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Development of small-molecule P-gp inhibitors of the *N*-benzyl 1,4-dihydropyridine type: Novel aspects in SAR and bioanalytical evaluation of multidrug resistance (MDR) reversal properties

Christiane Baumert [a], Marianne Günthel [a], Sören Krawczyk [a], Marc Hemmer [a], Tom Wersig [a], Andreas Langner [a], Joséf Molnár [b], Hermann Lage [c], Andreas Hilgeroth [a,*]

[a] Institute of Pharmacy, Martin Luther University, 06120 Halle, Germany
[b] Department of Medical Microbiology, University of Szeged, 6720 Szeged, Hungary
[c] Institute of Pathology, University Hospital Charité, 10117 Berlin, Germany

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ABSTRACT

Novel series of *N*-benzyl 1,4-dihydropyridines have been prepared by facile syntheses. All relevant substituents of the molecular scaffold have been varied. The resulting compounds were biologically evaluated as P-glycoprotein (P-gp) inhibitors. Substitutions of the *N*-benzyl residue favour biological activity beside respective 3-ester functions. Most active compounds were further evaluated as multidrug resistance (MDR) modulators to restore the cytotoxic properties of varying daunorubicin applications.

1. Introduction

Multidrug resistance (MDR) against various anticancer drugs of different compound classes has maintained the central problem in anticancer drug therapies over the last decades.[1–3] Although recent anticancer drug developments resulted in novel compound classes like tyrosine kinase inhibitors as monoclonal antibodies or small-molecule inhibitors, the improvement in drug-resistant therapies remained limited because these novel compounds were also affected by the MDR phenomenon.[4–6] Multidrug efflux pumps of various types have been identified to be mainly causative agents in the multidrug affected process of MDR by transporting anticancer drugs out of the cells so that effective intracellular drug levels are no longer reached.[3,7,8] As the hopes of novel drugs to overcome the MDR phenomenon have been disappointed in general, other strategies have been followed over the last years.[2,4,9] The inhibition of the efflux pump activity by drugs turned out as the most perspective approach to combate MDR because successful alternatives of influencing the efflux pump protein formation by a transcriptional control are still limited to in vitro studies.[2,10] Drugs of various families have early been observed to modulate the MDR phenomenon.[9,11] With a limited application range of these drugs because of their originally pharmacological properties as antihypertensive or immunosuppressive drugs, second generation inhibitors with reduced original effects were developed. However, they showed a partial loss of their MDR modulating properties.[9,11,12] Following inhibitors which presently undergo clinical trials suffer from observed toxic problems.[4,13]

Intensive efforts have been taken to develop small-molecule inhibitors with a promising favourable resorption in contrast to recent complex natural compounds with high and critical molecular weights which are unfavourable also because the synthetic access to such natural compounds is difficult and expensive.[4,14,15]

We recently described pyridine-2-ones as novel class of MDR modulators.[16] They have been developed from our earlier *N*-acyloxy-1,4-dihydropyridines which suffered from a critical hydrolysis sensitivity of the *N*-acyloxy substituent (Fig. 1).[4]

The pyridine-2-ones partly showed merely in vitro activities in the range of verapamil which was used as one of the best described in vitro inhibitors. The *N*-acyloxy function of our former 1,4-dihydropyridines was placed within the dihydropyridine scaffold leading to a 1,2-dihydro structure of the pyridine-2-ones instead of the former 1,4-dihydro structure.

We now present 1,4-dihydropyridines without a ring-carbonyl function. We maintained the *N*-benzyl function of the pyridine-2-ones. In our novel series we varied the substitution patterns of the 4-phenyl and the *N*-benzyl residue to find out which structural

* Corresponding author. Tel.: +49 345 55 25168; fax: +49 345 55 27207.
E-mail address: andreas.hilgeroth@pharmazie.uni-halle.de (A. Hilgeroth).

