Bioorganic & Medicinal Chemistry 19 (2011) 6309–6315

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Discovery of pyridine-2-ones as novel class of multidrug resistance (MDR) modulators: First structure–activity relationships

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ARTICLE INFO

Article history:
Received 10 May 2011
Revised 1 September 2011
Accepted 5 September 2011
Available online 10 September 2011

Keywords:
Structure–activity relationships (SAR)
Multidrug resistance (MDR)
P-glycoprotein (P-gp)
Lead structure
Efflux pump inhibition

ABSTRACT

A novel facile synthesis led to pyridine-2-one target structures of which first series with varying substituents have been yielded and biologically characterized as novel multidrug resistance (MDR) modulators inhibiting P-glycoprotein (P-gp). Structure–activity relationships prove a dependency of the MDR-modulating properties from the kind and positioning of hydrogen bond acceptor functions within the molecular skeleton. Cyano functions turned out as biologically effective substituents for a potential hydrogen bonding to the protein target structure.

1. Introduction

Multidrug resistance (MDR) is an ongoing main problem in anticancer therapies.[1–3] Main causative agents for this problem have been multidrug efflux pumps which are found overexpressed in tumor cells.[4,5] Such efflux pumps transport anticancer drugs out of the tumor cells so that insufficient intracellular concentrations result and reason a tumor cell resistance.[3] Various efflux pump proteins have been identified meanwhile.[3–5] P-glycoprotein (P-gp) is the most spread efflux pump in MDR-resistant tumor cells.[6] Numerous cytostatic agents have been found as substrates of P-gp.[6–8] Also novel cytostatics like tyrosine kinase inhibitors or monoclonal antibodies turned out as P-gp substrates.[9–11]

So there have been ongoing efforts to find strategies to circumvent the MDR problem. Although novel methods like gene silencing have been perspective in vitro techniques the in vivo realization remains doubtful due to various reasons.[2,12,13]

The main perspective strategy has been the development of MDR modulators which effectively inhibit the efflux pump activity.[6,14,15] However, most of the identified MDR modulators are unfortunately substrates of the efflux pumps so that their effectiveness is given only at higher concentrations which easily reach toxic ranges.[7,8,16]

Such toxic problems concern also representatives of MDR modulators of the third generation which presently undergo clinical trials.[6,17] With many problems of the presently developed MDR modulators there is still a great challenge to discover novel MDR lead structures. Many of the recently reported MDR modulators have been derived from natural sources like plants.[6,18,19] They have complex structures and their high molecular weights are unfavorable molecular properties because molecules with high molecular weights often have bioavailability-problems. Moreover, natural products are often difficult to synthesize because of many necessary reaction steps. Thus, such resynthesized compounds will finally be expensive for a therapeutical use.

Pharmacologically used 1,4-dihydropyridines like nifedipin have been identified to act as MDR modulators beside their blood pressure-influencing properties (Fig. 1).[13,20,21] The introduction of more lipophilic phenyl substituents into first lead structures led to increased MDR-modulating properties.[22,23] However, the water solubility of such lipophilic derivatives with various phenyl substituents was limited. Such a lowered water solubility was unfavorable for in vivo applications. Moreover, essential structural changes within the molecular skeleton were necessary to reduce or avoid the originally pharmacological properties of the early 1,4-dihydropyridines. First attempts to design novel MDR-modulating 1,4-dihydropyridines led to *N*-phenoxycarbonyl substituted compounds (Fig. 1).[6]

However, the *N*-carbamide ester function in these structures was sensitive to hydrolysis. This structural sensitivity made a

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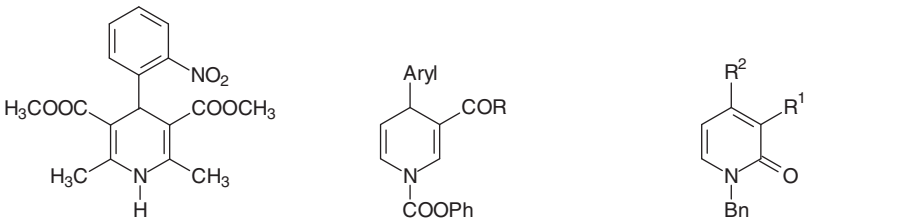
doi:10.1016/j.bmc.2011.09.005



Nifedipin | ***N*-Phenoxycarbonyl-1,4-dihydropyridines** | **Pyridine-2-one Target Structure**

Figure 1.

further in vivo application to a critical procedure because of the early inactivation of the bioactive molecules by such an easy carbamide ester hydrolysis.

We had the idea to change the compound structure by maintaining two molecular features: A lipophilic *N*-substituent was maintained by changing the phenoxy substituent by a benzyl substituent and, furthermore, the ester amide function should be kept. Such ester functions are potential hydrogen bond acceptor functions within MDR modulators and discussed to be important for the biological activities although the contribution of such functions to the extent of the biological activity is unknown.[24,25] The knowledge of such hydrogen bond acceptor functions is limited to few investigated structures and their effects to reverse MDR.[26,27]

If the *N*-phenoxycarbonyl function was involved in the mode of the biological activity to reverse MDR we thought to place it next to the nitrogen atom of the 1,4-dihydropyridine scaffold and so the novel pyridine-2-one structure with a *N*-benzyl substituent was favored with all the suggested molecular properties: the maintained lipophilic *N*-substituent contributing to the lipophilicity of the molecule, the amide function replacing the ester carbamide function near the nitrogen atom and the two double bonds in the 1,2-dihydropyridine-2-one structure instead of the non-conjugated double bonds within the reported 1,4-dihydropyridines.

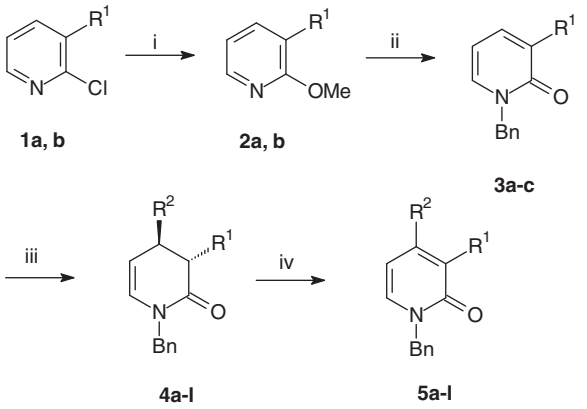
Two other important structural features have also been maintained within the novel MDR-modulating lead structure: the 4-aryl substituent and, finally, the 3-carbonyl function which is found in almost all biologically active 1,4-dihydropyridines.

2. Results and discussion

2.1. Chemistry

The 2-methoxy substituted pyridines **2a** and **2b** with R^1 = COOMe (**2a**) and R^1 = CN (**2b**) have been given by the reaction of the 2-chloro substituted starting compounds **1a** (R^1 = COOMe) and **1b** (R^1 = CN) with freshly prepared sodium methoxide under reflux conditions in methanolic solutions via a nucleophilic substitution reaction (Scheme 1).

The *N*-benzylation of the pyridine nucleus in compounds **2a** and **2b** to **3a** and **3b** succeeded in toluene under reflux conditions with benzyl bromide as alkylating agent. The 2-methoxy function in the primary yielded *N*-benzyl pyridinium cation underwent a bond cleavage by the attack of the bromide anion so that the alkylation reaction product finally owned a pyridine-2-one structure after the rearrangement of the pyridine nucleus bonds. The 3-benzyloxy ester substituted product **3c** (R^1 = COOBn) was given as a side product of the *N*-alkylation reaction of derivative **2a** by a transesterification of the 3-methoxy ester **3a** which reacted with benzyl alcohol as product of a partial benzyl bromide hydrolysis during the reaction course.



