Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 38 (2003) 19–26
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Original article

Synthesis of aza mono, bi and tricyclic compounds. Evaluation of their anti MDR activity

Sandrine Gallo [a], Siham Atifi [a], Abdallah Mahamoud [a], Christiane Santelli-Rouvier [a,*], Krisztina Wolfárt [b], Jozsef Molnar [b], Jacques Barbe [a]

[a] *GERCTOP-UMR CNRS 6009, faculté de pharmacie, Université de la Méditerranée, 27, bd Jean Moulin, 13385 Marseille cedex 05, France*
[b] *Institute of Microbiology, University of Szeged, Szeged, Hungary*

Received 17 June 2002; received in revised form 4 October 2002; accepted 8 October 2002

Abstract

Anti MDR activity of a series of acridine, pyridoquinoline, quinoline and pyridine analogous amines was evaluated. Interesting activity is displayed by tricyclic compounds. Besides ring size, influence of the side chain was studied.

Keywords: P-gp inhibitors; Acridinic compounds; Pyridoquinolines

1. Introduction

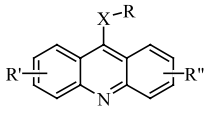
Broad spectrum resistance to chemotherapy in human cancer has been called multidrug resistance [1]. Human P-glycoprotein (P-gp) is related to multidrug resistance and causes a decrease of drug accumulation by an energy ATP dependent efflux. Besides verapamil (VP) [2], a large number of substances have shown the capacity—when added to the anticancer drug—to restore its former activity. Those agents acting as resistance reversal agents have unrelated chemical structures and their mode of interaction with P-gp is still under investigation. Statistical studies with respect to a large number of known P-gp inhibitors led to propose a general pattern for the substrate recognition [3,4]. Aromatic moieties, nitrogen atom, frequently encountered in reversal agents, have been supposed to be essential but the presence of electron donor groups is a more general feature as non ionic compounds, although less numerous than amino compounds, are P-gp inhibitors as well [5].

2. Chemistry

Acridinic compounds **1** (Fig. 1) have already been tested and several derivatives have demonstrated interesting anti MDR activity [6]. The aim of this study is to clarify the functional groups, which actually interact with the protein. Several variations have been made concerning:

- the aromatic moiety: pyridine, quinoline, acridine and pyridoquinoline derivatives have been investigated,
- the side chain has been modified: X atom nature (O, N or S) and/or size of the side chain.

Aminopyridines **2**, **3**, aminoquinolines **4** [7], **5**, aminoacridines **6** [8], **7**, have been prepared by action of primary diethylaminoethylamine or secondary amine (X = NMe) on the corresponding chloro aromatic compound (Fig. 2).



1 X = S, SO

Fig. 1. 9-Thio and 9-sulfoacridines **1**.

Abbreviations: MDR, multidrug resistance; VP, verapamil; PTC, phase transfer catalysis.

* Corresponding author
E-mail address: christiane.santelli@pharmacie.univ-mrs.fr (C. Santelli-Rouvier).

0223-5234/02/$ - see front matter
PII: S0223-5234(02)01422-8

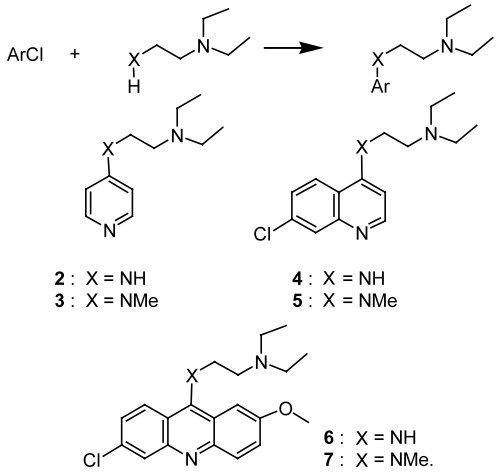


Fig. 2. Synthesis of 4-aminoquinolines **4**–**5** and 9-aminoacridines **6**–**7**.

Thioquinoline **8** (A. Mahamoud, unpublished results) and aminothioacridine **9** [9] were prepared by alkylation with diethylaminoethyl chloride (in butanone and sodium hydroxide for **8**, in toluene and sodium hydroxide for **9**) (Fig. 3).

Monosubstituted pyridoquinoline **14** was prepared (Fig. 4) by alkylation of pyridoquinolinone **13** obtained by thermocyclisation of quinoline **10.** This procedure was previously used to afford **11** [10]. Disubstituted pyridoquinolines **15** and **16** were prepared by alkylation of pyridoquinoline dione **11** or dithione **12** under PTC conditions with tetrabutylammonium bromide (TBAB) as catalyst [10]. Alkylation of **11** (or **12)** by chlorobromoethane gives the intermediates **18** (or **19**) [11], as a mixture of chloro, bromo derivatives used as crude for the next step. The reaction of primary or secondary amines with **18** or **19** leads to amines **20** [11], **21** or **22**. When diethoxy pyridoquinoline **18** is treated by a primary amine (diethylaminoethylamine), aromatic nucleophilic substitution occurs, leading to the diamino compound **20**. By contrast, when **18** or **19** are treated by a secondary amine (diethylaminoethylmethylamine), substitution of alkyl halide occurs, instead of aromatic

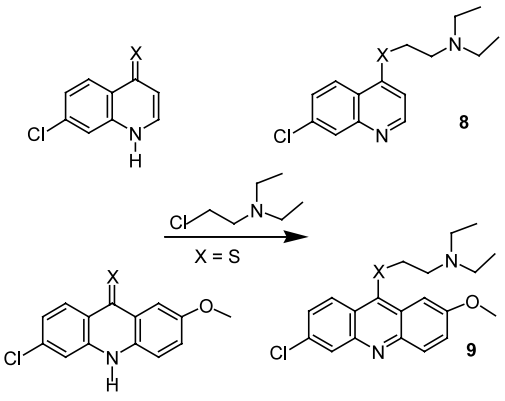


Fig. 3. Synthesis of 4-thioquinoline **8** and 9-thioacridine **9**.

substitution and yield **21** or **22**. Pyridoquinolines **17** [10], **21**, **22** have an extended chain including additional electron donor group compared with **15**, **16**.

