ELSEVIER

Il Farmaco 53 (1998) 139–149

IL FARMACO

# Quinoxaline chemistry
# Part 9. Quinoxaline analogues of trimetrexate (TMQ) and 10-propargyl-5,8-dideazafolic acid (CB 3717) and its precursors. Synthesis and evaluation of in vitro anticancer activity

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Received 10 October 1997

**Abstract**

Eighteen quinoxalines bearing a methyleneanilino or methyleneaminobenzoylglutamate group on position 6 of the ring and various lipophilic substituents on positions 2 and 3 were prepared in order to discover if their structural analogy with both trimetrexate (TMQ) and 10-propargyl-5,8-dideazafolic acid (CB 3717) might display in vitro anticancer activity. Among these, 12 compounds were selected at the National Cancer Institute, Bethesda, MD, USA; they exhibited moderate (**4b,d,i,l,m** and **8**) to strong (**4f,h** and **5a,e**) cell-growth inhibition at a concentration of $10^{-4}$ M. Interesting selectivities were also recorded between $10^{-8}$ and $10^{-6}$ M. These analogues proved to be less potent inihibitors of tumor cells than other classical and non-classical antifolate analogues previously described by us. 

*Keywords:* Anticancer activity; Quinoxaline derivatives; Trimetrexate analogues; Propargyl-dideazafolic acid analogues

## 1. Introduction

Our contributions on quinoxaline derivatives acting as possible classical and non-classical antifolate agents have considered so far three series of compounds which are referred to as structures **1**, **2** and **3** in Fig. 1.

From the preliminary in vitro screening of all the series examined we found some interesting results at the most dilute concentrations, whereas in general at a concentration of $10^{-4}$ M the percent growth inhibition activity was moderate to high in all subpanel cell lines [1–3].

Our program has now focused attention on some quinoxaline isosters of trimetrexate (TMQ) and 10-propargyl-5,8-dideazafolic acid (CB 3717) (Fig. 2) which, in the series of quinazoline derivatives, represent the most important and promising antifolic experimental drugs to be used in the treatment of cancer and *Pneumocystic carinii* and *Toxoplasma gondii* opportunistic infections [4].

In this context we have designed the series of compounds of formulae **4a–o** and **5a–g** (Figs. 3 and 4) which are structurally related to the above-mentioned leads, preliminary results of which have been recently reported [5].

In particular, from the comparison of the structures of our compounds with those of Fig. 2 it is hopefully reasonable to predict a similar action on the same targets by TMQ and CB 3717, either according to the quinazoline–quinoxaline isosterism or to the lipophilicity of the considered substituents.

## 2. Chemistry

The synthetic approach to obtain the desired compounds **4a–o** and **5a–g** is depicted in Scheme 1. The bromomethylquinoxalines (**6a–c**), according to route (a), were reacted with the appropriate aniline (**7**) (R, $R^1$, $R^2$, $R^3$ of Fig. 3) to give compounds **4a–e,g,i,j,k,m,o**. In the case of condensation of **6b** with 3,4,5-trimethoxyaniline we have isolated compound **8** from the reaction mixture (13% yield) along with the expected **4i**. Compound **4g** could not be isolated as a pure product but on hydrolysis it gave pure **4h**. Compounds **4f,l,n** were in turn obtained by hydrolysis of the corresponding **4e,k,m**.

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0014-827X/98/$19.00 
*PII* S0014-827X(98)00002-0

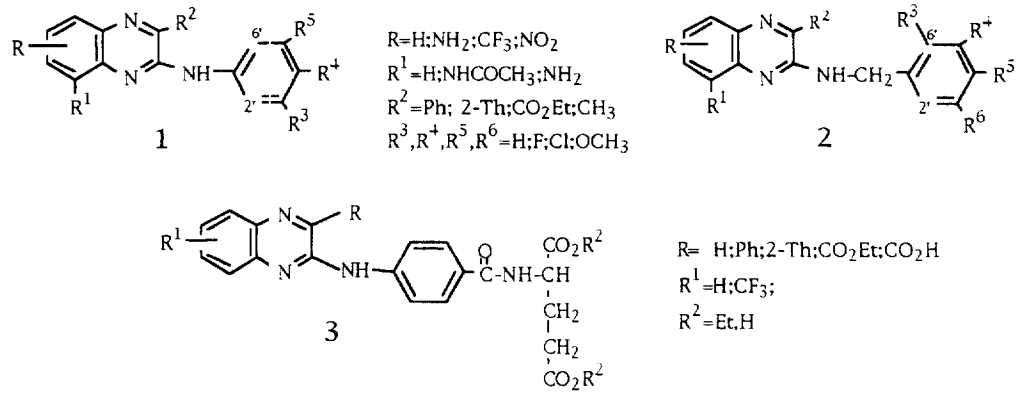



Fig. 1. Quinoxaline derivatives: the three series of compounds studied previously.

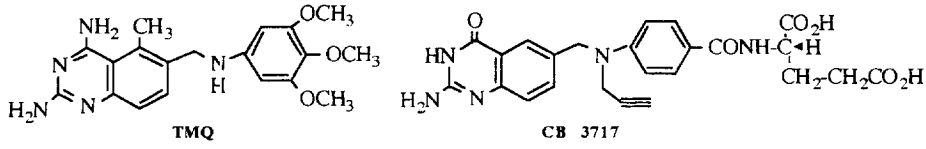
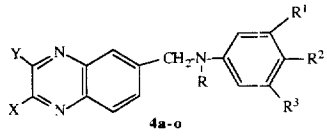


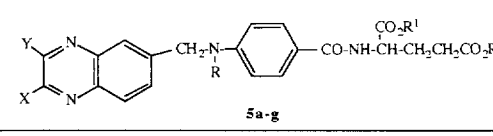

Fig. 2. Trimetrexate (TMQ) and 10-propargyl-5,8-dideazafolic acid (CB 3717).




| Compound | X | Y | R | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|---|---|
| **a** | Ph | Cl | H | OMe | OMe | OMe |
| **b** | Ph | Cl | propargyl | OMe | OMe | OMe |
| **c** | Ph | Cl | propargyl | Cl | Cl | H |
| **d** | Ph | Cl | propargyl | H | F | H |
| **e** | Ph | NH-Piv | propargyl | OMe | OMe | OMe |
| **f** | Ph | $NH_2$ | propargyl | OMe | OMe | OMe |
| **g** | Ph | NH-Piv | H | OMe | OMe | OMe |
| **h** | Ph | $NH_2$ | H | OMe | OMe | OMe |
| **i** | OMe | OMe | H | OMe | OMe | OMe |
| **j** | OMe | OMe | propargyl | OMe | OMe | OMe |
| **k** | Ph | NH-Piv | H | H | COOEt | H |
| **l** | Ph | $NH_2$ | H | H | COOH | H |
| **m** | OMe | OMe | H | H | COOEt | H |
| **n** | OMe | OMe | H | H | COOH | H |
| **o** | Ph | Cl | H | H | COOEt | H |

Fig. 3. Series of compounds of formula **4a–o**.