	R^1	R^2
1a, **2a**, **3a**	COOMe	-
1b, **2b**, **3b**	CN	-
3c	COOBn	-
4a, **5a**	COOMe	Ph
4b, **5b**	COOMe	Bn
4c, **5c**	COOMe	2-tolyl
4d, **5d**	COOMe	4-tolyl
4e, **5e**	COOMe	4-MeOPh
4f, **5f**	CN	Ph
4g, **5g**	CN	Bn
4h, **5h**	CN	2-tolyl
4i, **5i**	CN	4-tolyl
4j, **5j**	CN	2-MeOPh
4k, **5k**	CN	4-MeOPh
4l, **5l**	COOBn	Ph

Scheme 1. Reagents and conditions: (i) MeOH, NaOMe, reflux, 6 h; (ii) BnBr, 120 °C, 20 h; (iii) THF, Cu(I)I, LiCl, ArylMgBr, −40 °C to rt, 4 h; (iv) toluene, MnO_2, 130 °C, 6–8 h.

The varying 3-substituted pyridine-2-ones **3a**–**c** were then treated with the various grignards reagents at low temperatures using copper(I) iodide as catalyst in dried THF as solvent. The solubility of the used copper(I) iodide was improved by the use of lithium chloride. The pyridine-2-one nucleus may formally be attacked at two favored positions by the grignard reagents, namely the 4- and the 6-position. We exclusively found the 4-grignard addition products. The preferred attack at the 4-position of the pyridine nucleus has been observed to proceed under similar reaction conditions as in the reported reaction of *N*-acylated pyridinium cations with corresponding grignard reagents yielding 4-substituted 1,4-dihydropyridines from pyridines with 3-substituents similar to our pyridine-2-ones.[28–30] The only formation of 4-addition products by the use of copper(I) iodide is much more favorable than the formation of both 4- and 6-addition products described in

a previous procedure to functionalize pyridine-2-ones by nucleophilic addition reactions using varying lewis acids as catalysts.[31]

The stereochemistry of the resulting 4-aryl tetrahydropyridine-2-ones **4a**–**l** was suggested by comparison of the coupling constants of the hydrogen atoms at C3 and C4 making about 5–8 Hz each for a vicinal *trans* proton coupling with those of the vicinal *cis* proton coupling. In the case of the 3-cyano substituted pyridine-2-ones few amounts of diastereomers with such a *cis* coupling of the corresponding C3 and C4 protons and a higher coupling constant of about 11 Hz were isolated in mixtures with the C3 and C4 *trans*-substituted compounds **4f**–**k**. In the case of the sterically more demanding 3-ester derivatives the only formation of C3 and C4 *trans*-substituted derivatives was plausible.

The rearomatized final target structures **5a**–**l** were yielded by heating of the tetrahydro derivatives **4a**–**l** in refluxing toluene with excess amounts of heat-activated manganese dioxide using a previously described method to form diphenyl derivatives from phenyl substituted cyclohexadienes.[32]

Table 1
Target compound (**5a**–**l**) activities in the concentration-dependent inhibition of P-gp as determined *FAR* values for each three measurements, p <0.1

Compd	Residues		*FAR*-values	
	R^1	R^2	P-gp inhibition	
			1 mM	10 mM
5a	COOMe	Ph	1.06 ± 0.13	1.74 ± 0.27
5b	COOMe	Bn	1.09 ± 0.19	1.68 ± 0.38
5c	COOMe	2-Tolyl	1.10 ± 0.54	1.51 ± 0.25
5d	COOMe	4-Tolyl	0.94 ± 0.20	1.75 ± 0.34
5e	COOMe	4-MeOPh	0.94 ± 0.13	1.91 ± 0.43
5f	CN	Ph	0.99 ± 0.02	1.71 ± 0.51
5g	CN	Bn	1.06 ± 0.06	1.63 ± 0.08
5h	CN	2-Tolyl	1.05 ± 0.05	1.83 ± 0.20
5i	CN	4-Tolyl	0.98 ± 0.08	1.56 ± 0.28
5j	CN	2-MeOPh	1.29 ± 0.06	2.16 ± 0.37
5k	CN	4-MeOPh	1.05 ± 0.08	1.35 ± 0.13
5l	COOBn	Ph	1.22 ± 0.39	2.59 ± 0.88
Verapamil			0.89 ± 0.21	2.94 ± 0.78

2.2. Biological evaluation and structure–activity relationships (SAR) of the P-gp inhibition

The in vitro evaluation of the target structures **5a**–**l** has been carried out in both, a mouse T-lymphoma model cell line and a P-gp expressing subline which resulted from retrovirus transfection with the human *MDR1* gene. This model system allows the determination of the P-gp specific inhibiting potential of a MDR modulator, because the P-gp expressing subline exclusively overexpresses P-gp. The used marker for the determination of the inhibiting potential has been rhodamine 123 as a fluorescent dye which is transported by P-gp as a substrate. So if both cell lines are treated with rhodamine 123 the fluorescence accumulation in the P-gp expressing subline is much lower than the accumulation in the non-expressing parental subline. The effective inhibition of P-gp leads to an increase in the intracellular rhodamine 123 uptake in the P-gp expressing subline. The inhibition potential is calculated as *FAR* (fluorescence activity ratio) value. By dividing the uptaken fluorescence amount in the P-gp expressing subline with the fluorescence amount uptaken into the non-P-gp expressing parental cell line a resulting *FAR* value of more than 1.0 will mean that a compound is active as an inhibitor of P-gp. The fluorescence uptake assay has been used in various cell line models with inhibitors like verapamil and cyclosporine A and also the fluorescent anticancer drug daunorubicin as P-gp substrate beside rhodamine 123.[33] Similar determined *FAR* values for both fluorescent substrates daunorubicin and rhodamin 123 reflect similar P-gp inhibiting properties of the used inhibitors. As the *FAR* value directly reflects the P-gp inhibition potential of a modulator it is of an important significance to estimate the benefit of a modulator for further therapeutical usage. If an inhibitor also functions as a P-gp substrate the determined MDR modulating effects have to be considered critically as recently discussed for macrolide antibiotics.[34]

First the P-gp inhibiting potential of the 3-ester substituted derivatives **5a**–**e** has been determined as *FAR* values at each 1 and 10 μmol as used inhibitor concentrations (Table 1). Verapamil has been used as control inhibitor because verapamil is a proven in vitro P-gp inhibitor.

However, verapamil was not active at the low concentration of 1 μmol with a *FAR* value of 0.89 (Table 1). From the various 4-aryl substituted esters **5a**–**e** the phenyl and the 2-tolyl derivatives **5a** and **5c** were found active with *FAR* values of more than 1.0. Also the 4-benzyl residue led to P-gp inhibiting properties of the compound **5b**. So far only 4-aryl substituted 1,4-dihydropyridines have been characterized as P-gp inhibitors.

The inhibitory activity of the compounds increased at the higher used concentrations of 10 μmol with the compound **5e** showing the best activities. However, verapamil is more active than compound **5e** with a 4-methoxyphenyl residue.

In the next series of derivatives we decided to change the 3-ester substituent by a cyano function. Ester groups are known to function as potential hydrogen bond acceptor functions in MDR modulators as discussed above. Cyano functions have been discovered as interesting substituents in enzyme inhibitors with the ability to replace basic substituents.[35] Such cyano functions may undergo hydrogen bonding to amide functions of a potential P-gp binding site similar to ester functions. Interestingly, cyano functions are also found as original structural elements in verapamil. However, so far cyano functions have not been investigated as structural elements in MDR modulators.