Finally treatment of dichloroquinoline **23** by a secondary amine affords diamino pyridoquinoline **24** analogue to **20** (Fig. 5). A small amount of mono amine **25** was separated by chromatography, the phenoxy (or diphenoxy) compound is an intermediate when phenol is used as solvent in aromatic nucleophilic substitution by amines.

3. Results and discussion

As drug efflux is a major characteristic of P-gp mediated MDR and as rhodamine 123 is more sensitive to efflux than other anticancer drugs [12], we evaluated the anti MDR activity by the inhibition rate of rhodamine 123 efflux. Blind tests have been made to confirm that this method is suitable with compounds like acridines or pyridoquinolines, which display their own fluorescence. pK_a, log P, log D were calculated with PALLAS 2.0 [13]. The drug concentration necessary to a 50% decrease efflux is the IC_{50} value. These data are summarised in Table 1.

The pyridine derivatives **2** and **3** are inactive. Quinolines **4**, **5**, **8** are as active as VP, **15** and **20** are slightly better than VP. The amino substituted compound **5** has a slightly better activity than the non substituted **4** and the best result is obtained with the S-alkylated derivative **8** (Figs. 2 and 3). Interesting activity is only observed for tricyclic derivatives. Acridine derivatives **6** and **7** present a similar activity at low concentration but the non substituted compound **6** could be toxic at high doses (at 50 μM, percentage of reversal activity is near 100 for **7** and decreases from 90 to 35 when concentration of **6** varies from 20 to 50 μM). Likewise thioquinoline **8**, but in an enhanced way, thioether **9** displays a better activity than the corresponding amino compounds **6** or **7** (Figs. 2 and 3).

For disubstituted pyridoquinolines, **15**, **16**, **20**, **24** in which X atom varies, we observed an increasing activity in the order: O, NH < NR < S (Figs. 4 and 5).

Unsymmetrical pyridoquinolines **14** and **25** have a better activity than symmetrical derivatives, so one amino side chain is enough to confer a good activity ($IC_{50} = 10.5$ and 8.7 μM, respectively). Moreover, comparison between **14** and **15** points out that a second amino side chain is detrimental. Pyridoquinoline **25** and the aminoacridine **7** have a similar activity ($IC_{50} = 8.7$ and 9.2 μM, respectively) (Figs. 2, 4 and 5).

For compounds **17**, **21** in which side chains are longer with additional electron donor groups (diethylaminoethyl or morpholino ring), a better activity ensues: **21** > **15**; **17** and **22** > **16** (Fig. 4).