Compounds **5a,c,g** were obtained in a similar fashion according to route (b) from **6a,c** with ethyl *p*-amino-substituted-benzoylglutamates (**9**) in dimethylacetamide (DMA) at room temperature, while compound **5e** was obtained by reaction of the acid **4n** with the commercially available ethyl glutamate hydrochloride. Saponification of the ester **5a** yielded the acid **5b**, thus indicating that chloroquinoxaline was undergoing nucleophilic ethanolysis. The attempts at saponification of the ester **5g** were unsuccessful and a pure product was not isolated from the reaction mixture.




| Compound | X | Y | R | $R^1$ |
|---|---|---|---|---|
| **a** | Ph | Cl | H | Et |
| **b** | Ph | OEt | H | H |
| **c** | Ph | NH-Piv | H | Et |
| **d** | Ph | $NH_2$ | H | H |
| **e** | OMe | OMe | H | Et |
| **f** | OMe | OMe | H | H |
| **g** | Ph | Cl | propargyl | Et |

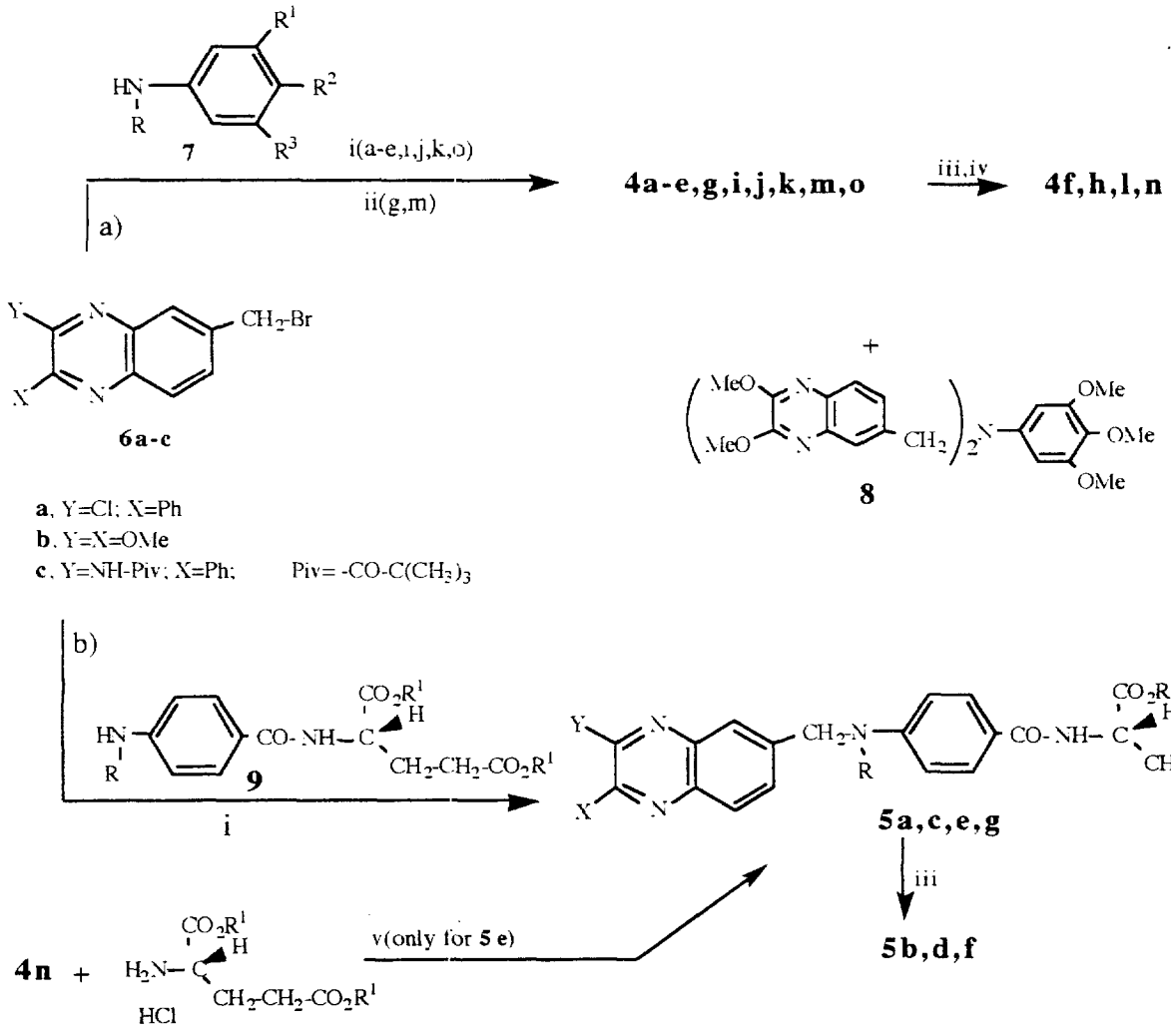
Fig. 4. Series of compounds of formula **5a–g**.

The necessary intermediates **6a–c** have been obtained according to the reaction conditions of Scheme 2. Bromination of 7-methylquinoxaline (**13**) did not occur but it was successful after protection of the amino group to give **14**. In this case we were able to isolate the dibromo derivative **15** formed during the reaction (22% yield). Only compound **6b** is mentioned in a Patent [6] but neither physical data nor preparation methods were reported.

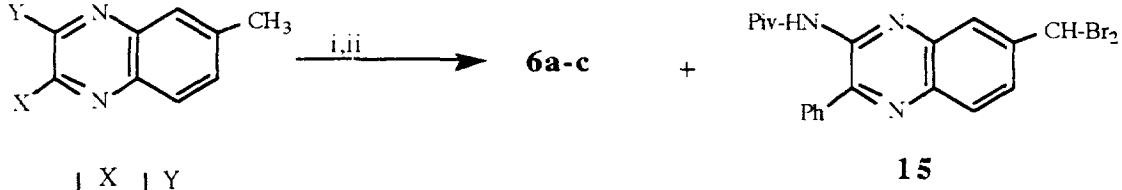
## 3. Experimental

### 3.1. Chemistry

Melting points are uncorrected and were recorded on a Kofler or electrothermal melting point apparatus. UV spectra




Scheme 1. Synthetic approach to obtain compounds **4a–o** and **5a–g**: i, dimethylacetamide (DMA) or dimethylformamide (DMF) at room temperature for 72 h; ii, DMF, $CsHCO_3$ at 65°C for 2 h; iii, a mixture of EtOH and 1 M NaOH; iv, EtOH and 2M HCl at room temperature for 72 h; v, DMF, $(EtO)_2POCN$, $N_2$ and triethylamine (TEA) at room temperature for 2 h




| | X | Y |
|---|---|---|
| **10** | Cl | Cl |
| **11** | Ph | Cl |
| **12** | OMe | OMe |
| **13** | Ph | $NH_2$ |
| **14** | Ph | NH-Piv |

Scheme 2. Reaction conditions necessary to obtain intermediates **6a–c**: i, *N*-bromosuccinimide (NBS), benzoyl peroxide, and $CCl_4$ under reflux for 16 h; ii, 1,3-dibromo-5,5-dimethylhydantoin (DDH), $CCl_4 + h\nu$ under reflux for 24 h.

are qualitative and were recorded in nm for solutions in ethanol with a Perkin-Elmer Lambda 5 spectrophotometer. IR spectra are for nujol mulls and were recorded on Perkin-Elmer 781 instruments. $^1H$ NMR spectra were recorded at 200 MHz with a Varian XL-200 instrument using tetramethylsilane (TMS) as internal standard. Elemental analyses were performed at the Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, Università di Padova (Padua). The analytical results for C, H, and N were within $\pm 0.4\%$ of the theoretical values.

*3.1.1. Intermediates*

Most of the quinoxalines necessary to obtain the starting material (**6a–c**) were known compounds and have been prepared according to the procedures described in the literature. In particular, compounds **10** and **12** were prepared according to the procedure described by Curd et al. [7]. Compound **11** was obtained (2% yield) by Meth-Cohn et al. [8] by an alternative route, whereas in our case it was prepared by $POCl_3$ chlorination of the known compound 6-methylquinoxalin-2-one [9] as previously described [10]. Compound **13** is a new derivative obtained by us by ammonolysis of **11** in a sealed tube and then converted into pivaloyl amide as described below.