Almost all cyano derivatives **5f**–**k** were active as P-gp inhibitors at the tested inhibitor concentrations of 1 μmol with a tendency of the 2-phenyl substituted derivatives **5h** and **5j** to be slightly more active than the corresponding 4-phenyl substituted derivatives **5i** and **5k**.

This tendency was more strengthened at the higher inhibitor concentrations of 10 μmol with the 2-methoxyphenyl derivative **5j** being the most active one which almost reaches the activity of verapamil. Methoxy functions are known hydrogen bond acceptor functions in MDR modulators so that it can be assumed that the methoxy functions may be involved in hydrogen bonding to the potential P-gp binding site.

We have been interested to investigate a possible increase in the P-gp inhibiting properties of the methoxy substituted derivatives **5j** and **5k** and so we tested inhibitor concentrations of 20 μmol in the assay system. At this concentration the 2-methoxyphenyl derivative **5j** reaches a *FAR* value of 3.3 and thus it shows a P-gp inhibiting potential better than verapamil. It was obvious from the flow cytometric analysis of the cells that all the tested inhibitors were non-toxic up to the tested concentrations of 20 μM. As this is a high cellular in vitro concentration the compounds can be classified as non-toxic as far as evaluated. As similar non-toxic effects have been observed in both the P-gp expressing subline and the parental cell line potential P-gp substrate properties of our novel modulators are not suggested because in this case different cytotoxic effects were expected in both cell lines as has been recently demonstrated for ritonavir as P-gp inhibitor with P-gp substrate properties.[36]

The 3-benzyloxy ester derivative **5l** with more lipophilic properties than the other either 3-methoxy ester or 3-cyano substituted compounds has finally been tested as P-gp inhibitor. The

compound was found as active as the 2-methoxyphenyl substituted derivative **5j** with the 3-cyano function at the lower concentration of 1 μmol. At 10 μmol it showed the highest activity within this first pyridine-2-one compound series similar to verapamil. So a favored influence of lipophilic substituents in the increase of the MDR-modulating properties could also be demonstrated within this novel class of MDR modulators.

3. Conclusions

A novel class of perspective MDR modulators has been discovered. First derivatives are active as P-gp inhibitors in concentration ranges at which early MDR modulators of the 1,4-dihydropyridine type like nifedipin have been reported to be inactive.[37] Furthermore, the small-sized pyridine-2-ones promise improved molecular properties with respect to recent hydrolysis-sensitive 1,4-dihydropyridines because the hydrolysis-sensitive carbamide ester functions of those 1,4-dihydropyridines was replaced by a more hydrolysis-stable amide function. So our novel compound class is more active and more stable than previously reported 1,4-dihydropyridines with respect to further clinical applications.

It could be demonstrated that 4-aryl and 4-alkyl–aryl substituents at the molecular skeleton led to biologically active P-gp inhibiting compounds. A favored influence of methoxy functions within the 4-aryl substituents was shown. Moreover, the positioning of the methoxy function within the aryl residue plays an important role with respect to the biological activity suggesting a contribution to hydrogen bonding to the potential P-gp binding site. Additionally, a favorite influence of a first more lipophilic substituent could be demonstrated. Cyano functions are effective substituents as potential hydrogen bond acceptor functions, alternatively to ester groups. Pyridine-2-ones turned out as a perspective class of MDR modulators and are an alternative to the partly critical class of 1,4-dihydropyridines. However, following investigations in structure-dependent properties will help to further develop the biological activities.

4. Experimental

4.1. Chemistry

All the chemical agents used were either synthesized or have been commercially available. Melting points were determined using a Boetius melting desk microscope and are uncorrected. Proton NMR and ^{13}C NMR spectra were recorded on a Varian Gemini 2000 at 400 and 100 MHz, respectively. Chemical shifts are reported in ppm units with tetramethylsilane as internal reference standard. Mass spectra were recorded on an AMD 402 mass spectrometer named AMD INTEGRA (EI masses) or on a Finnigan LCQ classic (ESI masses). Elemental analyses (C, H, N) were carried out with a Leco analyzer apparatus (CHN-932) and the results were within ±0.4% of the theoretical values.

4.1.1. General procedure for the preparation of the 2-methoxy substituted pyridines (2a, b)

Sodium (1.5 g, 65.2 mmol) was added to dried methanol (40 mL). After the dissolvation of the sodium the 2-chloro-pyridine **1** (59 mmol) was added and the solution was heated under reflux for 6 h. Then water (50 mL) and ethyl acetate (50 mL) were added. The organic phase was separated and the water phase was additionally extracted with three portions of ethyl acetate (each 50 mL). The organic layers were unified and dried over sodium sulfate. After filtration the solvent was evaporated to dryness and the remaining oil was purified using column chromatography over silica gel and a mixture of chloroform/ethyl acetate (75/25) as eluent solvent.

4.1.1.1. Methyl 2-methoxynicotinate (2a). Colorless oil; yield 61%; MS (ESI) *m/z* 168 ($M+H^+$, 100%). ^1H NMR ($CDCl_3$) δ 3.85 (s, 3H, $COOCH_3$), 4.05 (s, 3H, C2-OCH_3), 6.91 (dd, J = 7.5 Hz, 4.6 Hz, 1H, 5-H), 8.12 (dd, J = 7.5 Hz, 2.1 Hz, 1H, 4-H), 8.28 (dd, J = 4.6 Hz, 2.1 Hz, 1H, 6-H). Anal. Calcd for $C_8H_9NO_3$: C, 57.14; H, 5.39; N, 8.38. Found: C, 57.53; H, 4.99; N, 8.11.

4.1.1.2. 3-Cyano-2-methoxypyridine (2b). Colorless crystals; yield 93%; mp 70–73 °C; MS (EI) *m/z* 134 (M^+, 70%), 104 (M^+-CH_2O, 100%). ^1H NMR ($CDCl_3$) δ 4.04 (s, 3H, OCH_3), 6.95 (dd, J = 7.5 Hz, 5.0 Hz, 1H, 5-H), 7.86 (dd, J = 7.5 Hz, 2.0 Hz, 1H, 4-H), 8.32 (dd, J = 5.0 Hz, 2.0 Hz, 1H, 6-H). Anal. Calcd for $C_7H_6N_2O$: C, 62.68; H, 4.51; N, 20.88. Found: C, 62.39; H, 4.49; N, 20.48.

4.1.2. General procedure for the preparation of the *N*-benzyl pyridine-2-ones (3a, b, c)

Compound **2** (11 mmol) was dissolved in benzyl bromide (2.3 g, 14 mmol) under heating and the mixture was further heated at 120 °C for 20 h. Then petrol ether was added for the isolation of **3b** and the mixture was kept cooled in a refrigerator. The resulting precipitate of **3b** was purified by washing with cyclohexane and recrystallized from cyclohexane/ethyl acetate. Compounds **3a** and **3c** were purified by column chromatography of the final reaction mixture over silica gel and chloroform/ethyl acetate (25/75) as eluent mixture.

4.1.2.1. Methyl 1-benzyl-2-oxo-1,2-dihydronicotinate (3a). Brownish oil; yield 54%; MS (ESI) *m/z* 244 ($M+H^+$, 100%). ^1H NMR ($CDCl_3$) δ 3.88 (s, 3H, $COOCH_3$), 5.17 (s, 2H, NCH_2), 6.21 (dd, J = 7.1, 6.6 Hz, 1H, 5-H), 7.22–7.37 (m, 5H, aromatic H), 7.50 (dd, J = 6.6, 2.1 Hz, 1H, 4-H), 8.12 (dd, J = 7.1, 2.1 Hz, 1H, 6-H). Anal. Calcd for $C_{14}H_{13}NO_3$: C, 69.13; H, 5.39; N, 5.75. Found: C, 58.88; H, 5.12; N, 5.54.