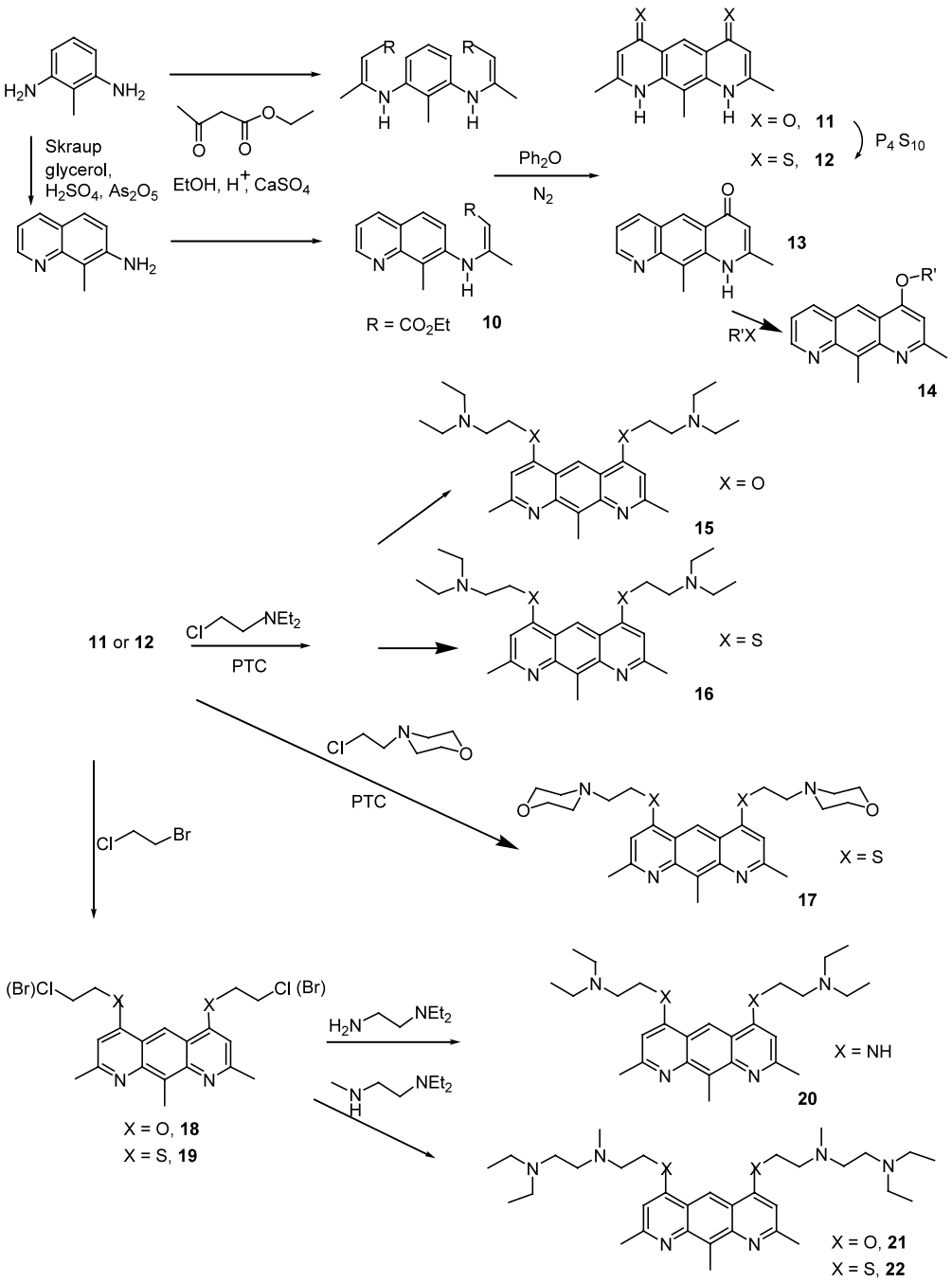


Fig. 4. Synthetic pathways to 4,6-substituted pyridoquinolines: ether, thioether and secondary amine derivatives **14**–**22**.

To summarise, anti MDR activity depends on the aromatic moiety, the bonded X heteroatom and nature of the side chain. A better activity is found:

a) when the aromatic moiety is acridine or pyridoquinoline compared with quinoline and pyridine. It can be questioned whether log *P* (or log *D*) are related to the activity. If the values for pyridine compounds are low (< 2) compared with all other products, the calculated values for quinoline compounds **4** and **5** (3.32 and 3.70) are in the same range of the **14** value (3.78) and even higher than that of **22** (2.38), the latter product is nevertheless active. The lack of direct correlation between log *P* and anti MDR activity has previously been underlined [14]. So, tricyclic aromatic ring seems to be required to fit necessary electrostatic interactions.

b) for S-alkylated derivatives compared with O, NH or NR analogues, the hypothesis that a strong, favourable interaction exists in protein crystal structures between aromatic rings (in histidine, tryptophane, tyrosine or phenylalanine) and sulphur-bearing

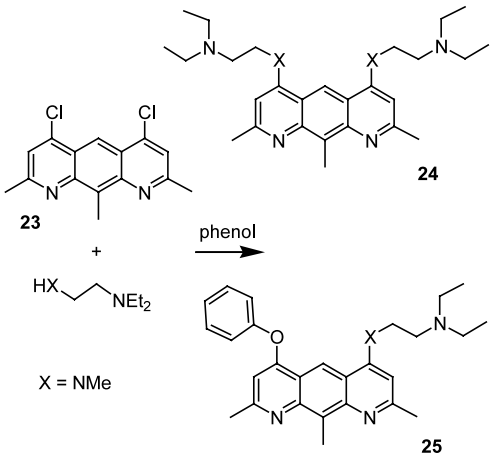


Fig. 5. Synthesis of 4,6-pyridoquinolines: tertiary amines **24** and **25**.

Table 1
Physical and biological data

Products	pK_a	log P	log D	IC_{50} [a]
2	10.11; 8.15	1.32	−2.72	–
3	10.11; 5.17	1.70	−1.19	–
4	10.11; 7.66	3.32	−0.4	< VP
5	10.11; 4.10	3.70	0.72	~ VP
6	10.11; 8.59	4.76	0.14	8.0
7	10.11; 6.32	5.15	2.04	9.2
8	9.29; 3.59	3.94	1.66	~ VP
9	9.29; 5.84	5.39	3.07	1.9
14	9.22; 6.11	3.78	1.51	10.5
15	9.52; 8.92	4.75	0.06	> VP
16	9.59; 8.99	5.59	1.02	4.7
17	7.40; 6.80	2.38	1.68	0.85
20	10.41; 9.81	4.34	−3.22	> VP
21	9.94; 9.34	4.32	−1.51	11.7
22	9.94; 9.34	5.16	−0.09	0.75
24	10.41; 9.81	5.11	−0.92	11.1
25	10.11; 7.05	5.95	2.58	8.7

[a] IC_{50} (μM): 50% reversal activity(cf. biology). VP reversal activity at 20 μM 6%.

amino acids (in cysteine and methionine), was first proposed by Morgan et al. [15] and evidence brought about from crystallographic data [16]. A similar favourable interaction between S-derivatives and aromatic aminoacid residues of P-gp may be inferred to explain their relative high activity.

Finally, if we consider the compound activity related to the number and the length of the side chain-keeping the same X atom on the ring-activities are in the following increasing order

X = O, **15** < **21** < **14**
X = N, **20** < **24** < **25**
X = S, **16** < **9** < **17**, **22**,

that is: 2 side 'short' chains < one side 'short' chain or two side 'long' chains.

4. Conclusion

Besides substrates, which are translocated by P-gp, several other compounds, not structurally related, impede P-gp functions. Mechanism by which reversing agent interactions occurs, is still unknown. Nevertheless at least two drug interaction sites have been distinguished [17]. Considering 100 compounds already tested [3], activities have been correlated with the presence of two or three hydrogen bond acceptor (HBA) groups with a spatial separation of 2.5 or 4.6 Å. Additionally, activities of propafenone derivatives have been correlated with strength of HBA groups [5]. For the products studied here, possible HBA groups are tertiary amino groups, heterocyclic nitrogen whose potency can be described by pKHb scale [18]. When X = N, the heterocyclic nitrogen potency is increased by a push–pull effect [19]. These values are close and included between 1.99 and 2.80. In addition to its nitrogen HBA group, the heterocycle can interact with P-gp by aromatic hydrophobic interaction. The lack of activity of pyridine and quinoline derivatives **2**–**5** seems to indicate that hydrophobic interactions are rather involved. A further study replacing aromatic heterocyclic moiety by the corresponding aromatic structure would give useful information. Differences in the observed activities of the compounds can be attributed to the spatial position of the amino HBA groups and aromatic rings. The results display that two long chains allow the best interactions between compound pharmacophoric groups on one hand and P-gp binding sites on the other hand. However, considering also that one side short chain affords an activity would suggest that in this case two molecules are required to account for the effect observed.

