 Hz, 1H, H-5), 7.55–7.60 (m, 1H, pyridine CH) 8.15–8.20 (m, 1H, pyridine CH), 8.30 ppm (d, J = 8.4 Hz, 1H, H-4).

6-[4-(1,3-Benzothiazol-2-yl)-1-piperazinyl]-*N*-methyl-5-nitro-2-pyridinamine (46). A mixture of compound **44** (0.10 g, 0.26 mmol) in EtOH (3 mL) and a 33% solution of $MeNH_2$ in EtOH (0.03 g, 0.39 mmol) were irradiated in a microwave oven at 75 °C with three 3 min cycles. The reaction mixture was evaporated to dryness to give a residue which was treated with Et_2O to give **46** (0.098 g, 99%); mp: 180–181 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 2.90 (d, J = 4.0 Hz, 3H, CH_3), 3.50–3.55 and 3.60–3.70 (m, each 4H, piperazine CH_2), 6.00 (d, J = 9.0 Hz, 1H, H-5), 7.05 and 7.30 (t, J = 8.0 Hz, each 1H, benzothiazole CH), 7.50 and 7.80 (d, J = 7.7 Hz, each 1H, benzothiazole CH), 8.00–8.10 ppm (m, 2H, NH and H-4).

***N*-methyl-5-nitro-6-[4-(2-pyridinyl)-1-piperazinyl]-2-pyridinamine (47).** Compound **47** was prepared from **45** following the procedure used for the synthesis of compound **46**, in 97% yield; mp: 208–209 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 2.90 (d, J = 4.0 Hz, 3H, CH_3), 3.50–3.60 and 3.65–3.75 (m, each 4H, piperazine CH_2), 6.00 (d, J = 9.0 Hz, 1H, H-5), 6.60–6.70 (m, 1H, pyridine CH), 6.85 (d, J = 8.5 Hz, 1H, pyridine CH), 7.50–7.60 (m, 1H, pyridine CH), 7.95 (bs, 1H, NH), 8.05–8.20 ppm (m, 2H, H-4 and pyridine CH).

Ethyl 7-[4-(1,3-benzothiazol-2-yl)-1-piperazinyl]-1-methyl-6-nitro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (48). A mixture of compound **46** (0.82 g, 2.21 mmol) and diethyl 2-(ethoxymethylene)malonate (5 mL) was heated at 140 °C for one week. After cooling the reaction mixture was purified by flash chromatography eluting with CH_2Cl_2 to give diethyl 2-{[{6-[4-(1,3-benzothiazol-2-yl)-1-piperazinyl]-5-nitro-2-pyridinyl}(methyl)amino]methylene}malonate, which was used in the next step without further purification. A mixture of this compound (0.70 g, 1.29 mmol) and polyphosphoric acid (3.5 g) was heated at 90 °C for 2 h. After cooling, the reaction mixture was poured into ice/H_2O and neutralized with 10% NaOH, obtaining a precipitate which was filtered and purified by flash chromatography eluting with MeOH/$CHCl_3$ (2%), to give **48** (0.31 g, 28% overall yield); mp: 256–257 °C; ^{1}H NMR ($CDCl_3$): δ = 1.25 (t, J = 7.0 Hz, 3H, CH_2CH_3), 3.80–3.95 (m, 11H, piperazine CH_2 and CH_3), 4.45 (q, J = 7.0 Hz, 2H, CH_2CH_3), 7.10 and 7.30 (t, J = 8.0 Hz, each 1H, benzothiazole CH), 7.55–7.65 (m, 2H, benzothiazole CH), 8.50 (s, 1H, H-5), 9.10 ppm (s, 1H, H-2).

Ethyl 1-methyl-6-nitro-4-oxo-7-[4-(2-pyridinyl)-1-piperazinyl]-1,4-dihydro-1,8-naphthyridine-3-carboxylate (49). Compound **49** was prepared from **47** following the procedure used for the synthesis of compound **48** in 10% overall yield, through the intermediate diethyl 2-[(methyl{5-nitro-6-[4-(2-pyridinyl)-1-piperazinyl]-2-pyridinyl}amino)methylene]malonate; mp: 182–183 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 1.30 (t, J = 7.0 Hz, 3H, CH_2CH_3), 3.65–3.75 (m, 8H, piperazine CH_2), 3.90 (s, 3H, CH_3), 4,20 (q, J = 7.0 Hz, 2H, CH_2CH_3), 6.65–6.70 (m, 1H, pyridine CH), 6.85 (d, J = 8.6 Hz, pyridine CH) 7.50–7.60 and 8.15–8.20 (m, each 1H, pyridine CH), 8.75 (s, 1H, H-2), 8.80 ppm (s, 1H, H-5).