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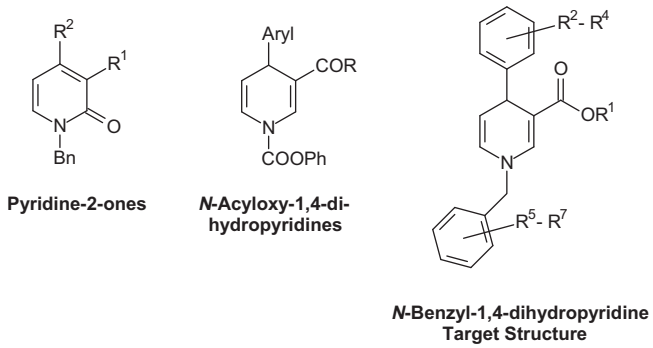


Figure 1. Pyridine-2-ones starting structure, *N*-acyloxy-1,4-dihydropyridine precursor structure and *N*-benzyl 1,4-dihydropyridine target structure.

element is more sensitive to substitution with respect to the biological activity. Moreover, we varied the 3-ester substituent.

2. Results and discussion

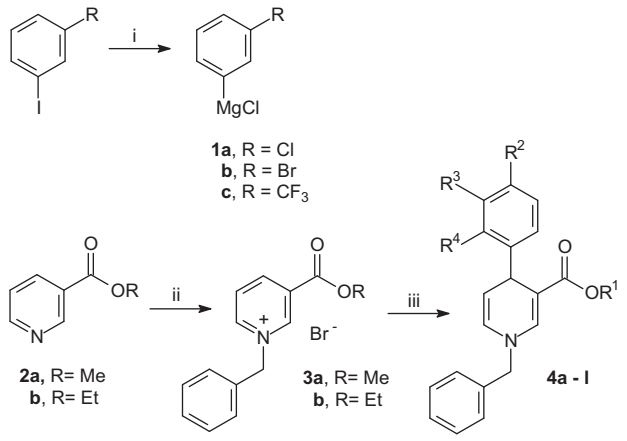
2.1. Chemistry

The synthesis of the 4-aryl substituted 1,4-dihydropyridines **4a**–**l** followed a simple two-step procedure starting with the *N*-alkylation of the commercially available pyridines **2a** and **b** (Scheme 1).

Both pyridines **2a** and **b** were dissolved in a minimum volume of isopropanol. Then 1.5 equiv of benzyl bromide were added dropwise under stirring which continued for 18 h. The resulting partly oily products of **3a** and **b** were washed with diethyl ether and then evaporated in vaccuum. After additional washing of the formed semi-solid compounds with diethyl ether they were stored in vacuum to be used for the following reactions without further purification.

The 4-arylation of the *N*-benzyl pyridinium ions of the crude compounds **3a** and **b** was carried out in dried THF with 1.5 equiv of Grignard reagents under copper(I) iodide catalysis and under addition of lithium chloride to improve the catalyst solubility in the reaction mixture.

In the case of the 3-halogen substituted derivatives **4a**–**f**, the Grignard reagents were freshly prepared from isopropyl magnesium chloride and the corresponding 3-chloro, 3-bromo and 3-trifluoromethyl iodopyridines **1a**–**c**, respectively, at −40 °C in dried THF under stirring for 1 h (Scheme 1). Each 1.5 equiv of the resulting or of the otherwise commercially available Grignard reagents were added dropwise to a stirring suspension of the *N*-benzyl pyridinium salts **3a** and **b** at low temperatures of −20 °C. The catalyst directed the nucleophilic aryl residue into the 4-position of the pyridinium nucleus so that the 4-aryl 1,4-dihydropyridines **4a**–**l** were yielded. This method has been successfully used for the preparation of corresponding 4-phenyl substituted *N*-acetyl 1,4-dihydropyridines.[17] However, the isolated reaction products after the workup procedure were mainly not pure and made another column chromatography necessary.



Scheme 1. Reagents and conditions: (i) $(CH_3)_2CHMgCl$, THF, −40 °C, 1 h; (ii) BnBr, $(CH_3)_2CHOH$, rt, 18 h; (iii) ArylMgBr, Cu(I)I, LiCl, THF, −20 °C—rt.

As the described favourable two-step procedure led to impure reaction products which afforded additional purifications we practiced a second access to the 1,4-dihydropyridines target structures by a simple two component reaction of previously prepared compounds **7a**–**j** of both the benzylamine and the varying ester moieties and of **9a** and **b** bearing the later 4-aryl substituent of the resulting 1,4-dihydropyridines **10a–j** (Scheme 2).