For compounds with two short amino side chains, although same pharmacophoric groups responsible of the effect are present they should not have the right reciprocal position to reach the targets. A possible arrangement of acridinic and pyridoquinoline compounds is proposed in Fig. 6.

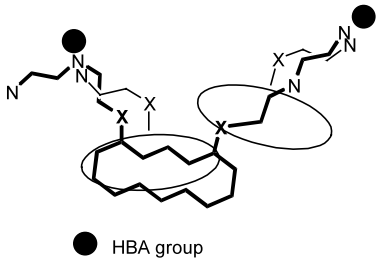


Fig. 6. A possible superimposition of pyridoquinoline derivatives (in bold) and acridinic derivatives when interacting with the P-gp.

5. Experimental

5.1. Chemistry

5.1.1. General methods

Liquid chromatography was performed on silica gel 60 (230–400 Mesh) and TLC on silica gel 60 F_{254}. Melting points were determined on a Büchi apparatus and are given uncorrected. ^{1}H and ^{13}C-NMR spectra were performed on a Brüker ARX 200 spectrometer with TMS as internal reference; chemical shifts are given on the δ (ppm) scale with J values in Hertz.

*5.1.1.1. 4-[(2′-Diethylaminoethyl)amino]-pyridine (**2**).* Chloropyridine, hydrochloride (3 g, 20 mmol), 2-diethylaminoethylamine (3.2 g, 27 mmol) in pyridine (15 mL) were heated at 90 °C for 13 h. Excess pyridine was eliminated in vacuo, the product was extracted with CH_2Cl_2, washed with NaOH 10% and dried over drierite. After elimination of the solvent in vacuo, the product was chromatographed on silica gel with MeOH–ammonia 0–5%. Yield 42%; melting point (m.p.) (hydrochloride): 199–201 °C.

^{1}H-NMR ($CDCl_3$): 8.01 (d, $J = 6.1$ Hz, 2H), 6.30 (dd, $J = 5.0$ Hz, $J = 1.3$ Hz, 2H), 5.14 (m, 1H), 2.99 (td, $J = 6.1$ Hz, $J = 5.3$ Hz, 2H), 2.51 (t, $J = 6.1$ Hz, 2H), 2.4 (q, $J = 7.1$ Hz, 4H), 0.88 (t, $J = 7.1$ Hz, 6H). ^{13}C ($CDCl_3$): 153.22 (s), 149.05 (d), 107.15 (d), 50.51 (t), 46.07 (t), 39.31 (t), 11.17 (q). ^{1}H-NMR (D_2O) (hydrochloride): 8.15 (br.d, 2H), 7.0 (d, $J = 7.5$ Hz, 2H), 3.87 (t, $J = 6.6$ Hz, 2H), 3.51 (t, $J = 6.6$ Hz, 2H), 3.35 (q, $J = 7.2$ Hz, 4H), 1.35 (t, $J = 7.3$ Hz, 6H).

*5.1.1.2. 4-[(2′-Diethylaminoethyl)methylamino]-pyridine (**3**).* A similar procedure to the previous one is used, the corresponding secondary amine is heated for 4 h. Yield 70%; m.p.: 189–190 °C. ^{1}H-NMR ($CDCl_3$): 8.17 (br.s, 2H), 6.47 (dd, $J = 5.1$Hz, $J = 1.3$ Hz, 2H), 3.42 (t, $J = 7.1$, 2H), 2.97 (s, 3H), 2.54 (q, $J = 7.1$ Hz, 6H), 1.01 (t, $J = 7.1$ Hz, 6H). ^{13}C ($CDCl_3$): 153.17 (s), 148.82 (d), 106.10 (d), 49.91 (t), 49.09 (t), 47.17 (t), 37.66 (q), 11.60 (q).

*5.1.1.3. 7-Chloro-4-[(2′-diethylaminoethylamino]-quinoline (**4**).* 4,7-Dichloroquinoline (3 g, 15 mmol), *N*,*N*-diethylaminoethylamine (1.74 g, 15 mmol) and phenol (10 g) are heated at 90 °C for 11 h. Excess phenol is removed in vacuo, the residue extracted with CH_2Cl_2, washed with NaOH, 10%. The organic phase is dried over drierite. The solvent is then removed in vacuo and the product is chromatographed on silica gel and ether–methanol. Yield 35%; m.p.: 102–103 °C (94–97 °C [7]). ^{1}H-NMR ($CDCl_3$): 8.50 (d, $J = 5.3$ Hz, 1H), 7.93 (d, $J = 2.1$ Hz, 1H), 7.64 (d, $J = 8.9$ Hz, 1H), 7.34 (dd, $J = 8.9$ Hz, $J = 2.1$ Hz, 1H), 6.34 (m, 1H), 3.22 (br.q, 2H), 2.79 (t, $J = 6.0$ Hz, 2H), 2.58 (q, $J = 7.1$ Hz, 4H), 1.05 (t, $J = 7.1$ Hz, 6H). ^{13}C ($CDCl_3$): 152.03 (d), 149.80 (s), 149.02 (s), 134.64 (s), 128.58 (d), 125.13 (d), 121.16 (d), 117.37 (s), 99.20 (d), 50.46 (t), 46.37 (t), 39.61 (t), 11.99 (q).