*3.1.1.1. 3-Amino-6-methyl-2-phenylquinoxalines (**13**)*

A mixture of **11** (4 g, 15.7 mmol), prepared as described in Ref. [10], and ethanol saturated with gaseous ammonia (40 ml) was heated in a sealed tube at 150°C for 7 h. On cooling, the solid product formed was filtered off and washed with ethanol to give **13** (2.9 g, 78% yield), m.p. 150–152°C, as yellow crystals from methanol. *Anal.* ($C_{15}H_{13}N_3$). C, H, N; IR ($cm^{-1}$): 3400, 3300, 3150; UV (nm): 365, 300, 256, 209; $^1H$ NMR ($CDCl_3$) $\delta$: 7.85 (1H, d, $J_{7,8}=8.6$ Hz, H-8), 7.82–7.75 (2H, m, H-2′,6′), 7.60–7.50 (3H, m, H-3′,4′,5′), 7.47 (1H, d, $J_{5,7}=1.8$ Hz, H-5), 7.29 (1H, dd, $J_{7,8}=8.6$ and $J_{5,7}=1.8$ Hz, H-7), 5.08 (2H, s, $NH_2$), 2.53 (3H, s, Me).

*3.1.1.2. 6-Methyl-2-phenyl-3-trimethylacetylamidoquinoxaline (**14**)*

A mixture of **13** (1 g, 4.24 mmol) and pivaloyl chloride (0.66 g, 5.45 mmol) in DMA (10 ml) in the presence of pyridine (0.67 g, 8.48 mmol) was heated at 110°C under stirring for 2.5 h. On cooling, the solution was poured onto water and a yellow–cream product filtered off to give **14** (1.1 g, 82% yield), m.p. 163–164°C, as white dust from ethanol. *Anal.* ($C_{20}H_{21}N_3O$). C, H, N; IR ($cm^{-1}$): 3490, 3380, 3220, 1660, 1640; UV (nm): 344, 245, 207; $^1H$ NMR ($CDCl_3$) $\delta$: 8.54 (1H, br s, NH), 8.05 (1H, d, $J_{7,8}=8.6$ Hz, H-8), 7.84 (1H, a s, H-5), 7.76–7.68 (2H, m, H-2′,6′), 7.60–7.48 (4H, m, H-3′,4′,5′ + H-7), 2.59 (3H, s, Me), 1.17 (9H, s, $Me_3$–C).

*3.1.1.3. General procedure for preparation of the bromomethylquinoxalines **6a–c** and **15***

(i) A mixture of 2,3-disubstituted-6-methylquinoxalines (**11** and **12**) (1 g, 3.92 mmol), *N*-bromosuccinimide (NBS) (0.75 g, 4.21 mmol) and benzoyl peroxide (20 mg) in tetrachloromethane (30 ml) was heated under reflux for 16 h. On cooling, the precipitate was collected and washed with tetrachloromethane to give the desired compounds **6a,b**.

**6a** (0.75 g, 58% yield), m.p. 190–191°C, as white crystals from ethanol. *Anal.* ($C_{15}H_{10}BrClN_2$): C, H, N; UV (nm): 337, 246, 204; $^1H$ NMR ($CDCl_3$) $\delta$: 8.13 (1H, d, $J_{7,8}=8.5$ Hz, H-8), 8.04 (1H, d, $J_{5,7}=2.05$ Hz, H-5), 7.88–7.84 (2H, m, H-2′,6′), 7.81 (1H, dd, $J_{7,8}=8.5$ and $J_{5,7}$ 2.05 Hz, H-7), 7.63–7.50 (3H, m, H-3′,4′,5′), 4.69 (2H, s, $CH_2$).

**6b** (76% yield), m.p. 159–160°C, from $CCl_4$. *Anal.* ($C_{11}H_{11}BrN_2O_2$): C, H, N; UV (nm): 331, 317, 250, 217; $^1H$ NMR ($CDCl_3$) $\delta$: 7.79 (1H, d, $J_{5,7}=2.0$ Hz, H-5), 7.75 (1H, d, $J_{7,8}=8.6$ Hz, H-8), 7.52 (1H, dd, $J_{7,8}=8.6$ and $J_{5,7}=2.0$ Hz, H-7), 4.65 (2H, s, $CH_2$), 4.15 (3H, s, OMe), 4.14 (1H, s, OMe).

(ii) Benzoyl peroxide (6 × 16 mg) was added at regular intervals of 1 h to a mixture of **14** (1 g, 3.12 mmol) and 1,3-dibromo-5,5-dimethylhydantoin (DDH) (0.44 g, 1.56 mmol) in $CCl_4$ (120 ml) irradiated with a 300 W lamp. Then the mixture was stirred under reflux for an additional 24 h. On cooling, a solid was removed and the organic mother liquors were evaporated in vacuo to give a glassy residue that was purified by flash chromatography on a silica gel column (eluant: ethyl acetate/petrol ether, ratio 2:8, b.p. 40–60°C). On evaporation, the most mobile fraction ($R_f=0.44$) gave the product **15** (0.34 g, 22% yield), m.p. 165–167°C, from ethanol. *Anal.* ($C_{20}H_{19}Br_2N_3O$): C, H, N; IR ($cm^{-1}$): 3290, 1660; UV (nm): 345, 249, 207; $^1H$ NMR ($CDCl_3$) $\delta$: 8.31 (1H, br s, NH), 8.14 (1H, d, $J_{7,8}=8.8$ Hz, H-8), 8.13 (1H, d, $J_{5,7}=2.0$ Hz, H-5), 7.97 (1H, dd, $J_{7,8}=8.8$ and $J_{5,7}=2.0$ Hz, H-7), 7.80–7.70 (2H, m, H-2′,6′), 7.65–7.45 (3H, m, H-3′,4′,5′), 6.84 (1H, s, $CHBr_2$), 1.84 (9H, s, $Me_3$–C).

Successive fractions ($R_f=0.3$) gave **6c** (0.8 g, 64% yield), m.p. 131–133°C, from ethanol. *Anal.* ($C_{20}H_{20}BrN_3O$): C, H, N; IR ($cm^{-1}$): 3280, 1660; UV (nm): 344, 247, 207; $^1H$ NMR ($CDCl_3$) $\delta$: 8.14 (1H, br s, NH), 8.07 (1H, d, $J_{7,8}=8.2$, H-8), 8.06 (1H, s, H-5), 7.78–7.68 (3H, m, H-2′,6′ + H-7), 7.60–7.56 (3H, m, H-3′,4′,5′), 4.68 (2H, s, $CH_2$), 1.18 (9H, s, $Me_3$–C).

*3.1.1.4. General procedure for preparation of the methylanilinoquinoxalines **4a–e,g,i,j,k,m,o***

(i) A mixture of bromomethylquinoxaline (**6a–c**) (2 mmol) and the appropriate aniline **7** (Fig. 3) in the molar ratio 1:2, in dry DMA (20 ml), was stirred at room temperature for 72 h. (In the case of **4d** an equimolar amount of reactants was used in dimethylformamide). Water was then added to the mixture and the precipitates formed were collected and washed with water to give crude products of **4a–e,i,j,k,o**. Purification was accomplished by either recrystallization or column chromatography on silica gel with the solvent indicated in the second column of Table 1. The melting points, yields, analytical and spectroscopic (IR, UV, $^1H$ NMR) data are also reported in Table 1. In the case of purification of **4i** by fractional recrystallization from acetonitrile we isolated compound **8** (Table 1).

(ii) An equimolar amount (1.8 mmol) of **6b**, ethyl *p*-aminobenzoate and $CsHCO_3$ in dry DMF (10 ml) was heated at 65°C under stirring for 2 h. On cooling, the inorganic precipitate was removed by filtration and the mother liquors were evaporated in vacuo. A solid was taken up with ethanol and recrystallized from the same solvent to give **4m** (Table 1). Compound **4g** was obtained from **6c** in an identical manner but the isolated product was impure and is not reported in Table 1. It was used as it was to obtain **4h**.