The β-enaminocarbonyl compounds **7a–j** were given by a quantitative reaction of the propiolic acid esters **5a** and **5b** with the respective benzylamines **6a**–**e** in dried THF under stirring for 5 h at rt. After evaporation of the solvent the residual oil of compounds **7a**–**j** was used without further purification. The compounds were given as a 1:3 mixture of *E*/*Z* isomers according to the ^{1}H NMR data which showed coupling constants of the methine protons of the *E* isomer in the range of ^3J = 13 Hz while the neighboured methine protons in the *Z* isomer had a coupling constant of about ^3J = 8 Hz.

The aldehyde compounds **9a** and **9b** were given by the reaction of the corresponding aryl iodide **8a** and **8b** with acrolein diethyl acetale in DMF at 90 °C in a palladium catalyzed *Heck* coupling reaction using palladium(II) acetate as primary catalyst, tetrabutyl ammonium acetate as phase transfer catalyst and, finally, potassium carbonate as basic component.

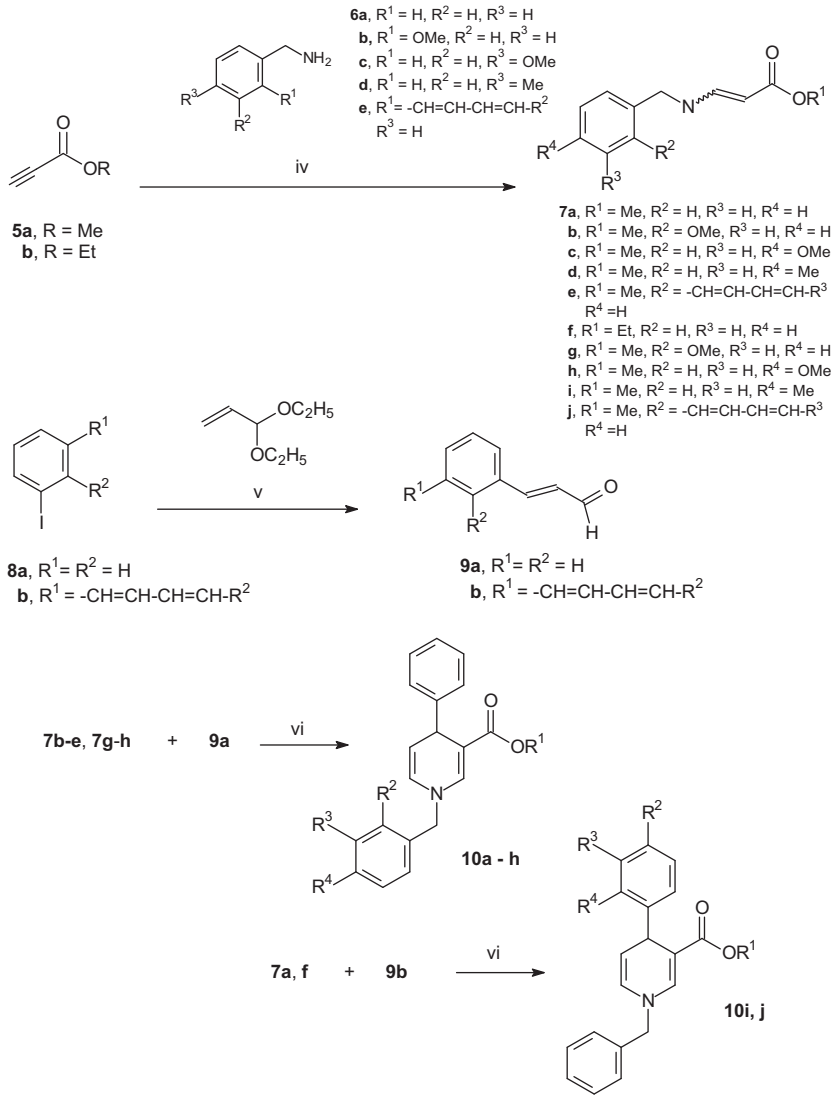
The resulting aldehyde compounds **9a** and **9b** exclusively consisted of the *E* isomer with high coupling constants of the methine protons of about ^3J = 16 Hz according to a selective *trans* formation of the double bond within the complex reaction pathway in a thermodynamically controlled reaction as described in literature.[18]