*5.1.1.4. 7-Chloro-4-[(2′-diethylaminoethyl)-methylamino]-quinoline (**5**).* *N*,*N*-diethylaminoethylmethylamine and 4,7-dichloroquinoline are treated according to the same procedure used for **4**.Yield 47%, m.p. (HCl): 152–153 °C. ^{1}H ($CDCl_3$): 8.57 (d, $J = 5.1$ Hz, 1H), 8.05 (d, $J = 9.0$ Hz, 1H), 7.95 (d, $J = 2.0$ Hz, 1H), 7.32 (dd, $J = 9.0$ Hz, $J = 2.1$ Hz, 1H), 6.72 (d, $J = 5.1$ Hz, 1H), 3.30 (dd, $J = 7.4$ Hz, $J = 6.6$ Hz, 2H), 2.94 (s, 3H), 2.70 (dd, $J = 7.3$ Hz, $J = 6.7$ Hz, 2H), 2.47 (q, $J = 7.1$ Hz, 4H), 0.95 (t, $J = 7.1$ Hz, 6H). ^{13}C ($CDCl_3$): 157.16 (s), 151.26 (d), 150.21 (s), 134.44 (s), 128.44 (d), 125.75 (d), 125.11 (d), 121.50 (s), 108.13 (d), 54.83 (t), 50.53 (t), 47.26 (t), 40.49 (q), 11.69 (q).

*5.1.1.5. 6-Chloro-2-methoxy-9-[(2′-diethylaminoethylamino]-acridine (**6**).* 6,9-Dichloro-2-methoxy-acridine (2.7 g, 10 mmol), *N*,*N*-diethylaminoethylamine (1.24 g, 10.6 mmol), phenol (8 g) are heated at 120 °C for 2 h. After elimination of phenol in vacuo, the residue is extracted with CH_2Cl_2 washed with NaOH, 10%. After chromatography (silica gel, ether–methanol 1–10% and triethylamine 1%) a solid is recovered. Yield: 67%; m.p.: 100 °C (hydrochloride, m.p.: 257–259 °C [8]). ^{1}H-NMR ($CDCl_3$): 8.12 (d, $J = 9.3$ Hz, 1H), 8.02 (d, $J = 2.1$ Hz, 1H), 7.96 (d, $J = 9.4$ Hz, 1H), 7.39 (dd, $J = 9.4$ Hz, 2.7 Hz, 1H), 7.25 (m, 2H), 6.45 (m, 1H), 3.95 (s, 3H), 3.78 (m, 2H), 2.75 (m, 2H), 2.67 (q, $J = 7.1$ Hz, 4H), 1.10 (t, $J = 7.1$ Hz, 6H). ^{13}C ($CDCl_3$): 155.54 (s), 150.12 (s), 148.63 (s), 134.57 (s), 131.23 (d), 127.94 (d), 124.81 (d), 124.29 (d), 123.47 (d), 117.02 (s), 114.87 (s), 99.04 (d), 55.30 (q), 52.35 (t), 46.22 (t), 45.89 (t), 11.75 (q).

*5.1.1.6. 6-Chloro-2-methoxy-9-[(2′-diethylaminoethyl)-methylamino]-acridine (**7**).* A similar procedure to the latter is used with the corresponding secondary amine. Yield: 62%; m.p. (hydrochloride): 216 °C. ^{1}H-NMR ($CDCl_3$): 8.18 (d, $J = 9.2$ Hz, 1H), 8.13 (d, $J = 2.0$ Hz, 1H), 8.03 (d, $J = 10.2$ Hz, 1H), 7.43–7.31 (m, 3H), 3.95 (s, 3H), 3.62 (dd, $J = 8.9$ Hz, $J = 5.7$ Hz, 2H), 3.26 (s, 3H), 2.68 (dd, $J = 8.9$ Hz, $J = 5.7$ Hz, 2H), 2.45 (q, $J = 7.1$ Hz, 6H), 0.91 (t, $J = 7.1$ Hz, 6H). ^{13}C ($CDCl_3$): 156.58 (s), 153.65 (s), 148.70 (s), 148.00 (s), 134.39 (s), 131.49 (d), 128.38 (d), 126.14 (d), 125.78 (d), 125.65 (s), 125.10 (d), 123.43 (s), 100.51 (d), 55.38 (q), 52.24 (t), 47.04 (t), 42.71 (q), 11.55 (q).

*5.1.1.7. 8-Methyl-7-[(2′-carboethoxy-1′-methylvinyl)amino)]-quinoline (**10**).* 7-Amino-8-methylquinoline (4.87 g, 30.7 mmol), ethyl acetoacetate (4.0 g, 30.7 mmol), absolute ethanol (35 mL), anhydrous

$CaSO_4$ (12 g) and few drops of acetic acid are heated at 80 °C under stirring for 5 days. After filtration, the solvent is eliminated in vacuo and the crude product used without further purification for the next step. ^{1}H-NMR ($CDCl_3$): 11.5 (s, 1H), 8.9 (d, $J = 4.2$ Hz, $J = 1.6$ Hz, 1H), 8.1 (d, $J = 8.2$ Hz, $J = 1.6$ Hz, 1H), 7.6 (d, $J = 8.6$ Hz, 1H), 7.3 (dd, $J = 8.2$ Hz, $J = 4.2$ Hz, 1H), 7.2 (d, $J = 8.7$ Hz, 1H), 4.8 (s, 1H), 4.2 (q, $J = 7.2$ Hz, 2H), 2.8 (s, 3H), 1.9 (s, 3H), 1.25 (t, $J = 7.2$ Hz, 3H).