Table 1
Yields, physical and spectroscopic data of the compounds of Scheme 1

| Compound | M.p. (°C) [a] | Yield (%) | Analysis for C, H, N | IR $\nu_{max}$(nujol) ($cm^{-1}$) | UV $\lambda_{max}$(EtOH) (nm) | $^1$H NMR, $\delta_H$ ($J$ in Hz) Solvent: [A] = $CDCl_3$, [B] = $CDCl_3$–DMSO-$d_6$ (3:1), [C] = DMSO-$d_6$ |
|---|---|---|---|---|---|---|
| **4a** | 109–111 (k) | 46 | $C_{24}H_{22}ClN_3O_3$ | 3380 | 342, 250, 210 | [A] 8.13 (1H, d, $J_{7,8}$ = 8.6, H-8), 8.04 (1H, d, $J_{5,7}$ = 1.6, H-5), 7.87–7.80 (2H, m, H-2″,6″), 7.79 (1H, dd [c], $J_{7,8}$ = 8.6 and $J_{5,7}$ = 1.6, H-7), 7.55–7.52 (3H, m, H-3″,4″,5″), 5.89 (2H, s, H-2′,6′), 4.59 (2H, s, $CH_2$NH), 4.37 (1H, s, N$H$$CH_2$), 3.77 (6H, s, 3′,5′-$OCH_3$), 3.76 (3H, s, 4′-$OCH_3$) |
| **4b** | 178–180 (b) | 81 | $C_{27}H_{24}ClN_3O_3 + 0.25H_2O$ | 3280, 2120 | 340, 249, 211 | [A] 8.14 (1H, d, $J_{7,8}$ = 8.6, H-8), 8.03 (1H, d, $J_{5,7}$ = 1.8, H-5), 7.92–7.82 (2H, m, H-2″,6″), 7.83 (1H, dd [c], $J_{7,8}$ = 8.6 and $J_{5,7}$ = 1.8, H-6), 7.60–7.52 (3H, m, H-3″,4″,5″), 6.17 (2H, s, H-2′,6′), 4.75 (2H, s, 9-$CH_2$N), 4.08 (2H, d, $J$ = 2.4, $CH_2$C≡CH), 3.81 (6H, s, 3′,5′-$OCH_3$), 3.79 (3H, s, 4′-$OCH_3$), 2.33 (1H, t, $J$ = 2.4, $CH_2$C≡C$H$) |
| **4c** | 148–153 (k) | 20 | $C_{24}H_{16}Cl_4N_4 + 0.25H_2O$ | 3220, 2120 | 340, 349, 209 | [A] 8.14 (1H, d, $J$ = 8.6, H-8), 7.93 (1H, d, $J_{5,7}$ = 1.8, H-5), 8.92–8.82 (2H, m, H-2″,6″), 7.72 (1H, dd, $J_{7,8}$ = 8.6 and $J_{5,7}$ = 1.8, H-7), 7.60–7.50 (3H, m, H-3″,4″,5″), 7.26 (1H, d, $J$ = 9.0, H-6′), 6.95 (1H, d, $J$ = 3.0, H-2′), 6.71 (1H, dd, $J_{5',6'}$ = 9.0 and $J_{2',6'}$ = 3.0, H-6′), 4.77 (2H, s, 9-$CH_2$N), 4.11 (2H, d, $J$ = 2.2, $CH_2$C≡CH), 2.32 (1H, t, $J$ = 2.2, $CH_2$C≡C$H$) |
| **4d** | 117–119 (a) | 96 | $C_{24}H_{17}ClFN_3$ | 3230, 2100 | 341, 348, 205 | [A] 8.13 (1H, d, $J_{7,8}$ = 8.6, H-8), 7.88 (1H, d, $J_{5,7}$ = 1.8, H-5), 7.86–7.82 (2H, m, H-2″,6″), 7.78 (1H, dd, $J_{7,8}$ = 8.6 and $J_{5,7}$ = 1.8, H-7), 7.55–7.52 (3H, m, H-3″,4″,5″), 6.93–6.84 (4H, m, H-2′,3′,5′,6′), 4.71 (2H, s, $CH_2$N), 4.05 (2H, d, $J$ = 2.2, $CH_2$C≡CH), 2.29 (1H, t, $J$ = 2.2, $CH_2$C≡C$H$) |
| **4e** | 154–156 (k,a) | 83 | $C_{32}H_{34}N_4O_4$ | 3280, 3240, 2120, 1650 | 344, 248, 212 | [A] 8.07 (1H, d [c], $J_{7,8}$ = 8.4, H-8), 8.05 (1H, a, s, H-5), 7.77–7.60 (3H, m, H-2″,6″ + H-7), 6.60–7.50 (3H, m, H-3″,4″,5″), 6.17 (2H, s, H-2′,6′), 4.72 (2H, s, $CH_2$), 4.07 (2H, d, $J$ = 1.8, $CH_2$C≡CH), 3.80 (6H, s, 3′,5′-$OCH_3$), 3.79 (3H, s, 4′-$OCH_3$), 2.30 (1H, t, $J$ = 1.8, $CH_2$C≡CH), 1.73 (9H, s, C($CH_3$)$_3$) |
| **4f** | 178–179 (j,a) | 63 | $C_{27}H_{26}N_4O_3$ | 3480, 3320, 3210, 2120 | 368, 292 infl, 254, 215 | [A] 7.95 (1H, d, $J_{7,8}$ = 8.4, H-8), 7.83–7.75 (2H, m, H-2″,6″), 7.65 (1H, d, $J_{5,7}$ = 1.8, H-5), 7.52–6.62 (3H, m, H-3″,4″,5″), 7.45 (1H, dd, $J_{7,8}$ = 8.4 and $J_{5,7}$ = 1.8, H-7), 6.18 (2H, s, H-2′,6′), 5.12 (2H, s, $NH_2$ [b]), 4.67 (2H, s, 9-$CH_2$), 4.06 (2H, d, $J$ = 2.2, $CH_2$C≡CH), 3.81 (6H, s, 3′,5′-$OCH_3$), 3.78 (3H, s, 4′-$OCH_3$), 2.30 (1H, t, $J$ = 2.2, $CH_2$C≡C$H$) |
| **4h** | 181–183 (l) | 42 | $C_{24}H_{24}N_4O_3$ | 3490, 3320 | 367, 292 infl, 255, 213 | [A] 7.94 (1H, d, $J_{7,8}$ = 8.4, H-8), 7.80–7.76 (3H, m, H-3′,4′,5′), 7.67 (1H, d, $J_{5,7}$ = 2.2, H-5), 7.56–7.52 (2H, m, H-2″,6″), 7.46 (1H, dd, $J_{7,8}$ = 8.4 and $J_{5,7}$ = 2.2, H-7), 5.91 (2H, s, H-2′,6′), 5.12 (2H, s, $NH_2$), 4.49 (2H, s, $CH_2$NH), 3.78 (6H, s, 3′,5′-$OCH_3$), 3.76 (3H, s, 4′-$OCH_3$) |
| **4i** | 139–140 (b) | 39 | $C_{20}H_{23}N_3O_5 + 0.25H_2O$ | 3380 | 329, 314, 249, 212 | [A] 7.78 (1H, d, $J_{5,7}$ = 2.0, H-5), 7.76 (1H, d, $J_{7,8}$ = 8.6, H-8), 7.51 (1H, dd, $J_{7,8}$ = 8.6 and $J_{5,7}$ = 2.0, H-7), 5.91 (2H, s, H-2′,6′), 4.46 (2H, s, $CH_2$), 4.15 (3H, s, 3-$OCH_3$), 4.14 (3H, s, 2-$OCH_3$), 4.09 (1H, br s, NH), 3.78 (6H, s, 3′,5′-$OCH_3$), 3.76 (3H, s, 4′-$OCH_3$) |
| **4j** | 104–105 (n) | 63 | $C_{23}H_{25}N_3O_5$ | 3270 | 329, 315, 249, 213 | [A] 7.75 (1H, d, $J_{7,8}$ = 8.8, H-8), 7.75 (1H, d, $J_{5,7}$ = 2.0, H-5), 7.48 (1H, dd, $J$ = 8.8 and 2.0, H-7), 6.19 (2H, s, H-2′,6′), 4.68 (2H, 9-$CH_2$), 4.15 (3H, s, 3-$OCH_3$), 4.14 (3H, s, 2-$OCH_3$), 4.04 (2H, a s, $CH_2$C≡CH), 3.81 (6H, s, 3′,5′-$OCH_3$), 3.79 (3H, s, 4′-$OCH_3$), 2.30 (1H, t, $CH_2$C≡C$H$) |