The final 1,4-dihydropyridine formation took place under smooth reaction conditions of 40 °C using iron(III) chloride hexahydrate as lewis acid for the activation of the non-saturated aldehyde components **9a** and **9b** to react in a following michael addition with the β-enaminocarbonyl components **7a–j**. A concluding condensation reaction led to the 1,4-dihydropyridine ring closure giving the 1,4-dihydropyridine target structures **10a–j**.

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

The P-gp inhibition of our target compounds has been carried out by the determination of the uptaken amount of the fluorescent P-gp substrate rhodamine 123 in both a mouse T lymphoma cell line without P-gp and another one with overexpressing P-gp. The later cell line has been derived fom the non-P-gp expressing cell line after retroviral transfection with the human *mdr1* gene and a consequent cell culturing in a colchicine-containing medium to select only the P-gp expressing cells which survive the colchicine-culturing by the extracellular colchicine efflux. Thus both cell lines exclusively differ in the expression of human P-gp so that a difference in the uptake of the fluorescent rhodamine 123 can be directly reasoned with the expression of this efflux pump. By the way the use of different P-gp inhibitor concentrations in pretreated cells caused differences in the fluorescence uptake so that the inhibition of the efflux pump activity increased the fluorescence uptake in the P-gp expressing cell line.

The P-gp inhibition-characterizing size is the fluorescence activity ratio named as *FAR* value which was calculated by dividing the



Scheme 2. Reagents and conditions: (iv) THF, rt, 5 h; (v), $(C_4H_9)4NAc$, K_2CO_3, KCl, Pd(II)AC_2, DMF, 90 °C, 2–3 h;(VI) Na_2SO_4, Fe(III)$Cl_3 \times 6\ H_2O$, CH_2Cl_2, 40 °C, 12 h.

fluorescence uptake in the P-gp expressing subline after inhibitor addition through the fluorescence uptake in the non-P-gp expressing subline after inhibitor addition. Each used values have been related to the untreated control values without inhibitor before final division. Verapamil has been used as established standard for in vitro assays because of its reliable high in vitro activity. This was confirmed in comparison with tariquidar which proved to be only 1.2-fold more active than verapamil in our assay system at the used inhibitor concentration of 10 μM.

In our earlier *N*-acyloxy 1,4-dihydropyridines we varied the substitution patterns in the 4-aryl residue by introducing methoxy functions into both the 2- and the 4-position of the aryl residue. In our present series of *N*-benzyl derivatives we varied all positions of the 4-aryl residue starting with the introduction of synthetically accessible 3-halogen substituents. We characterized the introduction of the halogen substituents chloro, bromo and, finally, trifluoromethyl within the compounds **4a**–**f** under additional variation of the 3-ester substituents of the molecular scaffold as will be discussed. We preferred 3-ester derivatives because 3-acetyl derivatives were less active within the series of *N*-acyloxy compounds.[4]

The 3-chlorophenyl derivative **4a** showed P-gp inhibiting activities at both the lower concentration of 1 μM and the higher concentration of 10 μM with *FAR* values >1 (Table 1).

Verapamil for comparison was not active at the lower inhibitor concentration. The activity data of the 3-bromophenyl derivative **4b** were similar to those of the 3-chlorophenyl compound **4a**. The 3-trifluorophenyl compound **4c** resulted in the best activity with a *FAR* value of 1.53 at the higher concentration. Nifedipine as used 1,4-dihydropyridine reference showed a poorer activity with a *FAR* value of only 1.0.

The change of the 3-methyl ester in compounds **4d**–**f** each led to main increases in the P-gp inhibiting properties, namely at the higher inhibitor concentration. All 3-ethyl ester derivatives were slightly better inhibitors than the 3-methyl ester derivatives with each improved *FAR* values at the lower concentration. The *FAR* value of the 3-chlorophenyl 3-ethyl ester **4d** at the higher concentration almost duplicated if compared to that of the 3-methyl ester compound **4a** and in the case of the 3-bromophenyl 3-ethyl ester **4e** more than the twofold *FAR* value with 2.76 was reached.