*5.1.1.8. 2,10-Dimethylpyrido-[3,2g]quinoline-4-one (**13**).* Quinoline **10** (6 g, 22 mmol), diphenylether (80 mL) are heated rapidly to 250 °C under nitrogen and left for 1 h at this temperature. After cooling to 60 °C, the mixture is poured onto petroleum ether (150 mL). The red precipitate is filtered, washed thoroughly with ethylether and dried. Yield: 56%, m.p.: 255 °C. ^{1}H-NMR (DMSO-d_6): 10.5 (s,1H), 9.0 (dd, $J = 3.9$Hz, $J = 1.8$Hz, 1H), 8.6 (s, 1H), 8.5 (dd, $J = 8.3$Hz, $J = 1.8$Hz, 1H),7.5 (dd, $J = 8.3$ Hz, $J = 3.9$Hz, 1H), 5.9 (s, 1H), 3.0 (s, 3H), 2.5 (s, 3H).

*5.1.1.9. 2,10-Dimethylpyrido-4-(2′-diethylaminoethoxy)-[3,2-g]quinoline (**14**).* Quinoline **13** (1 g, 4.46 mmol), 1-chloro-2-diethylaminoethane, hydrochloride (1.54 g, 8.92 mmol, toluene (70 mL), KOH, 50% (35 mL), tetrabutylammonium bromide (0.3 g, 0.9 mmol) are heated at 110 °C under stirring for 24 h, then filtered warm. The aqueous phase is extracted three times with toluene. The organic layers are dried over anhydrous Na_2SO_4. After elimination of the solvent, the oily product is kept in the refrigerator and then the solid is washed with cold toluene. Yield: 30%; m.p.: 142–144 °C. ^{1}H ($CDCl_3$): 9.0 (dd, $J = 3.9$ Hz, $J = 1.9$ Hz, 1H), 8.5 (s,1H), 8.3 (dd, $J = 8.5$ Hz, $J = 1.8$ Hz, 1H), 7.3 (dd, $J = 6.5$ Hz, $J = 3.9$ Hz, 1H), 6.5 (s, 1H), 4.25 (t, $J = 6.2$ Hz, 2H), 3.3 (s, 3H), 3.05 (t, 2H), 2.7 (s, 3H), 2.7 (q, $J = 6.3$ Hz, 4H), 1.1 (t, $J = 2.1$ Hz, 6H). ^{13}C ($CDCl_3$): 161.6 (s), 160.7 (s), 150.8 (d), 145.9 (s), 145.6 (s), 137.3 (d), 134.1 (s), 125.2 (s), 120.15 (s), 119.9 (d), 118.9 (d), 99.9 (d), 67.4 (t), 51.04 (t), 48.1 (t), 27.0 (q), 12.4 (q), 12.1 (q).

*5.1.1.10. 2,8,10-Trimethylpyrido-4,6-bis(2′-halogenoethoxy)-[3,2-g]quinoline (**18**).* Pyridinoquinoline dione **11** (1.27 g, 5 mmol), 1-bromo-2-chloroethane (2.87 g, 20 mmol), K_2CO_3 (1.38 g, 10 mmol) and DMF (20 mL) are heated at 100 °C for 4 h under stirring. After cooling, water (300 mL) is added yielding a precipitate, which is filtered, washed thoroughly and recrystallised with methanol. The product (0.58 g) is a mixture of chloro and bromo compound used without further separation for the next step. ^{1}H-NMR ($CDCl_3$): 8.9 (s, 2H), 6.55 (s, 4H), 4.55 (t, $J = 6.4$ Hz, 4H), 4.5 (t, $J = 6.0$ Hz, 4H), 4.00 (t, $J = 6.0$ Hz, 4H), 3.85 (t, $J = 6.3$ Hz, 4H), 3.30 (s, 6H), 2.75 (s, 12H). ^{13}C ($CDCl_3$): 161.45 (s), 160.70 (s), 146.85 (s), 130.15 (s), 118.25 (s), 113.15 (d), 99.85 (d), 68.35 (t), 68.10 (t), 41.75 (t), 28.75 (t), 27.30 (q), 13.00 (q).

*5.1.1.11. 2,8,10-Trimethyl-4,6-bis(2′-diethylaminoethylamino)-pyrido-[3,2-g]quinoline (**20**).* Compound **18** (0.3 g) and 1-amino-2-diethylaminoethane (1.5 mL) are heated at 90 °C for 2 h 15 min. KOH, 10% (50 mL) is added under stirring for 2 h to obtain a solid which is filtered and recrystallised in ethanol. Yield: 80%; m.p.: 151–153 °C, (260-262 °C, hydrochloride). ^{1}H-NMR ($CDCl_3$): 9.35 (s, 1H), 6.65 (s, 2H), 3.90 (t, $J = 6.3$ Hz, 4 H), 3.50 (t, $J = 6.3$Hz, 4H), 3.20 (q, $J = 7.3$ Hz, 8H), 2.7 (s, 3H), 2.60 (s, 6H), 1.10 (t, $J = 7.3$ Hz, 12H).^{13}C ($CDCl_3$): 159.90 (s), 150.55 (s), 145.90 (s), 132.20 (s), 115.80 (s), 109.80 (d), 96.90 (d), 51.35 (t), 45.95 (t), 40.15 (t), 26.80 (q), 12.90 (q), 10.80 (q).