Table 1 (*continued*)

| Compound | M.p. (°C) [a] | Yield (%) | Analysis for C, H, N | IR $\nu_{max}$(nujol) ($cm^{-1}$) | UV $\lambda_{max}$(EtOH) (nm) | $^1$H NMR, $\delta_H$ ($J$ in Hz) Solvent: [A] = $CDCl_3$, [B] = $CDCl_3$–DMSO-$d_6$ (3:1), [C] = DMSO-$d_6$ |
|---|---|---|---|---|---|---|
| **4k** | 67 (k) | 50 | $Cd_{29}H_{30}N_4O_3 + 0.5H_2O$ | 3340, 1700, 1685 | 344, 302, 244, 224, 204 | [A] 8.11 (1H, s, NHCO), 8.02 (1H, d, $J_{7,8}$ = 8.6, H-8), 7.96 (1H, d, $J_{5,7}$ = 1.0, H-5), 7.84 (2H, d, $J$ = 8.8, H-2′,6′), 7.74–7.69 (2H, m, H-2″,6″), 7.63 (1H, dd, $J_{7,8}$ = 8.6 and $J_{5,7}$ = 1.0, H-7), 7.55–7.51 (3H, m, H-3″,4″,5″), 6.58 (2H, d, $J$ = 8.8, H-3′,5′), 4.92 (1H, br s, N*H*$CH_2$), 4.59 (2H, d, $J$ = 3.2, N*H*$CH_2$), 4.29 (2H, q, C*H*$_2$$CH_3$), 1.34 (3H, t, C*H*$_3$$CH_2$), 1.16 (9H, s, C($CH_3$)$_3$) |
| **4l** | 290–294 (d) | 69 | $C_{22}H_{18}N_4O_2$ | 3460, 3430, 3280, 1675 | 365, 300, 258, 217 | [B] 7.81–7.72 (4H, m, H-2″,6″ + H-2′,6′), 7.70 (1H, d, $J_{7,8}$ = 8.4, H-8), 7.60–7.50 (4H, m, H-3″,4″,5″ + H-5), 7.36 (1H, dd, $J_{7,8}$ = 8.4 and $J_{5,7}$ = 1.6, H-7), 6.87 (1H, t, $J$ = 5.0, N*H*$CH_2$), 6.62 (2H, d, $J$ = 8.8, H-3′,5′), 6.12 (2H, s, $NH_2$ [b]), 4.53 (2H, d, $J$ = 5.0, C*H*$_2$NH), 3.89 (1H, br s, COOH [b]) |
| **4m** | 148–150 (i) | 60 | $C_{20}H_{21}N_3O_4$ | 3390, 1700 | 328, 314, 307, 246, 209 | [A] 7.87 (2H, d, $J$ = 8.6, H-2′,6′), 7.75 (1H, d, $J_{7,8}$ = 8.6, H-8), 7.73 (1H, d, $J_{5,7}$ = 1.8, H-5), 7.46 (1H, dd, $J_{7,8}$ = 8.6 and $J_{5,7}$ = 1.8, H-7), 6.62 (2H, d, $J$ = 8.6, H-3′,5′), 4.66 (1H, br s, N*H*$CH_2$), 4.55 (2H, d, $J$ = 4.7, $CH_2$NH), 4.30 (2H, q, C*H*$_2$$CH_3$), 4.14 (3H, s, 3-$OCH_3$), 4.13 (3H, s, 2-$OCH_3$), 1.35 (3H, t, C*H*$_3$$CH_2$) |
| **4n** | 259–260 (a) | 83 | $C_{18}H_{17}N_3O_4 + 0.5H_2O$ | 3370, 1710, 1680 | 326, 312, 303, 230, 211 | [B] 7.80–7.70 (4H, m, H-2′,6′ + H-5 + H-8), 7.49 (1H, dd, $J_{7,8}$ = 8.4 and $J_{5,7}$ = 1.8, H-7), 6.62 (2H, d, $J$ = 8.6, H-3′,5′), 4.53 (2H, d, $J$ = 4.8, C*H*$_2$NH), 4.09 (3H, s, 3-$OCH_3$), 4.08 (3H, s, 2-$OCH_3$) |
| **4o** | 172–175 (a) | 57 | $C_{24}H_{20}ClN_2O_3$ | 3400, 1680 | 341, 303, 248, 205 | [C] 8.15 (1H, d, $J_{7,8}$ = 8.8, H-8), 7.98 (1H, a s, H-5), 7.95 (1H, d, $J$ = 8.8, H-7), 7.86–7.80 (2H, m, H-2″,6″), 7.68 (2H, d, $J$ = 8.2, H-2′,6′), 7.58–7.52 (3H, m, H-3″,4″,5″), 7.41 (1H, t, $J$ = 4.8, N*H*$CH_2$), 6.68 (2H, d, $J$ = 8.2, H-3′,5′), 4.66 (2H, d, $J$ = 4.8, C*H*$_2$NH), 4.19 (2H, q, C*H*$_2$$CH_3$), 1.25 (3H, t, C*H*$_3$$CH_2$) |
| **5a** | 145–147 (n,b) | 33 | $C_{31}H_{31}ClN_4O_5 + 0.25\ H_2O$ | 3420, 3300, 1740, 1720 | 336, 294, 245, 204 | [A] 8.12 (1H, d, $J_{7,8}$ = 8.6, H-8), 7.99 (1H, d, $J_{5,7}$ = 1.9, H-5), 7.92–7.83 (2H, m, H-2″,6″), 7.77 (1H, dd, $J_{7,8}$ = 8.6 and $J_{5,7}$ = 1.8, H-7), 7.70 (2H, d, $J$ = 8.7, H-2′,6′), 7.60–7.50 (3H, m, H-3″,4″,5″), 6.80 (1H, d, $J$ = 7.6, CHN*H*CO), 6.62 (2H, d, $J$ = 8.7, H-3′,5′), 4.85–4.70 (1H, m, NHC*H*$CH_2$), 4.67 (2H, s, C*H*$_2$NH), 4.21 (2H, q, C*H*$_2$$CH_3$), 4.12 (2H, q, C*H*$_2$$CH_3$), 2.60–2.05 (4H, m, $CH_2CH_2$), 1.29 (3H, t, C*H*$_3$$CH_2$), 1.21 (3H, t, C*H*$_3$$CH_2$) |
| **5b** | 135–140 (i) | 89 | $C_{29}H_{28}N_4O_6$ | 3430, 1710, 1620 | 344, 299, 260, 216, 206 | [B] 8.14–8.08 (2H, m, H-2″,6″), 7.98 (1H, d, $J_{7,8}$ = 8.4, H-8), 7.79 (1H, d, $J_{5,7}$ = 1.8, H-5), 7.68 (2H, d, $J$ = 8.6, H-2′,6′), 7.64–7.55 (1H, dd [c], $J_{7,8}$ = 8.4 and $J_{5,7}$ = 1.8, H-7), 7.55–7.45 (3H, m, H-3″,4″,5″), 6.65 (2H, d, $J$ = 8.6, H-3′,5′), 4.60 (2H, q, C*H*$_2$$CH_3$), 4.59 (2H, s, C*H*$_2$NH), 4.59 (1H, m, NHC*H*$CH_2$), 2.60–2.00 (4H, m, $CH_2CH_2$), 1.48 (3H, t, C*H*$_3$$CH_2$) |
| **5c** | 70 (dec.) (o,j) | 48 | $C_{36}H_{41}N_5O_6 + 0.5H_2O$ | 3320 br, 1730, 1630 | 340, 294, 243, 203 | [A] 8.09 (1H, s, N*H*CO [b]), 8.03 (1H, d, $J_{7,8}$ = 8.8, H-8), 7.99 (1H, d, $J_{5,7}$ = 1.4, H-5), 7.76–7.70 (2H, m, H-2″,6″), 7.64 (2H, d, $J$ = 8.6, H-2′,6′), 7.64 (1H, dd [c], $J_{7,8}$ = 8.8 and $J_{5,7}$ = 1.4, H-7), 7.60–7.50 (3H, m, H-3″,4″,5″), 6.79 (1H, d, $J$ = 7.6, CHN*H*CO), 6.60 (2H, d, $J$ = 8.6, H-3′,5′), 4.82–4.72 (1H, m, NHC*H*$CH_2$), 4.60 (2H, s, C*H*$_2$NH), 4.21 (2H, q, C*H*$_2$$CH_3$), 4.09 (2H, q, $CH_2$–$CH_3$), 4.09 (2H, q, $CH_2CH_3$), 2.55–2.05 (4H, m, $CH_2CH_2$), 1.28 (3H, t, C*H*$_3$$CH_2$), 1.20 (3H, t, C*H*$_3$$CH_2$), 1.16 (9H, s, C($CH_3$)$_3$) |