Ethyl 6-amino-7-[4-(1,3-benzothiazol-2-yl)-1-piperazinyl]-1-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (50). A stirred solution of compound **48** (0.27 g, 0.54 mmol) in DMF (30 mL) was hydrogenated over a catalytic amount of Raney nickel at RT and atmospheric pressure for 1 h. The mixture was then filtered over Celite, and the filtrate was evaporated to dryness to give a residue which was purified by flash chromatography eluting with MeOH/$CHCl_3$ (3%), to give **50** (0.08 g, 32%); mp: 301–302 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 1.30 (t, J = 7.0 Hz, 3H, CH_2CH_3), 3.40–3.50 and 3.65–3.75 (m, each 4H, piperazine CH_2), 3.85 (s, 3H, CH_3), 4.20 (q, J = 7.0 Hz, 2H, CH_2CH_3), 5,30 (bs, 2H, NH_2), 7.10 and 7.30 (t, J = 8.0 Hz, each 1H, benzothiazole CH), 7.50 (d, J = 7.7 Hz, 1H, benzothiazole CH), 7.65 (s, 1H, H-5), 7.80 (d, J = 7.7 Hz, 1H, benzothiazole CH), 8.55 ppm (s, 1H, H-2).

Ethyl 6-amino-1-methyl-4-oxo-7-[4-(2-pyridinyl)-1-piperazinyl]-1,4-dihydro-1,8-naphthyridine-3-carboxylate (51). Compound **51** was prepared from **49** following the procedure used for the synthesis of compound **50** in 81% yield; mp: 276–277 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 1.30 (t, J = 7.0 Hz, 3H, CH_2CH_3), 3.40–3.50 and 3.65–3.75 (m, each 4H, piperazine CH_2), 3.85 (s, 3H, CH_3), 4.20 (q, J = 7.0 Hz, 2H, CH_2CH_3), 5,30 (bs, 2H, NH_2), 6.70 (t, J = 6.5 Hz, pyridine CH), 6.80 (d, J = 8.8 Hz, pyridine CH) 7.55 (t, J = 7.5 Hz, pyridine CH),

7.70 (s, 1H, H-5), 8.15–8.20 (m, 1H, pyridine CH), 8.55 ppm (s, 1H, H-2).

6-Amino-7-[4-(1,3-benzothiazol-2-yl)-1-piperazinyl]-1-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (12). Compound **12** was prepared from intermediate **50** by Method C (12 h). After cooling, the reaction mixture was filtered, the solid was suspended in 2 N HCl, and stirred for ~1 h. The precipitate obtained was then filtered and washed with Et_2O to give **12** in 76% yield; mp 337–338 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 3.55–3.65 and 3.75–3.85 (m, each 4H, piperazine CH_2), 4.00 (s, 3H, NCH_3), 5.80 (bs, 2H, NH_2), 7.10 and 7.30 (t, J = 7.0, each 1H, benzothiazole CH), 7.50 (d, J = 7.5 Hz, 1H, benzothiazole CH), 7.70 (s, 1H, H-5), 7.80 (d, J = 7.5 Hz, 1H, benzothiazole CH), 8.90 (s, 1H, H-2), 15.90 ppm (bs, 1H, COOH); ^{13}C NMR ([D$_6$]DMSO): δ = 39.4, 46.8, 48.0, 107.3, 114.5, 116.9, 119.0, 121.7, 121.8, 126.5, 130.6, 136.2, 141.5, 146.3, 152.4, 153.4, 167.0, 168.6, 176.9 ppm; Anal. calcd (%) for $C_{21}H_{20}N_6O_3S$: C 57.79, H 4.62, N 19.25, found: C 58.09, H 4.48, N 19.07.