*5.1.1.12. 2,8,10-Trimethyl-4,6-bis[N-(2′-diethylaminoethyl)-N-methylamino) ethoxy] pyrido-[3,2-g] quinoline (**21**).* Diethoxy pyridoquinoline **18** (0.37 g), *N′*,*N′*-diethyl, *N*-methylethyl diamine (1.5 mL) are heated at 80 °C for 12 h. After cooling a KOH 10% solution is added. After extraction with $CHCl_3$, the organic phase is dried over drierite. After elimination of solvent in vacuo the product is chromatographed on silica gel and MeOH–ammonia 1%.Yield: 240 mg ($\sim$40%) as an oil. ^{1}H-NMR ($CDCl_3$): 8.85 (s, 1H), 6.54(s, 2H), 4.32 (t, $J = 6.0$ Hz, 4H), 3.28 (s, 3H), 3.04 (t, $J = 6.0$Hz, 4H), 2.73 (s, 6H), 2.69-2.42 (m, 22H), 1.0 (t, $J = 7.0$ Hz, 12H). ^{13}C ($CDCl_3$):170.36 (s), 161.82 (s), 160.38 (s), 146.40 (s), 118.03 (s), 99.39 (d), 66.98 (t), 56.36 (t), 56.19 (t), 50.92 (t), 47.34 (t), 43.60 (q), 29.67 (t), 26.85 (q), 12.52 (q), 11.55 (q).

*5.1.1.13. 2,8,10-Trimethyl-4,6-bis(2′-halogenoethylthio)-pyrido-[3,2-g]quinoline (**19**).* Pyridoquinolinethione **12** (0.72 g, 2.5 mmol), 1-bromo-2-chloroethane (1.7 g, 12 mmol), K_2CO_3 (1.4 g, 10 mmol), DMF (30 mL) are heated at 60 °C under stirring for 6 h. After cooling, the mixture is poured onto H_2O. The precipitate is filtered and washed. The solid is dried and used without further purification. ^{1}H-NMR ($CDCl_3$): 8.73 (s, 1H), 7.11 (s, 2H), 3.79 (m, 4H), 3.50 (m, 4H), 3.32 (s, 3H), 2.78 (s, 6H).

*5.1.1.14. 2,8,10-Trimethyl-4,6-bis-[N′-(2′-diethylaminoethyl)-N-methylamino)-thioethyl]-pyrido[3,2-g] quinoline (**22**).* Pyridoquinoline **19** (0.3 g), *N′*,*N′*-diethyl-*N*-methylethylenediamine (1.2 g, 9.3 mmol) are heated at 80 °C for 6 h. After cooling KOH, 10% is added and the mixture is extracted with chloroform and then the organic layers are dried over drierite. After elimination of the solvent in vacuo the crude product is chromatographed on silica gel, solvent:

ether–methanol giving an oil. Yield: 53%. ^{1}H-NMR ($CDCl_3$): 8.75 (s, 1H), 7.08 (s, 2H), 3.31 (s, 3H), 3.26 (m, 4H), 2.85 (m, 4H), 2.76 (s, 6H), 2.61 (br.s) and 2.57 (q, $J = 7.0$ Hz)(16 H), 2.39 (s, 6H), 1.03 (t, $J = 7.0$ Hz, 12 H). ^{13}C-NMR ($CDCl_3$): 157.89 (s), 147.62 (s), 144.71(s), 142.79 (s), 134.96 (s), 122.73 (s), 116.16 (d), 115.98 (d), 56.12 (t), 55.76 (t), 50.83 (t), 47.38 (t), 42.65 (q), 29.29 (t), 26.31 (q), 12.68 (q), 11.60(q).

*5.1.1.15. 4,6-Dichloro-2,8,10-trimethylpyrido-[3,2-g]quinoline (**23**).* To $POCl_3$ (25 mL) at 50 °C is added dropwise pyridoquinolinedione **11** (1.27 g, 5 mmol) under stirring. The mixture is heated for 2 h 30 min at 70–80 °C. After cooling, the mixture is added cautiously under stirring to ammonia and ice, keeping a basic medium. The precipitate is filtered, washed with water, dissolved in warm $CHCl_3$, filtered and dried over $MgSO_4$. Yield: 1.47 g. ^{1}H-NMR ($CDCl_3$): 8.77 (s, 1H), 7.36 (s, 2H), 3.26 (s, 3H), 2.76 (s, 6H). ^{13}C ($CDCl_3$): 159.24(s), 145.72 (s), 142.95 (s), 135.60 (s), 122.94 (s), 121.78 (d), 117.71 (d), 26.09 (q), 12.82 (q).

*5.1.1.16. Preparation of **24** and **25**.* (0.28 g, 0.96 mmol), and *N′*,*N′*-diethylamino-*N*-methylethylenediamine (0.3 g, 2.3 mmol), phenol (4 g) are heated at 90 °C for 14 h. Excess phenol is eliminated in vacuo and extracted with CH_2Cl_2, washed with NaOH, 10%. The organic phase is dried over drierite. The solvent is eliminated in vacuo and the residue chromatographed on silica gel with methanol–ammonia 0–10% giving **24** and **25**.

*5.1.1.17. 2,8,10-Trimethyl-bis-4,6-[N-(2′-diethylaminoethyl)-N-methylamino] pyrido-[3,2-g] quinoline (**24**).* Major fraction isolated as an oil (230 mg).^{1}H-NMR ($CDCl_3$): 8.60 (s, 1H), 6.67 (s, 2H), 3.35 (m, 4H), 3.30 (s, 3H), 3.02 (s, 6H), 2.81 (m, 4H), 2.71 (s, 6H), 2.48 (q, $J = 7.1$ Hz, 8H), 0.95 (t, $J = 7.1$ Hz, 12H). ^{13} C-NMR ($CDCl_3$): 159.17 (s), 157.65 (s), 146.44 (s), 133.00 (s), 119.17 (s), 116.61 (d), 107.96 (d), 54.59 (t), 50.54 (t), 47.28 (q), 41.05 (q), 26.42 (q), 12.70 (q), 11.71 (q).