Table 1 (*continued*)

| Compound | M.p. (°C) [a] | Yield (%) | Analysis for C, H, N | IR $\nu_{max}$ (nujol) ($cm^{-1}$) | UV $\lambda_{max}$ (EtOH) (nm) | $^1H$ NMR, $\delta_H$ ($J$ in Hz) Solvent: [A] = $CDCl_3$, [B] = $CDCl_3$–DMSO-$d_6$ (3:1), [C] = DMSO-$d_6$ |
|---|---|---|---|---|---|---|
| **5d** | 163–164 (i) | 83 | $C_{27}H_{25}N_5O_5 + 0.75H_2O$ | 3340 br, 3190, 1720, 1680 | 364, 293, 259, 207 | [B] 7.85 (1H, d, $J_{7,8}$ = 8.4, H-8), 7.80–7.70 (2H, m, H-2″,6″), 7.65 (2H, d, $J$ = 8.6 H-2′,6′), 7.59 (1H, d, $J_{5,7}$ = 1.4, H-5), 7.56–7.48 (3H, m, H-3″,4″,5″), 7.39 (1H, dd, $J_{7,8}$ = 8.4 and $J_{5,7}$ = 1.4, H-7), 6.62 (2H, d, $J$ = 8.6, H-3′,5′), 4.70–4.55 (1H, m, NHC*H*$CH_2$), 4.54 (2H, s, $CH_2$NH), 2.65–2.00 (4H, m, $CH_2CH_2$) |
| **5e** | 111–113 (m,a) | 49 | $C_{27}H_{32}N_4O_7$ | 3410, 3320, 1740 | 330, 316, 308 sh, 302, 247, 207 | [A] 7.75–7.65 (2H, m, H-5,8), 7.67 (2H, d, $J$ = 8.6, H-2′,6′), 7.45 (1H, dd, $J_{7,8}$ = 8.4 and $J_{5,7}$ = 1.8, H-7), 6.77 (1H, d, $J$ = 7.4, N*H*CH), 6.64 (2H, d, $J$ = 8.6, H-3′,5′), 4.78 (1H, m, NHC*H*$CH_2$), 4.54 (1H, s, $CH_2$NH), 4.22 (2H, q, $CH_2CH_3$), 4.15 (3H, s, 3-$OCH_3$), 4.14 (3H, s, 2-$OCH_3$), 4.09 (2H, q, $CH_2CH_3$), 2.60–2.00 (4H, m, $CH_2CH_2$), 1.30 (3H, t, $CH_3CH_2$), 1.21 (3H, t, $CH_3CH_2$) |
| **5f** | 70 (dec.) | 63 | $C_{27}H_{24}N_4O_7 + 2H_2O$ | 3380 br, 1720 br | 330, 316, 302, 246, 209 | [C] 8.12 (1H, d, $J$ = 7.8, N*H*CH [b]), 7.68 (1H, s, H-5), 7.64 (3H, m, H-2′,6′ + H-7), 7.52 (2H, d, $J_{7,8}$ = 7.8, H-8), 7.01 (1H, s, N*H*$CH_2$), 6.63 (2H, d, $J$ = 8.4, H-3′,5′), 4.51 (2H, d, $J$ = 3.4, $CH_2$NH), 4.25–4.14 (1H, m, NHC*H*$CH_2$), 4.02 (6H, s, 2,3-$OCH_3$), 2.42–1.91 (4H, m, $CH_2CH_2$) |
| **5g** | 60 (k) | 86 | $C_{34}H_{33}ClN_4O_5$ | 3250 br, 1720 br | 340, 292, 248, 205 | [A] 8.14 (1H, d, $J_{7,8}$ = 8.8, H-8), 7.94 (1H, a s, H-5), 7.90–7.80 (2H, m, H-2″,6″), 7.74 (2H, d, $J$ = 8.4, H-2′,6′), 7.55–7.52 (3H, m, H-3″,4″,5″), 6.90–6.84 (1H, m, H-7), 6.86 (2H, d, $J$ = 8.8, H-3′,5′), 6.69 (1H, d, $J$ = 7.6, N*H*CH), 4.89 (2H, s, $CH_2$N), 4.81–4.75 (1H, m, $CH_2CH$NH), 4.28–4.17 (4H, m, $CH_2CH_3 + CH_2C{\equiv}CH$), 4.10 (2H, q, $CH_2CH_3$), 2.60–2.05 (5H, m, $CH_2CH_2 + HC{\equiv}C{-}CH_2$), 1.29 (3H, t, $CH_3CH_2$), 1.22 (3H, t, $CH_3CH_2$) |
| **8** | 185–187 (b) | 13 | $C_{31}H_{33}N_5O_7 + H_2O$ | | 329, 315, 248, 213 | [A] 7.75 (2H, d, $J_{7,8}$ = 8.6, H-8), 7.69 (2H, d, $J_{5,7}$ = 1.4, H-5), 7.43 (2H, d, $J$ = 8.6, H-7), 6.03 (2H, s, H-2′,6′), 4.82 (4H, s, $CH_2NCH_2$), 4.15 (12H, s, 2,3-$OCH_3$), 3.76 (3H, s, 4′-$OCH_3$), 3.67 (6H, s, 3′,5′-$OCH_3$) |

[a] Purification procedure: (a) crystallized from ethanol; (b) crystallized from acetonitrile; (c) crystallized from a mixture of petroleum ether at 40–60°C and diethyl ether; (d) crystallized from acetic acid; (e) crystallized from $CCl_4$; (f) crystallized from a mixture of water and ethanol; (g) crystallized from methanol; (h) washed with diethyl ether; (i) washed with ethanol; (j) washed with a mixture of petroleum ether at 40–60°C and diethyl ether; (k) from flash chromatography (eluent: mixture of petroleum ether at 40–60°C and ethyl acetate); (l) from flash chromatography (eluent: mixture of diethyl ether and acetone; (m) from flash chromatography (eluent: mixture of $CH_2Cl_2$ and ethyl acetate); (n) from flash chromatography (eluent: diethyl ether); (o) from flash chromatography (eluent: mixture of $CH_2Cl_2$ and acetone).

[b] Exchanges with $H_2O$.

[c] Partially obscured by other resonances.

a s, apparent singlet; br, broad.

*3.1.1.5. General procedure for preparation of the acids* ***4f,h,l,n***

(i) Compound **4e** (0.15 g, 0.28 mmol) in a mixture of ethanol (4 ml) and 2 M HCl aqueous solution (3 ml) was stirred at room temperature for 72 h. Then the mixture was made alkaline with an acqueous solution of 8 M $NH_4OH$ to give a gummy mass that was extracted with chloroform. The chloroform layer was dried and evaporated to dryness. The residue was taken up with dry ether to give the yellow–cream solid of **4f** (Table 1).

(ii) Compound **4g** (0.4 g, 0.8 mmol) in a mixture of ethanol (5 ml) and 1 M NaOH aqueous solution (7 ml) was heated at 65°C for 18 h. On cooling, a precipitate was collected and flash-chromatographed on silica gel (eluant: ether/acetone, ratio 9:1) to give **4h**. The data are reported in Table 1. The preparation of **4l** from **4k** was identical, but after cooling the reaction mixture was made acidic with 1 M HCl aqueous solution.