We then introduced a methoxy function into the 4-aryl residue by the use of the corresponding available Grignard reagents. Methoxy functions are known to serve as hydrogen bond acceptor functions in P-gp inhibitors.[11,12] The introduction of a methoxy function into the 2-position of the 4-phenyl residue resulted in improved activity of compound **4h** at both the lower and the higher concentration if compared to the activity data of the 3-halogen

Table 1
Target compound (**4a–l** and **10a–j**) activities in the concentration dependent inhibition of P-gp as determined *FAR* values for each three measurements

Compd	Residues				*FAR*-Values	
	R^1	R^2	R^3	R^4	P-gp inhibition	
					1 μM	10 μM
4a	Me	H	Cl	H	1.12 ± 0.15	1.39 ± 0.16
4b	Me	H	Br	H	1.05 ± 0.08	1.22 ± 0.43
4c	Me	H	CF_3	H	1.05 ± 0.23	1.53 ± 0.19
4d	Et	H	Cl	H	1.25 ± 0.06	2.67 ± 0.17
4e	Et	H	Br	H	1.27 ± 0.07	2.76 ± 0.40
4f	Et	H	CF_3	H	1.17 ± 0.07	2.33 ± 0.27
4g	Me	Me	H	H	1.11 ± 0.03	1.88 ± 0.27
4h	Me	H	H	OMe	1.13 ± 0.09	1.76 ± 0.40
4i	Me	OMe	H	H	0.85 ± 0.04	2.95 ± 0.24
4j	Et	Me	H	H	1.34 ± 0.22	2.54 ± 0.95
4k	Et	H	H	OMe	1.18 ± 0.13	2.38 ± 0.21
4l	Et	OMe	H	H	1.52 ± 0.03	3.13 ± 0.33
10a	Me	OMe	H	H	1.82 ± 0.07	3.97 ± 0.47
10b	Me	H	H	OMe	1.50 ± 0.19	4.13 ± 1.12
10c	Me	H	H	Me	1.72 ± 0.26	7.80 ± 1.48
10d	Et	OMe	H	H	1.72 ± 0.27	4.64 ± 1.05
10e	Et	H	H	OMe	1.32 ± 0.09	4.89 ± 0.86
10f	Et	H	H	Me	2.54 ± 0.23	7.80 ± 1.42
10g	Me	–CH=CH–CH=CH–		H	1.19 ± 0.27	2.19 ± 0.63
10h	Et	–CH=CH–CH=CH–		H	1.28 ± 0.11	2.74 ± 0.61
10i	Me	H	–CH=CH–CH=CH		1.22 ± 0.09	2.35 ± 0.39
10j	Et	H	–CH=CH–CH=CH–		1.43 ± 0.05	3.45 ± 0.56
verapamil					0.89 ± 0.21	2.94 ± 0.78

substituted derivatives of the 3-methyl ester series. A movement of the methoxy function into the 4-position of the 4-phenyl residue again improved the activity at the higher concentration with a *FAR* value of 2.95 of derivative **4i**. The replacement of the 4-methoxy function with a 4-methyl group led to decreases in the activity of compound **4g** at the higher concentration. However, the activity of **4g** was again better than that of the 3-halogenphenyl substituted compounds of this 3-methyl ester series. The 4-position seems to be most favourable for the introduction of possible substituents either with size-enlarging effects in the case of methyl or methoxy groups or as potential hydrogen bond acceptor functions.

The same tendency of the effects of introduced 4-phenyl substituents was observed for the compounds of the 3-ethyl ester series **4j–l**. The 2-methoxyphenyl derivative **4k** showed similar activities than the 3-halogen substituted compounds. The movement of the methoxy function into the 4-position mainly increased activity up to a *FAR* value of 3.13 of compound **4l** at the higher inhibitor concentration which meant a higher activity than that of verapamil. The replacement of the 4-methoxy with a 4-methyl group lowered this activity but the 4-methylphenyl compound **4j** was more active than the 2-methoxyphenyl derivative **4k**. Again the 4-phenyl ring substitution was the most attractive one for the introduction of possible substituents.