*5.1.1.18. 2,8,10-Trimethyl-6-phenoxy-4-[N-(2′-diethylaminoethyl)-N-methylamino] pyrido-[3,2-g] quinoline (**25**).* Less polar fraction isolated as an oil (100 mg). ^{1}H ($CDCl_3$): 8.94 (s, 1H), 7.48 (m, 2H), 7.33–7.19 (m, 3H), 6.71 (s, 1H), 6.34 (s, 1H), 3.42 (m, 2H), 3.35 (s, 3H), 3.02 (s, 3H), 2.93 (m, 2H), 2.75 (s, 3H), 2.64 (s, 3H), 2.52 (q, $J = 7.1$ Hz, 4H), 0.96 (t, $J = 7.1$ Hz, 6H). ^{13}C ($CDCl_3$): 162.34 (s), 159.96 (s), 159.67 (s), 157.79 (s), 154.50 (s), 147.14 (s), 146.15 (s), 133.14 (s), 130.11 (d), 125.37 (d), 121.15 (d), 120.17 (s), 117.27 (s), 114.49 (d), 107.78 (d), 102.86 (d), 55.13 (t), 50.79 (t), 47.34 (t), 40.61 (q), 26.52 (q), 26.47 (q), 12.71 (q), 11.67 (q).

5.2. Biology

5.2.1. Cells

The L5178 Y mouse T-lymphoma parent cell line was infected with the pHa MDR1/A retrovirus as previously described by Pastan [20].

The L5178 MDR cell line and the L5178 Y parent cell line were grown in McCoy's 5A medium supplemented with 10% heat inactivated horse serum, L-glutamine and antibiotics (and 60 ng mL^{-1} colchicine for MDR cell line).

5.2.2. Rhodamine 123 (R123) uptake assay

The L5178 cells (2×10^6 mL^{-1}) were resuspended in serum-free medium and distributed (0.5 mL aliquot to Eppendorf tubes). Compounds to be tested were added at different concentrations and the samples were incubated for 10 min at room temperature. Then the R123 indicator was added to the samples at a final concentration of 5.2 μM and the cells were incubated for 20 min at 37 °C, washed twice and resuspended in 0.5 mL phosphate buffer saline (PBS) for analysis. The fluorescence of the cell populations was measured by flow cytometry using a Beckton Dickinson FACScan instrument. Since R123 is a substrate of P-gp, there was a significant fluorescence between MDR and parent cells. Untreated MDR cells accumulate only a low level of R123. VP was used as a reference drug. [21].

The fluorescence mean intensities (FL) were determined for the treated cells and were compared with those of untreated cells. The percentage of multidrug resistance reversion (% MDR reversion) was calculated as follows:

$$\%\text{MDR rev.} = \frac{\text{FL (MDR treated)} - \text{FL (MDR untreated)}}{\text{FL (Parent treated)} - \text{FL (MDR untreated)}} \times 100$$

References

[1] M.M. Gottesman, I. Pastan, Ann. Rev. Biochem. 62 (1993) 385–427.
[2] T. Tsuruo, H. Iida, S. Tsukagoshi, Y. Sakurai, Cancer Res. 41 (1981) 1967–1972.
[3] A. Seelig, Eur. J. Biochem. 251 (1998) 252–261.
[4] G. Klopman, M.S. Leming, A. Ramu, Mol. Pharmacol. 52 (1997) 323–334.
[5] G. Ecker, M. Huber, D. Schmid, P. Chiba, Mol. Pharmacol. 56 (1999) 791–796.
[6] A. Hevér, C. Santelli-Rouvier, P. Brouant, S. El Khyari, J. Molnar, Y. Barra, J. Barbe, Anticancer Res. 18 (1998) 3053–3058.
[7] A.R. Surrey, H.F. Hammer, J. Am. Chem. Soc. 68 (1946) 113–116.
[8] D.S. Breslow, H.G. Walker, R.S. Yost, J.C. Shivers, C.R. Hauser, J. Am. Chem. Soc. 68 (1946) 100–102.

[9] S. Bonse, C. Santelli-Rouvier, J. Barbe, R.L. Krauth-Siegel, J. Med. Chem. 42 (1999) 5448–5454.
[10] C. Matias, A. Mahamoud, J. Barbe, B. Pradines, J.-C. Doury, Heterocycles 43 (1996) 1621–1632.
[11] C. Matias, Ph.D., Marseille, 1997.
[12] J. Lee, K. Paull, M. Alvarez, C. Hose, A. Monks, M. Grever, A.T. Fojo, S.E. Bates, Mol. Pharmacol. 46 (1994) 627–638.
[13] Compudrug Chemistry, Ltd, 1994–1995.
[14] P. Chiba, G. Ecker, D. Schmid, J. Drach, B. Tell, S. Goldenberg, V. Gekeler, Mol. Pharmacol. 49 (1996) 1122–1130.
[15] R.S. Morgan, C.E. Tatsch, R.H. Gushard, J.M. McAdon, P.K. Warme, Int. J. Peptide Protein Res. 11 (1978) 209–217.
[16] R.J. Zauhar, C.L. Colbert, R.S. Morgan, W.J. Welsh, Biopolymers 53 (2000) 233–248.
[17] S. Dey, M. Ramachandra, I. Pastan, M.M. Gottesman, S.V. Ambudkar, Proc. Natl. Acad. Sci. USA 94 (1997) 10594–10599.
[18] C. Laurence, M. Berthelot, Perspect. Drug Discov. Des. 18 (2000) 39–60.
[19] M. Berthelot, C. Laurence, M. Safar, F. Besseau, J. Chem. Soc., Perkin Trans. 2 (1998) 283–290.
[20] I. Pastan, M.M. Gottesman, K. Ueda, E. Lovelace, A.V. Rutherford, M.C. Willingham, Proc. Natl. Acad. Sci. USA 85 (1988) 4486–4490.
[21] J.L. Weaver, G. Szabo, P.S. Pine, M.M. Gottesman, S. Goldenberg, A. Aszalos, Int. J. Cancer 54 (1993) 456–461.