(iii) Compound **4m** (0.22 g, 0.6 mmol), suspended in a mixture of ethanol (6 ml), water (6 ml) and 1 M NaOH aqueous solution (2 ml), was heated at 80°C under stirring for 5 h. The solution was then made acidic with 2 M HCl aqueous solution and a precipitate of **4n** was filtered off, washed out with water and purified as in Table 1.

*3.1.1.6. General procedure for preparation of compounds* ***5a,c,e,g***

(i) A mixture of compounds **6a,c** (2 mmol) and **9** (R and R′ as in Fig. 4) in the molar ratio 1:2 in DMA (10–15 ml) was stirred at room temperature for 72 h. On evaporation of the solvent, crude products **5a** and **c** were obtained and, after dilution with water, **5g**. Compounds **5a,c,g** were purified according to Table 1.

(ii) Diethyl cyanophosphonate (0.27 g, 1.62 mmol) dissolved in dry DMF (4 ml) was added to a mixture of **4n** (0.5 g, 1.47 mmol) and L-diethyl glutamate hydrochloride (0.39 g, 1.62 mmol) in dry DMF (20 ml). Then an excess of TEA (0.33 g, 3.25 mmol) was added to the mixture and stirred at room temperature under a stream of nitrogen for 2 h. The resulting suspension was poured onto a mixture of ethyl acetate and benzene in a 3:1 ratio. The organic phase was washed with water (100 ml), then with a saturated solution of sodium carbonate (120 ml) and again with water (100 ml) and, if necessary, with saturated sodium chloride solution. It was eventually dried over anhydrous sodium sulfate. On evaporation, the oily residue, constituted of **5e**, was purified as indicated in Table 1.

*3.1.1.7. Saponification of the esters* ***5a,c,e*** *into the acids* ***5b,d,f***

A small amount of the ester (**5a,c,e**) (0.7 mmol) suspended in a mixture of ethanol (8 ml) and 1 M NaOH aqueous solution (4 ml) was heated at 65°C under stirring for 2 h (**5a**), for 18 h (**5c**), and only stirred at room temperature for 3 h (**5e**). The work-up of the alkaline solution on acifidication (i) with 1 M HCl aqueous solution gave **5b,f**, purified as in Table 1, and (ii) with 1 M HCl aqueous solution up to pH = 6.7 gave **5d**, purified as indicated in Table 1.

*3.2. Pharmacology*

Evaluation of anticancer and anti-HIV activity was performed on 12 of the 18 compounds (structures **4b–d,f,h,i,l,m**, **5a,e,f** and **8** of Figs. 3 and 4 and Scheme 1) at the National Cancer Institute (NCI) of Bethesda, MD, USA, following the well-known [11] in vitro disease-oriented antitumor screening program against a panel of 60 human tumor cell lines and the anti-HIV drug testing system [12]. No compound exhibited anti-HIV activity. The anticancer activity of each compound is deduced from dose–response curves and is presented in three different tables according to the data provided by NCI. In Table 2 the response parameters $GI_{50}$, TGI and $LC_{50}$ refer to the concentration of the agent in the assay that produced 50% growth inhibition, total growth inhibition, and 50% cytotoxicity, respectively, and are expressed as mean graph midpoints.

In Table 3 we reported the activities of those compounds which showed percent growth inhibition greater than 40% on subpanel cell lines at $10^{-4}$ M. Compounds **4n** and **5d** were not tested at this molar concentration. The most dilute concentrations ($10^{-8}$, $10^{-7}$, and $10^{-6}$ M) were considered in the case of compounds **4b,h,m,n**, **5d,e** and **8** which showed high selectivity (Table 4).

## 4. Results and discussion

From the data of Table 2 we can deduce that the average sensitivity of all cell lines towards the tested agent, represented as mean graph midpoints, falls in the concentration range $10^{-4.5}$–$10^{-4}$ M. Mean graph midpoints for compounds **4b,c,f,h,i,l,m**, **5a,e,f** and **8** show that only $GI_{50}$ was significant

Table 2
$-\log_{10}GI_{50}$, $-\log_{10}TGI$, $-\log_{10}LC_{50}$ mean graph midpoints (MG-MID) of in vitro inhibitory activity tests for compounds **4b,c,f,h,i,l,m**, **5a,e,f** and **8** against human tumor cell lines [a]

| Compound | $-\log_{10}GI_{50}$ | $-\log_{10}TGI$ | $-\log_{10}LC_{50}$ |
|---|---|---|---|
| **4b** | 4.14 | 4.03 | 4.00 |
| **4c** | 4.02 | 4.00 | 4.00 |
| **4f** | 4.52 | 4.10 | 4.01 |
| **4h** | 4.59 | 4.13 | 4.01 |
| **4i** | 4.04 | 4.00 | 4.00 |
| **4l** | 4.03 | 4.00 | 4.00 |
| **4m** | 4.03 | 4.00 | 4.00 |
| **5a** | 4.52 | 4.12 | 4.04 |
| **5e** | 4.35 | 4.12 | 4.02 |
| **5f** | 4.06 | 4.00 | 4.00 |
| **8** | 4.47 | 4.32 | 4.30 |

MG-MID = mean graph midpoints, the average sensitivity of all cell lines towards the test agent.
[a] From NCI.

Table 3
Percent tumor growth inhibition recorded on subpanel cell lines at $10^{-4}$ M of compounds **4b–d,f,h,i,l,m**, **5a,e,f** and **8**