4.1.2.2. 1-Benzyl-3-cyano-2-oxo-1,2-dihydropyridine (3b). Brownish crystals; yield 56%; mp 110–112 °C; MS (EI) *m/z* 210 (M^+, 25%), 91 (Bn, 100%). ^1H NMR ($CDCl_3$) δ 5.15 (s, 2H, NCH_2), 6.22 ('t', J = 6.8 Hz, 1H, 5-H), 7.29–7.38 (m, 5H, aromatic H), 7.53 (dd, J = 6.8, 2.1 Hz, 1H, 4-H), 7.76 (dd, J = 6.8, 2.1 Hz, 1H, 6-H). Anal. Calcd for $C_{13}H_{10}N_2O$: C, 74.27; H, 4.79; N, 13.32. Found: C, 74.09; H, 4.85; N, 13.35.

4.1.2.3. Benzyl 1-benzyl-2-oxo-1,2-dihydronicotinate (3c). Brownish oil; yield 8%; MS (ESI) *m/z* 342 ($M+Na^+$, 100%). ^1H NMR ($CDCl_3$) δ 5.17 (s, 2H, NCH_2), 5.33 (s, 2H, OCH_2), 6.17 (dd, J = 7.1, 6.2 Hz, 1H, 5-H), 7.27–7.46 (m, 10H, aromatic H), 7.48 (d, J = 6.2 Hz, 1H, 4-H), 8.10 (d, J = 7.1 Hz, 1H, 6-H). Anal. Calcd for $C_{20}H_{17}NO_3$: C, 75.16; H, 5.37; N, 4.39. Found: C, 74.79; H, 5.05; N, 4.10.

4.1.3. General procedure for the 4-arylation of the pyridine-2-ones to the tetrahydropyridine-2-ones (4a–l)

One gram (4.1 mmol) of the corresponding pyridine-2-one **3** was dissolved in dried THF (30 mL). Copper(I) iodide (0.16 g, 0.8 mmol) and lithium chloride (0.034 g, 0.8 mmol) were added and the solution was cooled down to −40 °C. The corresponding grignard reagent (6.6 mmol) was added dropwise under stirring. After additional 6 h the stirred solution was warmed up to rt. Then a 20% solution of ammonium chloride (20 mL) and diethylether (25 mL) were added. The organic layer was separated and the water phase was extracted twice with diethylether (25 mL). The unified organic layer was extracted with a 20% solution of ammonium chloride (50 mL), water (50 mL), twice with a 10% solution of hydrochloric acid (50 mL), water (50 mL) and a saturated solution of sodium chloride. After drying over sodium sulfate the organic layer was evaporated to dryness and the remaining oil was purified

by column chromatography over silica gel using an eluent mixture of chloroform/ethyl acetate (70/30).

4.1.3.1. Methyl 1-benzyl-2-oxo-4-phenyl-1,2,3,4-tetrahydronicotinate (4a). Yellow oil; yield 16%; MS (EI) *m/z* 321 (M^+, 5%), 91 (Bn, 100%). ^{1}H NMR ($CDCl_3$) δ 3.69 (s, 3H, $COOCH_3$), 3.71 (d, J = 9.1 Hz, 1H, 3-H), 4.19 (ddd, J = 9.1 Hz, 4.2 Hz, 1.2 Hz, 1H, 4-H), 4.71 (d, J = 14.9 Hz, 1H, N–CH_B), 4.77 (d, J = 14.9 Hz, 1H, N–CH_A), 5.22 (dd, J = 7.9 Hz, 4.2 Hz, 1H, 5-H), 6.17 (dd, J = 7.9 Hz, 1.2 Hz, 1H, 6-H), 7.20–7.35 (m, 10H, aromatic H). Anal. Calcd for $C_{20}H_{19}NO_3$: C, 74.75; H, 5.96; N, 4.35. Found: C, 74.44; H, 5.68; N, 3.95.

4.1.3.2. Methyl 1,4-dibenzyl-2-oxo-1,2,3,4-tetrahydronicotinate (4b). Brownish oil; yield 38%; MS (EI) *m/z* 335 (M^+, 6%), 91 (Bn, 100%). ^{1}H NMR ($CDCl_3$) δ 2.63 (dd, J = 13.3 Hz, 8.3 Hz, 1H, C4-CH_B), 2.68 (dd, J = 13.3 Hz, 7.1 Hz, 1H, C4-CH_A), 3.19 (m, 1H, 4-H), 3.45 (d, J = 6.2 Hz, 1H, 3-H), 3.71 (s, 3H, $COOCH_3$), 4.65 (d, J = 14.9 Hz, 1H, N–CH_A), 4.75 (d, J = 14.9 Hz, 1H, N–CH_B), 5.06 (dd, J = 7.9 Hz, 4.6 Hz, 1H, 5-H), 5.99 (d, J = 7.9 Hz, 1H, 6-H), 7.21–7.34 (m, 10H, aromatic H). Anal. Calcd for $C_{21}H_{21}NO_3$: C, 75.14; H, 6.31; N, 4.18. Found: C, 74.85; H, 5.96; N, 3.79.

4.1.3.3. Methyl 1-benzyl-2-oxo-4-(2-methylphenyl)-1,2,3,4-tetrahydronicotinate (4c). Brownish oil; yield 23%; MS (EI) *m/z* 335 (M^+, 7%), 276 (M^+-$COOCH_3$, 100%). ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3H, Ph–CH_3), 3.72 (d, J = 5.7 Hz, 1H, 3-H), 3.73 (s, 3H, $COOCH_3$), 4.19 ('dt', J = 5.7 Hz, 1.1 Hz, 1H, 4-H), 4.73 (d, J = 14.7 Hz, 1H, N–CH_B), 4.82 (d, J = 14.7 Hz, 1H, N–CH_A), 5.17 (dd, J = 7.7 Hz, 5.7 Hz, 1H, 5-H), 6.23 (dd, J = 7.7 Hz, 1.1 Hz, 1H, 6-H), 7.12–7.37 (m, 9H, aromatic H). Anal. Calcd for $C_{21}H_{21}NO_3$: C, 75.20; H, 6.31; N, 4.18. Found: C, 74.95; H, 5.93; N, 3.78.

4.1.3.4. Methyl 1-benzyl-2-oxo-4-(4-methylphenyl)-1,2,3,4-tetrahydronicotinate (4d). Brownish oil; yield 42%; MS (EI) *m/z* 335 (M^+, 5%), 276 (M^+-$COOCH_3$, 100%). ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, Ph–CH_3), 3.69 (s, 3H, $COOCH_3$), 3.70 (d, J = 8.7 Hz, 1H, 3-H), 4.19 (ddd, J = 8.7, 4.2, 1.7 Hz, 1H, 4-H), 4.72 (d, J = 14.9 Hz, 1H, N–CH_B), 4.77 (d, J = 14.9 Hz, 1H, N–CH_A), 5.21 (dd, J = 7.9, 4.2 Hz, 1H, 5-H), 6.16 (dd, J = 7.9, 1.7 Hz, 1H, 6-H), 7.19–7.36 (m, 9H, aromatic H). Anal. Calcd for $C_{21}H_{21}NO_3$: C, 75.20; H, 6.31; N, 4.18. Found: C, 74.83; H, 6.00; N, 3.82.