6-Amino-1-methyl-4-oxo-7-[4-(2-pyridinyl)-1-piperazinyl]-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (13). Compound **13** was prepared from intermediate **51** by Method C (12 h). After cooling, the reaction mixture was filtered, the solid was suspended in 2 N HCl, and stirred for ~1 h. The precipitate obtained was filtered and washed with Et_2O to give **13** in 78% yield after crystallization by DMF: mp 297–298 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 3.60–3.70 and 3.80–3.90 (m, each 4H, piperazine CH_2), 4.00 (s, 3H, CH_3), 5.70 (bs, 2H, NH_2), 6.90 (t, J = 6.4 Hz, pyridine CH), 7.40 (d, J = 9.1 Hz, 1H, pyridine CH), 7.70 (s, 1H, H-5), 7.95 (t, J = 6.4 Hz, pyridine CH), 8.05 (d, J = 5.5 Hz, 1H, pyridine CH), 8.55 (s, 1H, H-2), 15.90 ppm (bs, 1H, COOH); ^{13}C NMR ([D$_6$]DMSO): δ = 31.1, 45.4, 46.4, 107.2, 112.4, 113.3, 114.6, 116.8, 135.9, 138.4, 141.5, 143.5, 146.5, 153.2, 159.1, 166.9, 176.9 ppm; Anal. calcd (%) for $C_{19}H_{20}N_6O_3$: C 59.99, H 5.30, N 22.09, found: C 60.13, H 5.15, N 22.07.

Biology

In vitro anti-HIV assays: Evaluation of the antiviral activity of the compounds against HIV-1 strain (III_B) and HIV-2 strain (ROD) in MT-4 cells was performed using the MTT assay as previously described.[25,26] Briefly, stock solutions (10 x final concentration) of test compounds were added in 25 μL volumes to two series of triplicate wells so as to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial fivefold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman instruments). Untreated control HIV- and mock-infected cell samples were included for each sample. HIV-1(III_B)[27] or HIV-2 (ROD)[28] stock (50 μL) at 100–300 $CCID_{50}$ (50% cell culture infectious dose) or culture medium was added to either the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of test compound on uninfected cells to assess the cytotoxicity of the test compound. Exponentially growing MT-4 cells[29] were centrifuged for 5 min at 1000 rpm (220 *g*) and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells mL^{-1} and 50 μL volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock- and HIV-infected cells was examined spectrophotometrically with the MTT assay.

The MTT assay is based on the reduction of yellow colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Acros Organics) by mitochondrial dehydrogenase of metabolically active cells to a blue-purple formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (Safire, Tecan), at two wavelengths (540 and 690 nm). All data were calculated using the median OD (optical density) value of three wells. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the test compound that reduced the absorbance (OD_{540}) of the mock-infected control sample by 50%. The concentration achieving 50% protection from the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC_{50}).

Acknowledgements

These studies were supported financially by the MIUR (PRIN 2008, Rome, Italy) and K.U. Leuven (GOA10/14). S.M. is supported by a postdoctoral fellowship from the Fondazione Cassa di Risparmio (Perugia, Italy). We thank Mr. Roberto Bianconi (Department of Chemistry and Technology of Drugs, Perugia), Ms. Kristien Erven, Ms. Cindy Heens, and Mr. Kris Uyttersprot (Rega Institute for Medical Research, Leuven, Belgium) for their excellent technical assistance.

Keywords: 1,6-naphthyridones · anti-HIV quinolones · antiviral agents · medicinal chemistry · transcription inhibitors