| Panel/cell lines | 4b | 4c | 4d | 4f | 4h | 4i | 4l | 4m | 5a | 5e | 5f | 8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Leukemia* | | | | | | | | | | | | |
| CCRF-CEM | 75 | | 90 | 144 | 115 | 55 | 45 | | 90 | nt | 94 | nt |
| HL-60(TB) | 63 | | 104 | 153 | 153 | | | 63 | 95 | nt | | |
| K-562 | 76 | | nt | 99 | 85 | 51 | 47 | | 80 | nt | 86 | nt |
| MOLT-4 | 52 | | 112 | 149 | 129 | 48 | 56 | | 126 | 100 | 98 | |
| RPMI-8226 | 142 | nt | 70 | 153 | nt | nt | | nt | 107 | 53 | | |
| SR | nt | | 69 | 145 | 88 | 53 | | 50 | 96 | 112 | | |
| *Non-small cell lung cancer* | | | | | | | | | | | | |
| A549/ATCC | 53 | | | 91 | 109 | | | | 65 | | | |
| EKVX | 39 | | | 89 | 97 | 56 | nt | | 72 | 47 | | 54 |
| HOP-62 | | | | 84 | 78 | | | 49 | 48 | 68 | | 90 |
| HOP-92 | | | 80 | nt | 107 | 44 | nt | 57 | 164 | nt | nt | nt |
| NCI-H226 | | nt | nt | 129 | 81 | 40 | | | 99 | 70 | | 47 |
| NCI-23 | | | 56 | 99 | 122 | 41 | 52 | 41 | 55 | | | 50 |
| NCI-H322M | | 53 | 52 | 66 | 62 | 55 | 40 | | 68 | nt | | nt |
| NCI-H460 | 68 | | 46 | 108 | 94 | | | | 92 | nt | | nt |
| NCI-H522 | 49 | 45 | 73 | 120 | 124 | 53 | 43 | 42 | 125 | 146 | | 46 |
| *Colon cancer* | | | | | | | | | | | | |
| COLO 205 | 66 | | 62 | 161 | 166 | 40 | | | 161 | 148 | | |
| HCC-2998 | | 102 | 40 | 71 | 75 | | | | 52 | 74 | | nt |
| HCT-116 | 75 | | | 125 | 188 | 40 | 57 | 52 | 66 | 42 | nt | 59 |
| HCT-15 | | 47 | 52 | 99 | 97 | | | | 56 | 45 | 66 | |
| HT29 | 43 | | 64 | 129 | 97 | | | | 46 | 69 | | |
| KM12 | | 43 | 45 | nt | 142 | | nt | | | 47 | | |
| SW-620 | 165 | | 42 | 75 | 70 | | | | | 52 | 75 | |
| *Central nervous system cancer* | | | | | | | | | | | | |
| SF-268 | 74 | | | 83 | 101 | | 41 | | 66 | 88 | | 59 |
| SF-295 | 40 | | | 116 | 104 | 53 | | | 55 | 52 | | 77 |
| SF-539 | | | | 90 | 104 | | 53 | 43 | 92 | 192 | nt | 76 |
| SNB-19 | | | | 86 | 71 | | | | 50 | | | 102 |
| SNB-75 | 40 | | | 109 | 111 | 59 | 54 | 95 | 96 | nt | | 171 |
| U251 | 85 | | | 92 | 90 | | 43 | 46 | 56 | | | 83 |
| *Melanoma* | | | | | | | | | | | | |
| LOX IMVI | 145 | 45 | | 130 | 92 | | 58 | | 87 | 83 | 76 | 47 |
| MALME-3M | | | | 108 | 121 | 47 | | | 197 | 170 | 52 | 44 |
| M14 | | | | 102 | 167 | | | | 153 | 171 | | 45 |
| SK-MEL-2 | | | | 121 | 97 | | | | 176 | 178 | | 65 |
| SK-MEL-28 | 42 | | | 89 | 120 | | | | nt | 147 | | 41 |
| SK-MEL-5 | 69 | | 84 | 188 | 197 | 46 | | | 197 | 179 | | |
| UACC-257 | 62 | | 42 | nt | 141 | | | | nt | 171 | | |
| UACC-62 | | | | 120 | 118 | | nt | | nt | 172 | | |
| *Ovarian cancer* | | | | | | | | | | | | |
| IGROV1 | | | | 80 | 86 | | | | 98 | nt | | nt |
| OVCAR-3 | | 59 | | 120 | 108 | 48 | | | 121 | 153 | | 59 |
| OVCAR-4 | | | 57 | 84 | 99 | | | 53 | 103 | 72 | | |
| OVCAR-5 | | | | 65 | 49 | | | | | | | |
| OVCAR-8 | | | | 95 | 138 | | | 55 | nt | 41 | | |
| SK-OV-3 | | | nt | nt | 87 | | | | | | | 65 |
| *Renal cancer* | | | | | | | | | | | | |
| 786-O | | | | 75 | 83 | | | 51 | 55 | 61 | nt | 71 |
| A498 | | | 44 | nt | 112 | | | | 110 | nt | | nt |
| ACHN | 55 | | 54 | 108 | 99 | | | 40 | 82 | 138 | | 54 |
| CAKI-1 | | | 42 | nt | 107 | | nt | | 83 | 41 | nt | |
| RXF-393 | 95 | nt | nt | 101 | 120 | | | nt | 74 | 84 | | 107 |
| SN12C | 41 | | | 95 | 73 | | | | 70 | 81 | 41 | |
| TK-10 | | | | 73 | 97 | nt | | nt | 53 | 56 | | 64 |
| UO-31 | | | 55 | 117 | 97 | | 53 | | 120 | 159 | | 56 |

Table 3 (*continued*)

| Panel/cell lines | **4b** | **4c** | **4d** | **4f** | **4h** | **4i** | **4l** | **4m** | **5a** | **5e** | **5f** | **8** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Prostate cancer* | | | | | | | | | | | | |
| PC-3 | nt | nt | | nt | 103 | 52 | | | nt | 63 | 68 | |
| DU-145 | nt | nt | 51 | nt | 100 | | 71 | | nt | 57 | | |
| *Breast cancer* | | | | | | | | | | | | |
| MCF7 | nt | nt | 59 | nt | 143 | 61 | | | nt | 64 | | 55 |
| MCF7/ADR-RES | nt | nt | | nt | 129 | 65 | | 49 | nt | | | |
| MDA-MB-231/ATCC | nt | nt | | nt | 90 | | 46 | 59 | nt | nt | | nt |
| HS-578T | nt | nt | | nt | nt | nt | 50 | nt | nt | 111 | | 124 |
| MDA-MB-435 | nt | nt | 44 | nt | 141 | | | | nt | 101 | | |
| BT-549 | nt | nt | | nt | nt | nt | | nt | nt | 49 | | 93 |
| T-47D | nt | nt | 69 | nt | 104 | 70 | 67 | 57 | nt | 125 | | |
| MDA-N | nt | nt | | nt | 95 | | | | nt | 92 | | |

in the case of **4f,h**, **5a,e** and **8**, whereas both TGI and $LC_{50}$ exhibited lower values of sensitivity. The data of Table 3 show that at $10^{-4}$ M the most active compound was **4h** (57 cell lines out of 60), endowed with high values of percent growth inhibition in all subpanel cell lines. In decreasing order of activity related to the number of subpanel cell lines inhibited we can record compound **4f > 5e > 5a > 4d = 8 > 4b > 4i > 4m = 4l > 5f > 4c**. Compounds **4f,h** and **5a,e** possess comparable percent tumor growth inhibition activity in all subpanel cell lines except for breast cancer cell lines that in the case of **4f** and **5a** were not tested. Interestingly, from the data of Table 4 we can observe that a certain number of derivatives (**4b,h,m,n**, **5d,e** and **8**) retain some cell line selectivity at the most dilute concentrations. Among these, compound **4n** exhibited the highest values of percent growth inhibition on the UO-31 renal cancer cell line between $10^{-8}$ and $10^{-6}$ M, while in the case of the NCI-H226 non-small cell lung (NSCL) cancer cell line this activity was low (20–24%) in the same range of concentration. The activities of the other compounds were within 30–40% of tumor growth inhibition for some subpanel cell lines such as central nervous system (CNS), melanoma, renal, breast and ovarian cancer.

Table 4
Comparison of the inhibitory activity of compounds **4b,h,m,n**, **5d,e** and **8** on some cell lines at the most dilute concentrations

| Cell line | Compound | Percent tumor growth inhibition at: | | |
|---|---|---|---|---|
| | | $10^{-8}$ M | $10^{-7}$ M | $10^{-6}$ M |
| *Non-small cell lung cancer* | | | | |
| NCI-H226 | **4n** | 20 | 27 | 24 |
| *Central nervous system cancer* | | | | |
| SNB-75 | **4m** | 31 | 41 | 24 |
| SNB-295 | **5d** | 26 | 27 | 38 |
| SNB-539 | **5e** | 47 | 47 | 45 |
| *Melanoma* | | | | |
| UACC-247 | **4b** | | 24 | 25 |
| *Renal cancer* | | | | |
| RXF-393 | **4h** | 19 | 32 | 49 |
| UO-31 | **4n** | 85 | 42 | 98 |
| *Ovarian cancer* | | | | |
| OVCAR-5 | **5d** | 29 | 25 | 27 |
| *Breast cancer* | | | | |
| HS-578T | **5e** | 36 | 39 | |
| MCF7/ADR-RES | **8** | 47 | 38 | 35 |

The limited number of compounds tested and the lack of many reference compounds does not allow us to establish structure–activity relationships. However, it seems evident that in the case of trimetrexate quinoxaline analogues the in vitro anticancer activity for compounds **4f,h** is associated with both similar lipophilicity (trimethoxyphenyl) and substitution pattern (2-amino and 3-phenyl groups), whereas when this condition is varied the activity decreases. In the series of dideazafolic analogues **5a–g**, two compounds (**5a,e**) seem to emerge at the same degree of activity as that of **4f**, both endowed with high lipophilicity. In conclusion, the supposed quinazoline–quinoxaline isosterism seems to hold in a few cases even though no compound was considered of further interest by NCI. Comparison of in vitro anticancer activity with other compounds previously described by us seems to indicate a lower inhibition activity in the series now examined.

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