4.1.3.5. Methyl 1-benzyl-2-oxo-4-(4-methoxyphenyl)-1,2,3,4-tetrahydronicotinate (4e). Brownish oil; yield 13%; MS (ESI) *m/z* 374 (M+Na^+, 100%). ^{1}H NMR ($CDCl_3$) δ 3.66 (d, J = 8.7 Hz, 1H, 3-H), 3.68 (s, 3H, $COOCH_3$), 3.76 (s, 3H, Ph–OCH_3), 4.14 (ddd, J = 8.7, 4.2, 1.7 Hz, 1H, 4-H), 4.70 (d, J = 14.9 Hz, 1H, N–CH_B), 4.76 (d, J = 14.9 Hz, 1H, N–CH_A), 5.20 (dd, J = 7.9, 4.2 Hz, 1H, 5-H), 6.15 (dd, J = 7.9, 1.7 Hz, 1H, 6-H), 6.78–7.35 (m, 9H, aromatic H). Anal. Calcd for $C_{21}H_{21}NO_4$: C, 71.78; H, 6.02; N, 3.99. Found: C, 71.66; H, 5.75; N, 3.68.

4.1.3.6. 1-Benzyl-3-cyano-4-phenyl-2-oxo-1,2,3,4-tetrahydropyridine (4f). Yellow semisolid compound; yield 14%; MS (EI) *m/z* 288 (M^+, 31%), 91 (Bn, 100%). ^{1}H NMR ($CDCl_3$) δ 3.77 (d, J = 6.1 Hz, 1H, 3-H), 4.00 (ddd, J = 6.1, 3.4, 2,1 Hz, 1H, 4-H), 4.70 (d, J = 15.0 Hz, 1H, N–CH_B), 4.76 (d, J = 15.0 Hz, 1H, N–CH_A), 5.27 (dd, J = 8.0, 3.4 Hz, 1H, 5-H), 6.25 (dd, J = 8.0, 2.1 Hz, 1H, 6H), 7.02–7.47 (m, 10H, aromatic H). Anal. Calcd for $C_{19}H_{16}N_2O$: C, 79.14; H, 5.59; N, 9.71. Found: C, 78.84; H, 5.79; N, 9.51.

4.1.3.7. 1,4-Dibenzyl-3-cyano-2-oxo-1,2,3,4-tetrahydropyridine (4g). Yellow semisolid compound; yield 56%; MS (EI) *m/z* 302 (M^+, 11%), 91 (Bn, 100%). ^{1}H NMR ($CDCl_3$) δ 2.75 (dd, J = 13.6, 8.7 Hz, 1H, C4-CH_B), 2.79 (dd, J = 13.6, 7.8 Hz, 1H, C4-CH_A), 3.03–3.11 (m, 1H, 4-H), 3.66 (d, J = 5.3 Hz, 1H, 3-H), 4.63 (d, J = 15.0 Hz, 1H, N–CH_B), 4.70 (d, J = 15.0 Hz, 1H, N–CH_A), 5.12 (dd, J = 7.8, 3.8 Hz, 1H, 5-H), 6.05 (dd, J = 7.8, 1.5 Hz, 1H, 6H), 7.18–7.36 (m, 10H, aromatic H). Anal. Calcd for $C_{20}H_{18}N_2O$: C, 79.44; H, 6.30; N, 9.26. Found: C, 79.04; H, 6.47; N, 9.36.

4.1.3.8. 1-Benzyl-3-cyano-4-(2-methylphenyl)-2-oxo-1,2,3,4-tetrahydropyridine (4h). Brownish semisolid compound; yield 50%; MS (EI) *m/z* 302 (M^+, 42%), 91 (Bn, 100%). ^{1}H NMR ($CDCl_3$) δ 1.41 (m, 3H, CH_3), 3.77 (d, J = 7.1 Hz, 1H, 3-H), 4.10 (dd, J = 7.1, 3.6 Hz, 1H, 4-H), 4.26 (d, J = 15.0 Hz, 1H, N–CH_B), 4.33 (d, J = 15.0 Hz, 1H, N–CH_A), 5.20 (dd, J = 7.9 Hz, 3.6 Hz, 1H, 5-H), 6.28 (dd, J = 7.9 Hz, 1H, 6H), 6.72–7.41 (m, 9H, aromatic H). Anal. Calcd for $C_{20}H_{18}N_2O$: C, 79.44; H, 6.0; N, 9.26. Found: C, 79.25; H, 5.98; N, 8.94.

4.1.3.9. 1-Benzyl-3-cyano-4-(4-methylphenyl)-2-oxo-1,2,3,4-tetrahydropyridine (4i). Brownish semisolid compound; yield 62%; MS (EI) *m/z* 302 (M^+, 34%), 91 (Bn, 100%). ^{1}H NMR ($CDCl_3$) δ 1.41 (m, 3H, CH_3), 2.97 (d, J = 7.9 Hz, 1H, 3-H), 3.74 (ddd, J = 7.9, 3.5, 2.1 Hz, 1H, 4-H), 4.56 (d, J = 14.9 Hz, 1H, N–CH_B), 4.93 (d, J = 14.9 Hz, 1H, N–CH_A), 5.25 (dd, J = 7.9, 3.5 Hz, 1H, 5-H), 6.23 (dd, J = 7.9, 2.1 Hz, 1H, 6H), 6.70–7.31 (m, 9H, aromatic H). Anal. Calcd for $C_{20}H_{18}N_2O$: C, 79.44; H, 6.0; N, 9.26. Found: C, 79.15; H, 6.04; N, 8.91.

4.1.3.10. 1-Benzyl-3-cyano-4-(2-methoxyphenyl)-2-oxo-1,2,3,4-tetrahydropyridine (4j). Yellow semisolid compound; yield 38%; MS (EI) *m/z* 318 (M^+, 66%), 91 (Bn, 100%). ^{1}H NMR ($CDCl_3$) δ 3.73–3.85 (m, 2H, 3-H, 4-H), 3.75 (s, OCH_3), 4.70 (d, J = 14.9 Hz, 1H, N–CH_B), 4.75 (d, J = 14.9 Hz, 1H, N–CH_A), 5.20 (dd, J = 7.9, 5.3 Hz, 1H, 5-H), 6.31 (dd, J = 7.9, 2.1 Hz, 1H, 6H), 6.71–7.31 (m, 9H, aromatic H). Anal. Calcd for $C_{20}H_{18}N_2O_2$: C, 75.45; H, 5.75; N, 8.80. Found: C, 75.78; H, 6.12; N, 8.70.

4.1.3.11. 1-Benzyl-3-cyano-4-(4-methoxyphenyl)-2-oxo-1,2,3,4-tetrahydropyridine (4k). Yellow semisolid compound; yield 12%; MS (EI) *m/z* 318 (M^+, 14%), 91 (Bn, 100%). ^{1}H NMR ($CDCl_3$) δ 2.95 (dd, J = 7.8, 3.8 Hz, 1H, 4-H), 3.75 (s, OCH_3), 4.00 (d, J = 7.8 Hz, 1H, 3-H), 4.73 (d, J = 14.9 Hz, 1H, N–CH_B), 4.76 (d, J = 14.9 Hz, 1H, N–CH_A), 5.25 (dd, J = 7.9, 3.8 Hz, 1H, 5-H), 6.23 (dd, J = 7.9 Hz, 1H, 6H), 6.75–7.42 (m, 9H, aromatic H). Anal. Calcd for $C_{20}H_{18}N_2O_2$: C, 75.45; H, 5.75; N, 8.80. Found: C, 75.34; H, 5.83; N, 8.34.

4.1.3.12. Benzyl 1-Benzyl-2-oxo-4-phenyl-1,2,3,4-tetrahydronicotinate (4l). Brownish oil; yield 12%; MS (EI) *m/z* 397 (M^+, 6%), 91 (Bn, 100%). ^{1}H NMR ($CDCl_3$) δ 3.71 (d, J = 9.1 Hz, 1H, 3-H), 4.19 (ddd, J = 9.1, 4.2, 1.2 Hz, 1H, 4-H), 4.71 (d, J = 14.9 Hz, 1H, N–CH_B), 4.77 (d, J = 14.9 Hz, 1H, N–CH_A), 5.13 (d, J = 12.5 Hz, O–CH_B), 5.17 (d, J = 12.5 Hz, O–CH_A), 5.22 (dd, J = 7.9, 4.2 Hz, 1H, 5-H), 6.17 (dd, J = 7.9, 1.2 Hz, 1H, 6-H), 7.20–7.35 (m, 10H, aromatic H). Anal. Calcd for $C_{26}H_{23}NO_3$: C, 78.57; H, 5.83; N, 3.52. Found: C, 78.25; H, 5.53; N, 3.12.