We then varied the substituents in the *N*-benzyl residue concentrating in the most effective methoxy and methyl substitutions as evaluated in the 4-phenyl residue of derivatives **4a–l**. Again we varied the 3-ester substituent with both methyl and ethyl ester functions.

We started with the introduction of the methoxy function into the 2-position of the phenyl ring of the *N*-benzyl substituent of the 3-methyl ester compound. Derivative **10a** showed increased activity if compared to all compounds of the 4-phenyl substituted series **4a–l** at both tested inhibitor concentrations reaching the highest *FAR* values so far. The *FAR* value at the lower concentration made almost twice as much than the *FAR* value of verapamil. A movement of the methoxy function from the 2- to the 4-position of the aromatic *N*-benzyl residue increased the activity at the higher concentration.

With the 4-methoxy function of **10b** being more favourable than the 2-methoxy we found a similar tendency as we observed for the methoxy positioning in the 4-aryl substituted compounds. The replacement of the 4-methoxy with a 4-methyl group further increased the activity. At the higher concentration the *FAR* value of 7.80 for derivative **10c** made much more than the double value of verapamil.

Within the 3-ethyl ester compound series **10d–f** we found the same substituent effects than those observed for the 3-methyl esters. By the way almost all resulting activities were better, especially at the higher inhibitor concentration. The 2-methoxy derivative **10d** was more active than the corresponding 3-methyl ester at the higher concentration. The 4-methoxy compound **10e** showed an increased *FAR* value if compared to the 2-methoxy compound **10d** and to the 4-methoxy 3-methyl ester derivative **10b** at the higher concentration. The replacement of the 4-methoxy by a 4-methyl group led to the most active derivative **10f** so far with a threefold increased *FAR* value at the lower concentration if compared to our verapamil standard.

As the methyl group effects on increasing activity turned out to be significant in the group of the varied aryl substituents of our compound series of both 3-methyl and 3-ethyl esters we extended our aromatic substitution patterns with the introduction of aryl-annelated residues. We started with the replacement of the 4-phenyl residue of our 1,4-dihydropyridine scaffold with a 1-naphthyl residue. Derivative **10i** showed increased activity if compared to the 4-tolyl derivative **4g** at both inhibitor concentrations. At the lower concentration it is the most active inhibitor of the 4-phenyl varied compound series with the 3-methyl ester. Again the corresponding 3-ethyl ester compound **10j** is more active at both concentrations and also the most active compound of the 4-aryl 3-ethyl ester series. Thus, the additional benzo-annelation led to compounds with highest activitis of all 4-aryl varied derivatives.

We have also been interested to investigate the influence of a benzo-annelation to the phenyl ring of the *N*-benzyl substituent. The resulting 3-methyl ester derivative **10g** almost reached the activity of the 4-(1-naphthyl) substituted compound **10i**. However, if compared to the excellent activity data of the *N*-(4-methylbenzyl) substituted compound **10c** the activity was found mainly reduced. The same tendency was observed for the corresponding 3-ethyl ester compound **10h** that was again more active than the 3-methyl ester compound **10g** but less active than the *N*-(4-methylbenzyl) substituted compound **10f**.

Summarising our novel structure-activity relationship (SAR) results we can conclude that variations of substitution patterns in both the 4-phenyl and the *N*-benzyl residue show similar tendencies. As far as evaluated the 2- and 4-substitutions are most favourable if substituted with single substituents. Most active derivatives have been given in the series of the *N*-benzyl substituted compounds. In all cases of our investigated compounds the ethyl esters tended to be more potent than the methyl ester derivatives.

2.3. MDR reversal studies

The observed compound effectiveness in inhibiting the P-gp activity in our in vitro assay encouraged to further studies. So we investigated the ability of our compounds to reverse the MDR in anticancer drug studies. The undertaken studies with rhodamine 123 were essential to characterize the SAR of the P-gp inhibition. However, the proof-of-concept would be a concentration dependent inhibition profile of selected compounds to really reverse the MDR of a cytostatic drug.