[1] V. Cecchetti, C. Parolin, S. Moro, T. Pecere, E. Filipponi, A. Calistri, O. Tabarrini, B. Gatto, M. Palumbo, A. Fravolini, G. Palù, *J. Med. Chem.* **2000**, *43*, 3799 – 3802.
[2] O. Tabarrini, M. Stevens, V. Cecchetti, S. Sabatini, M. Dell'Uomo, G. Manfroni, M. Palumbo, C. Pannecouque, E. De Clercq, A. Fravolini, *J. Med. Chem.* **2004**, *47*, 5567 – 5578.
[3] M. Stevens, J. Balzarini, O. Tabarrini, G. Andrei, R. Snoeck, V. Cecchetti, A. Fravolini, E. De Clercq, C. Pannecouque, *J. Antimicrob. Chemother.* **2005**, *56*, 847 – 855.
[4] D. Daelemans, R. Lu, E. De Clercq, A. Engelman, *J. Virol.* **2007**, *81*, 4381 – 4385.
[5] O. Tabarrini, S. Massari, D. Daelemans, M. Stevens, G. Manfroni, S. Sabatini, J. Balzarini, V. Cecchetti, C. Pannecouque, A. Fravolini, *J. Med. Chem.* **2008**, *51*, 5454 – 5458.
[6] S. Massari, D. Daelemans, G. Manfroni, S. Sabatini, O. Tabarrini, C. Pannecouque, V. Cecchetti, *Bioorg. Med. Chem.* **2009**, *17*, 667 – 674.
[7] O. Tabarrini, S. Massari, D. Daelemans, F. Meschini, G. Manfroni, L. Bottega, B. Gatto, M. Palumbo, C. Pannecouque, V. Cecchetti, *ChemMedChem* **2010**, *5*, 1880 – 1892.
[8] S. Massari, D. Daelemans, M. L. Barreca, A. Knezevich, S. Sabatini, V. Cecchetti, A. Marcello, C. Pannecouque, O. Tabarrini, *J. Med. Chem.* **2010**, *53*, 641 – 648.
[9] O. Tabarrini, S. Massari, V, Cecchetti, *Future Med. Chem.* **2010**, *2*, 1161 – 1180.
[10] S. Richter, C. Parolin, B. Gatto, C. Del Vecchio, A. Fravolini, G. Palù, M. Palumbo, *Antimicrob. Agents Chemother.* **2004**, *48*, 1895 – 1899.
[11] W. Coley, K. Kehn-Hall, R. Van Duyne, F. Kashanchi, *Expert Opin. Biol. Ther.* **2009**, *9*, 1369 – 1382.
[12] A. P. Rice, R. E. Sutton, *Future HIV Ther.* **2007**, *1*, 369 – 385.
[13] M. A. Khattab, *World J. Gastroenterol.* **2009**, *15*, 3472 – 3479.
[14] S. Ludwig, *J. Antimicrob. Chemother.* **2009**, *64*, 1 – 4.
[15] "Antibacterial Antibiotics": J. M. Beale, Jr., in *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, 11th ed.* (Eds.: J. H. Block, J. M. Beale, Jr.), Lipincott, Williams & Wilkins, Philadelphia, **2004**, p. 299.
[16] "Structure–Activity Relationships": L. A. Mitscher, P. Devasthale, R. Zavod, in *Quinolone Antimicrobial Agents, 2nd ed.* (Eds.: D. C. Hooper, J. S. Wolfson), *American Society for Microbiology*, Washington DC, **1998**, pp. 3 – 51.
[17] M. Verderame, *J. Med. Chem.* **1972**, *15*, 693 – 694.
[18] T. Hirose, S. Mishio, J. Matsumoto, S. Minami, *Chem. Pharm. Bull.* **1982**, *30*, 2399 – 2409.

[19] E. E. Glover, K. T. Rowbotton, *J. Chem. Soc. Perkin Trans. 1* **1976**, *4*, 367 – 371.
[20] S. Mishio, T. Hirose, A. Minamida, J. Matsumoto, S. Minami, *Chem. Pharm. Bull.* **1985**, *33*, 4402 – 4408.
[21] D. Anderson, B. Beutel, T. D. Bosse, R. Clark, C. Cooper, P. Dandliker, C. David, H. Yu-Gui, M. Todd, M. Hinman, D. Kalvin, D. P. Larson, L. Lynch, Z. Ma, C. Motter, F. Palazzo, T. Rosenberg, T. Rehm, W. Sanders, M. Tufano, R. Wagner, M. Weitzberg, H. Yong, T. Zhang, US 20030232818 A120031218, **2003** [*Chem. Abstr.* **2003**, *140*, 42160].
[22] E. Marzi, A. Bigi, M. Schlosser, *Eur. J. Org. Chem.* **2001**, *7*, 1371 – 1376.
[23] M. Hasegawa, K. Kano, M. Nakano, M. Yamabe, WO 2005070042A2 20050804, **2005** [*Chem. Abstr.* **2005**, *143*, 193995].
[24] T. Kimura, T. Katsube, EP 572259A1, **1993** [*Chem. Abstr.* **1994**, *121*, 57343].
[25] R. Pauwels, J. Balzarini, M. Baba, R. Snoeck, D. Schols, P. Herdewijn, J. Desmyter, E. De Clercq, *J. Virol. Methods* **1988**, *20*, 309 – 321.
[26] C. Pannecouque, D. Daelemans, E De Clercq, *Nat. Protoc.* **2008**, *3*, 427 – 434.
[27] M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *Science* **1984**, *224*, 497 – 500.
[28] F. Barrè-Sinoussi, J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Grest, C. Dauget, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum, L. Montagnier, *Science* **1983**, *220*, 868 – 871.
[29] I. Miyoshi, H. Taguchi, I. Kobonishi, S. Yoshimoto, Y. Ohtsuki, Y. Shiraishi, T. Akagi, *Gann. Monogr. Cancer Res.* **1982**, *28*, 219 – 228.

Received: February 8, 2011
Revised: April 1, 2011
Published online on May 12, 2011