4.1.4. General procedure for the synthesis of the aromatized pyridine-2-ones (5a–l)

One equivalent of the corresponding 1,2,3,4-tetrahydropyridine-2-one **4** is dissolved in toluene (50 mL). Two equivalents of heat-activated (120 °C, 12 h) manganese(IV) oxide were added. The suspension was heated under reflux for 6–8 h at 130 °C. Then the solution was filtered and the residue was washed for three times with ethyl acetate (50 mL each). The organic layers were unified and the solvent was evaporated to dryness in vacuum. The remaining oily residue was purified by column chromatography over silica gel using an eluent mixture of chloroform/ethyl acetate (40/60).

4.1.4.1. Methyl 1-Benzyl-2-oxo-4-phenyl-1,2-dihydronicotinate (5a). Yellow oil; yield 7%; MS (ESI) *m/z* 320 ($M+H^+$,100%). ^1H NMR ($CDCl_3$) δ 3.67 (s, 3H, $COOCH_3$), 5.15 (s, 2H, N–CH_2), 6.20 (d, J = 7.1 Hz, 1H, 5-H), 7.33 (d, J = 7.1 Hz, 1H, 6-H), 7.32–7.43 (m, 10H, aromatic H). Anal. Calcd for $C_{20}H_{17}NO_3$: C, 75.22; H, 5.37; N, 4.39. Found: C, 74.84; H, 5.22; N, 4.15.

4.1.4.2. Methyl 1,4-dibenzyl-2-oxo-4-phenyl-1,2-dihydronicotinate (5b). Brownish oil; yield 4%; MS (ESI) *m/z* 356 ($M+Na^+$, 100%). ^1H NMR ($CDCl_3$) δ 3.80 (s, 2H, C4-CH_2), 3.88 (s, 3H, $COOCH_3$), 5.06 (s, 2H, N–CH_2), 5.89 (d, J = 7.1 Hz, 1H, 5-H), 7.14 (d, J = 7.1 Hz, 1H, 6-H), 7.16–7.36 (m, 10H, aromatic H). Anal. Calcd for $C_{21}H_{19}NO_3$: C, 75.66; H, 5.74; N, 4.20. Found: C, 75.38; H, 5.51; N, 3.97.

4.1.4.3. Methyl 1-benzyl-2-oxo-4-(2-methylphenyl)-1,2-dihydronicotinate (5c). Yellow oil; yield 5%; MS (ESI) *m/z* 356 ($M+Na^+$, 100%). ^1H NMR ($CDCl_3$) δ 2.20 (s, 3H, Ph–CH_3), 3.55 (s, 3H, $COOCH_3$), 5.19 (s, 2H, N–CH_2), 6.04 (d, J = 7.1 Hz, 1H, 5-H), 7.09 (d, J = 7.1 Hz, 1H, 6-H), 7.14–7.40 (m, 9H, aromatic H). Anal. Calcd for $C_{21}H_{19}NO_3$: C, 75.66; H, 5.74; N, 4.20. Found: C, 75.44; H, 5.53; N, 3.98.

4.1.4.4. Methyl 1-benzyl-2-oxo-4-(4-methylphenyl)-1,2-dihydronicotinate (5d). Yellow oil; yield 6%; MS (ESI) *m/z* 356 ($M+Na^+$, 100%). ^1H NMR ($CDCl_3$) δ 2.36 (s, 3H, Ph–CH_3), 3.70 (s, 3H, $COOCH_3$), 5.14 (s, 2H, N–CH_2), 6.19 (d, J = 7.1 Hz, 1H, 5-H), 7.17–7.35 (m, 10H, aromatic H and 6-H). Anal. Calcd for $C_{21}H_{19}NO_3$: C, 75.66; H, 5.74; N, 4.20. Found: C, 75.35; H, 5.66; N, 4.05.

4.1.4.5. Methyl 1-benzyl-2-oxo-4-(4-methoxyphenyl)-1,2-dihydronicotinate (5e). Yellow oil; yield 11%; MS (ESI) *m/z* 372 ($M+Na^+$, 100%). ^1H NMR ($CDCl_3$) δ 3.72 (s, 3H, $COOCH_3$), 3.81 (s, 3H, Ph–OCH_3), 5.14 (s, 2H, N–CH_2), 6.19 (d, J = 7.1 Hz, 1H, 5-H), 6.90–7.35 (m, 9H, aromatic H), 7.29 (d, J = 7.1 Hz, 1H, 6-H). Anal. Calcd for $C_{21}H_{19}NO_4$: C, 75.66; H, 5.74; N, 4.20. Found: C, 75.44; H, 5.68; N, 3.99.

4.1.4.6. 1-Benzyl-3-cyano-4-phenyl-2-oxo-1,2-dihydropyridine (5f). Yellow crystals; mp 110–113 °C; yield 55%; MS (EI) *m/z* 286 (M^+, 53%), 91 (Bn, 100%). ^1H NMR ($CDCl_3$) δ 5.17 (s, 2H, N–CH_2), 6.3 (d, J = 7.1 Hz, 1H, 5-H), 7.51 (d, J = 7.1 Hz, 1H, 6-H), 7.30–7.61 (m, 10H, aromatic H). ^{13}C NMR ($CDCl_3$) δ 52.5 (t, N–CH_2), 104.5 (s, C-3), 107.2 (d, C-5), 115.0 (s, CN), 127.4 (d, C-4-Ph, C-4-CH_2–Ph), 128.5 (d, C-2/C-6-4-Ph), 128.6 (d, C-2/C-6-CH_2–Ph), 128.7 (d, C-3/C-5-CH_2–Ph), 129.2 (d, C-3/C-5-4-Ph), 134.8 (s, C-4-Ph), 136.2 (s, C1-CH_2–Ph), 140.5 (d, C-6), 159.9 (s, CO), 161.5 (s, C-4). Anal. Calcd for $C_{19}H_{14}N_2O$: C, 79.70; H, 4.93; N, 9.78. Found: C, 79.64; H, 4.67; N, 9.51.

4.1.4.7. 1,4-Dibenzyl-3-cyano-2-oxo-1,2-dihydropyridine (5g). Yellow solid; yield 47%; mp 108–112 °C; MS (EI) *m/z* 300 (M^+, 51%), 91 (Bn, 100%). ^1H NMR ($CDCl_3$) δ 4.02 (s, 2H, C4-CH_2), 5.08 (s, 2H, N–CH_2), 6.0 (d, J = 7.1 Hz, 1H, 5-H), 7.07–7.44 (m, 11H, aromatic H and 6-H). ^{13}C NMR ($CDCl_3$) δ 40.6 (t, C-4-CH_2), 52.5 (t, N–CH_2), 104.5 (s, C-3), 107.2 (d, C-5), 115.0 (s, CN), 127.4 (d, C-4-CH_2–Ph), 127.9 (d, C-4-N–CH_2–Ph), 128.0 (d, C-2/C-6-N–CH_2–Ph), 128.8 (d, C-3/C-5-N–CH_2–Ph), 128.9 (d, C-3/C-5-4-CH_2–Ph), 129.2 (d, C-2/C-6-4-CH_2–Ph), 134.8 (C-1-N–CH_2–Ph), 136.2 (s, C-1-4-CH_2–Ph), 140.5 (d, C-6), 159.9 (s, CO), 161.5 (s, C-4). Anal. Calcd for $C_{20}H_{16}N_2O$: C, 79.98; H, 5.37; N, 9.33. Found: C, 79.75; H, 5.47; N, 9.36.

4.1.4.8. 1-Benzyl-3-cyano-4-(2-methylphenyl)-2-oxo-1,2-dihydropyridine (5h). Yellow crystals; mp 115–117 °C; yield 45%; MS (EI) *m/z* 300 (M^+, 45%), 91 (Bn, 100%). ^1H NMR ($CDCl_3$) δ 2.26 (s, 3H, CH_3), 5.19 (s, 2H, N–CH_2), 6.15 (d, J = 7.0 Hz, 1H, 5-H), 7.15–7.50 (m, 10H, aromatic H and 6-H). ^{13}C NMR ($CDCl_3$) δ 19.9 (q, CH_3), 52.9 (t, N–CH_2), 105.3 (s, C-3), 108.2 (d, C-5), 114.8 (s, CN), 126.2 (d, C-4-4-Ph), 126.8 (d, C-6-4-Ph), 127.3 (d, C-4-N–CH_2–Ph), 127.4 (d, C-2/C-6-N–CH_2–Ph), 128.4 (d, C4-4-Ph), 129.1 (d, C-3/C-5-N–CH_2–Ph), 129.4 (d, C-3-4-Ph), 132.3 (s, C-1-4-Ph), 134.8 (s, *C*–CH_3), 135.5 (s, C-1-N–CH_2–Ph), 140.2 (d, C-6), 160.0 (s, CO), 161.1 (s, C-4). Anal. Calcd for $C_{20}H_{16}N_2O$: C, 79.98; H, 5.37; N, 9.33. Found: C, 79.77; H, 5.58; N, 9.11.

4.1.4.9. 1-Benzyl-3-cyano-4-(4-methylphenyl)-2-oxo-1,2-dihydropyridine (5i). Brownish crystals; mp 154–156 °C; yield 45%; MS (EI) *m/z* 300 (M^+, 63%), 91 (Bn, 100%). ^1H NMR ($CDCl_3$) δ 2.39 (m, 3H, CH_3), 5.16 (s, 2H, N–CH_2), 6.25 (d, J = 7.1 Hz, 1H, 5-H), 7.22–7.52 (m, 10H, aromatic H and 6-H). ^{13}C NMR ($CDCl_3$) δ 19.9 (q, CH_3), 52.9 (t, N–CH_2), 105.3 (s, C-3), 108.2 (d, C-5), 114.8 (s, CN), 126.2 (d, C-2/C-6-4-Ph), 126.7 (d, C-4-N–CH_2–Ph), 126.8 (d, C-2/C-6-N–CH_2–Ph), 128.5 (d, C-3/C-5-N–CH_2–Ph), 128.8 (d, C-3/C-5-4-Ph), 129.4 (s, C-1-4-Ph), 134.8 (s, *C*–CH_3), 135.5 (s, C-1-N–CH_2–Ph), 140.2 (d, C-6), 160.0 (s, CO), 161.1 (s, C-4). Anal. Calcd for $C_{20}H_{16}N_2O$: C, 79.98; H, 5.37; N, 9.33. Found: C, 79.58; H, 5.67; N, 8.95.

4.1.4.10. 1-Benzyl-3-cyano-4-(2-methoxyphenyl)-2-oxo-1,2-dihydropyridine (5j). Yellow crystals; mp 111–113 °C; yield 29%; MS (EI) *m/z* 316 (M^+, 66%), 91 (Bn, 100%). ^1H NMR ($CDCl_3$) δ 3.84 (s, 3H, OCH_3), 5.16 (s, 2H, N–CH_2), 6.26 (d, J = 7.1 Hz, 1H, 5-H), 6.96–7.45 (m, 10H, aromatic H and 6-H). ^{13}C NMR ($CDCl_3$) δ 52.7 (t, N–CH_2), 55.6 (q, CH_3), 105.2 (s, C-3), 108.8 (d, C-5), 115.4 (s, CN), 120.9 (d, C-3-4-Ph), 125.9 (s, C-1-4-Ph), 125.0 (d, C-5-4-Ph), 130.8 (d, C-4-N–CH_2–Ph), 130.9 (d, C-2/C-6-N–CH_2–Ph), 131.4 (d, C-6-4-Ph), 134.8 (d, C-3/C-5-N–CH_2–Ph), 135.0 (s, C1-N–CH_2–Ph), 135.1 (d, C-4-4-Ph), 139.7 (d, C-6), 156.2 (s, CO), 157.7 (s, *C*–OCH_3), 160.4 (s, C-4). Anal. Calcd for $C_{20}H_{16}N_2O_2$: C, 75.93; H, 5.10; N, 8.85. Found: C, 75.55; H, 5.50; N, 8.77.

4.1.4.11. 1-Benzyl-3-cyano-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine (5k). Yellow crystals; mp 113–117 °C; yield 58%; MS (EI) *m/z* 316 (M^+, 45%), 91 (Bn, 100%). ^1H NMR ($CDCl_3$) δ 3.75 (s, 3H, OCH_3), 5.16 (s, 2H, N–CH_2), 6.28 (d, J = 7.1 Hz, 1H, 5-H), 7.45 (d, J = 7.1 Hz, 1H, 6H), 7.00–7.60 (m, 9H, aromatic H). ^{13}C NMR ($CDCl_3$) δ 52.5 (t, N–CH_2), 55.5 (s, OCH_3), 101.8 (s, C-3), 107.1 (d, C-5), 116.0 (s, CN), 127.5 (d, C-3/C-5-4-Ph), 128.4 (s, C-1-4-Ph), 129.8 (d, C-2/C-6-N–CH_2–Ph), 129.9 (d, C-4-N–CH_2–Ph), 130.5 (d, C-2/C-6-4-Ph), 131.7 (d, C-3/C-5-N–CH_2–Ph), 135.1 (s, C-1-N–CH_2–Ph), 140.2 (d, C-6), 159.0 (s, CO), 160.6 (s, *C*–OCH_3) 161.7 (s, C-4). Anal. Calcd for $C_{20}H_{16}N_2O_2$: C, 75.93; H, 5.10; N, 8.85. Found: C, 75.80; H, 5.00; N, 8.52.

4.1.4.12. Benzyl 1-benzyl-2-oxo-4-phenyl-1,2-dihydronicotinate (5l). Yellow oil; yield 9%; MS (ESI) *m/z* 418 ($M+Na^+$, 100%). ^1H NMR ($CDCl_3$) δ 5.11 (s, 2H, OCH_2), 5.16 (s, 2H, N–CH_2), 6.18 (d, J = 7.9 Hz, 1H, 5-H), 7.19–7.36 (m, 16H, aromatic H and 6-H). Anal. Calcd for $C_{26}H_{21}NO_3$: C, 78.97; H, 5.35; N, 3.54. Found: C, 78.65; H, 5.28; N, 3.14.

4.2. Cell culture

Both cell lines the mouse T-lymphoma parental cell line L5178Y and the P-gp expressing subline L5178Y *mdr* which resulted from retrovirus-mediated gene transfection[38,39] were cultured in McCoys 5A medium containing 10% heat-inactivated horse serum, L-glutamine (2 mM) and antibiotics.[37] The cells of the P-gp expressing subline L5178Y have been yielded by precultivation in colchicine (60 ng/mL)-supplemented medium to ensure an ongoing extent of P-gp expression.

4.3. MDR reversal assay of P-gp inhibition

Cells of both cell lines were taken in an adjusted concentration of 2×10^6 cells per mL medium and resuspended in serum free Mc Coys 5A medium. 0.5 mL Aliquots were distributed in Eppendorf centrifuge tubes. The test compounds were taken from preprepared stock solutions (1.0 mg/mL). After 10 min of inhibitor preincubation at rt the P-gp substrate rhodamine 123 was added reaching a final concentration of 5.2 μM. Incubation was continued for 20 min at 37 °C. After that the cells were washed twice and then resuspended in phosphate-buffered saline (PBS) for measurement. The non-inhibitor containing cells were treated in the same way than the inhibitor-preincubated cells. The fluorescence uptake of rhodamine 123 was determined by flow cytometry using a Becton Dickinson FACScan flow cytometer within a number of 1×10^4 counted cells. The fluorescence activity ratio (*FAR*) value was calculated from the quotient of the determined fluorescence uptake ratios of the P-gp expressing cell line and the non-P-gp expressing parental cell line. Both ratios have been corrected by division with the fluorescence determined in the inhibitor-untreated control cell lines. Statistical significance was expressed using the student́s *t* test.

Acknowledgments

The authors gratefully acknowledge the financial support of their work by the EU within the COST B16 action, the BMBF and the DFG (Deutsche Forschungsgemeinschaft) within the projects HI 687/5-1 and HI 687/5-3 to Claudius Coburger, Sören Krawczyk and Andreas Hilgeroth.

Supplementary data

Supplementary data (inhibitor relevant fluorescence intensity data) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.09.005.

References and notes

1. Gottesman, M. M.; Ling, V. *FEBS Lett.* **2006**, *580*, 998.
2. Lage, H. *Cell Mol. Life Sci.* **2008**, *65*, 3145.
3. Eckford, P. D. W.; Sharom, F. J. *Chem. Rev.* **2009**, *109*, 2989.
4. Gillet, J.-P.; Efferth, T.; Remacle, J. *Biochim. Biophys. Acta* **2007**, *1775*, 237.
5. Dean, M.; Rzhetsky, A.; Allikmets, R. *Genome Res.* **2009**, *11*, 1156.
6. Baumert, C.; Hilgeroth, A. *Anti-Cancer Agents Med. Chem.* **2009**, *9*, 415.
7. Wang, R. B.; Kuo, C. L.; Lien, L. L.; Lien, E. J. *J. Clin. Pharm. Ther.* **2003**, *28*, 203.
8. Chang, C.; Bahadduri, P. M.; Polli, J. E.; Swaan, P. W.; Ekins, S. *Drug Metab. Disp.* **2006**, *34*, 1976.
9. Mahon, F. X.; Belloc, F.; Lagarde, V.; Chollet, C.; Moreau-Gaudry, F.; Reiffers, J.; Goldman, J. M.; Melo, J. V. *Blood* **2003**, *101*, 2368.
10. Matsui, H.; Takeshita, A.; Naito, K.; Shinjo, K.; Shigeno, K.; Maekawa, M.; Yamakaw, Y.; Tanimito, M.; Kobayashi, M.; Ohnishi, K.; Ohno, R. *Leukemia* **2002**, *16*, 813.
11. Bonavida, B. *Oncogene* **2007**, *26*, 3629.
12. Krühn, A.; Wang, A.; Fruehauf, J. H.; Lage, H. *Cell Cycle* **2009**, *20*, 3349.
13. Avendanó, C.; Menéndez, J. C. *Med. Chem. Rev.-Online* **2004**, *1*, 419.
14. Choi, C.-H. *Cancer Cell Int.* **2005**, *5*, 30.
15. Tain, Q.; Zhang, J.; Chan, E.; Duan, W.; Zhou, S. *Drug Dev. Res.* **2005**, *64*, 1.
16. Sheps, J. A.; Ling, V. *Eur. J. Physiol.* **2007**, *453*, 545.
17. Krishna, R.; Mayer, L. D. *Eur. J. Pharm. Sci.* **2000**, *11*, 265.
18. Kawase, M.; Motohashi, N. *Curr. Drug Targets* **2003**, *4*, 31.
19. Lage, H.; Duarte, N.; Coburger, C.; Hilgeroth, A.; Ferreira, M. J. U. *Phytomedicine* **2010**, *17*, 441.
20. Tanabe, H.; Tasaka, S.; Ohmori, H.; Gomi, N.; Sasaki, Y.; Machida, T.; Iino, M.; Kiue, A.; Naito, S.; Kuwano, M. *Bioorg. Med. Chem.* **1998**, *6*, 2219.
21. Tsuruo, T.; Iida, H.; Tukagoshi, S.; Sakurai, Y. *Cancer Res.* **1981**, *41*, 1967.
22. Kawase, M.; Shah, A.; Gaveriya, H.; Motohashi, N.; Sakagami, H.; Varga, A.; Molnár, J. *Bioorg. Med. Chem.* **2002**, *10*, 1051.
23. Shah, A.; Gaveriya, H.; Motohashi, N.; Kawase, M.; Saito, S.; Sakagami, H.; Satoh, K.; Tada, Y.; Solymosi, A.; Walfard, K.; Molnár, J. *Anticancer Res.* **2000**, *20*, 373.
24. Seelig, A.; Landwojtowicz, E. *Eur. J. Pharm. Sci.* **2000**, *12*, 31.
25. Ecker, G.; Huber, M.; Schmidt, D.; Chiba, P. *Mol. Pharmacol.* **1999**, *56*, 791.
26. Hilgeroth, A.; Molnár, J.; De Clercq, E. *Angew. Chem.* **2002**, *114*, 3772. *Angew. Chem. Int. Ed.* **2002**, *41*, 3623.
27. Seelig, A.; Blatter, L.; Wohnsland, F. *Int. J. Clin. Pharmacol. Ther.* **2000**, *38*, 111.
28. Hilgeroth, A.; Brachwitz, K.; Baumeister, U. *Heterocycles* **2001**, *55*, 661.
29. Commins, D. L.; Stroud, E. D.; Herrick, J. J. *Heterocycles* **1984**, *22*, 151.
30. Hilgeroth, A.; Baumeister, U. *Angew. Chem.* **2000**, *112*, 588. *Angew. Chem. Int. Ed.* **2000**, *39*, 576.
31. Hiroya, K.; Kawamoto, K.; Sakamoto, T. *Synlett* **2006**, *16*, 2636.
32. Kuckländer, U.; Hilgeroth, A. *Arch. Pharm.* **1994**, *327*, 287.
33. Coburger, C.; Lage, H.; Molnár, J.; Hilgeroth, A. *Pharm. Res.* **2008**, *26*, 182.
34. Munic, V.; Kelneric, Z.; Mikac, L.; Haber, V. E. *Eur. J. Pharm. Sci.* **2010**, *41*, 86.
35. Lee, S. L.; Alexander, R. S.; Smallwood, A.; Trievel, R.; Mersinger, L.; Weber, P. C.; Kettner, C. *Biochemistry* **1997**, *36*, 13180.
36. Coburger, C.; Lage, H.; Molnár, J.; Langner, A.; Hilgeroth, A. *J. Pharm. Pharmacol.* **2010**, *62*, 1704.
37. Coburger, C.; Wollmann, J.; Krug, M.; Baumert, C.; Seifert, M.; Molnár, J.; Lage, H.; Hilgeroth, A. *Bioorg. Med. Chem.* **2010**, *18*, 4983.
38. Weaver, J. L.; Szabo, D.; Pine, P. S.; Gottesmann, S.; Goldberg, A.; Aszalos, A. *Int. J. Cancer* **1993**, *54*, 456.
39. Cornwell, M. M.; Pastan, I.; Gottesmann, M. M. *J. Biol. Chem.* **1987**, *262*, 2166.