We used our model system of both mouse T lymphoma cell lines to further investigate the cell-profiled inhibitors in this

system for their activity to reverse MDR which meant that a reduced cytostatic drug activity in the P-gp overexpressing cell line had to be restored ideally reaching the same toxicity which was found for the cytostatic drug in the non-P-gp expressing cell line.

We chose the anthracycline drug daunorubicin for our anticancer studies. Daunorubicin is an established anticancer drug with well known P-gp substrate properties.[11] First we investigated the cytotoxic effect of the drug in both cell lines, the P-gp expressing and the non-expressing mouse T lymphoma cell line. The cytotoxic effects were determined in the MTT assay in which viable cells are characterized by the activity of mitochondrial dehydrogenases of reducing the MTT reagent to formazan which is determined UV spectroscopically after cell lysis.

The concentration dependent daunorubicin toxicity has been determined and sigmoidal curves resulted which are displayed in Figure 2.

The calculated corresponding IC_{50} values of the daunorubicin toxicity derived from the cell viability made 0.73 ± 0.03 μM for the non-P-gp expressing cell line and 7.73 ± 0.86 μM for the P-gp expressing cell line which meant a loss of the anticancer drug activity by a factor of more than 10 caused by the P-gp dependent efflux of the anticancer drug out of the P-gp expressing cell line.

We used one of our best inhibitors **10f** to investigate the effects of restoring the anticancer drug activity. We started with a concentration of 2.5 μM of **10f**. We observed a more than 50% reduction of the IC_{50} value of the daunorubicin toxicity with a value of 3.33 ± 0.36 μM which meant a more than 50% restoration of the cytotoxic anticancer drug effect. We then used 5 μM and, finally, 10 μM of compound **10f**. The resulting sigmoidal curves are shown in Figure 2. The shift of the curves towards the curve of the daunorubicin toxicity in the non-P-gp expressing cell line indicates the further restoration of the anticancer drug toxicity. A further reduced IC_{50} value of 1.96 ± 0.15 μM was calculated for the 5 μM P-gp inhibitor application if compared to the IC_{50} value at the 2.5 μM application. At the concentration 10 μM an almost complete restoration of the daunorubicin toxicity was observed with a resulting IC_{50} value of 0.96 ± 0.13 μM which meant an anticancer drug activity similar to that in the non-P-gp expressing cell line.

As anthracyclines show certain cardiotoxicity in anticancer drug therapies attempts have been made to reduce that undesired side effect by developing liposomal applications.[19,20] Moreover, anthracyclines are effected by the MDR phenomenon as known P-gp substrates so that such liposomal applications were encouraged by hopes to reduce the P-gp mediated resistance phenomenon.[21,22] Our encouraging P-gp inhibitor studies led to extended investigations including a liposomal daunorubicin application for consideration.

We used DaunoXome® as liposomal daunorubicin application and determined its toxicity, again in both mouse T lymphoma cell lines with and without P-gp expression. The resulting IC_{50} values are displayed in Figure 3.

We found different IC_{50} values with 0.3 μM in the non-P-gp expressing cell line and 2.32 μM in the P-gp expressing cell line. Both IC_{50} values are reduced if compared to those of the non-liposomal daunorubicin. Interestingly, also the liposomal daunorubicin turned out to have P-gp substrate properties although the cytotoxic effect is stronger in the P-gp expressing cell line if compared to the application of the non-liposomal daunorubicin.

We decided to investigate the potential of two of our P-gp inhibitors to reverse this determined MDR phenomenon of the liposomal daunorubicin application. We uniformly used two relevant concentrations in our SAR studies discussed above to characterize the activity of the P-gp inhibitors. For two of our best inhibitors **10c** and **10e** we found increased *FAR* values of 17.6 (**10c**) and 11.8 (**10e**) at higher concentrations which were tested to investigate possible saturation effects of the P-gp inhibition at the chosen concentration of 10 μM. Compound **10f** tested as our first investigated MDR reverser showed saturation effects at 10 μM. However this concentration led to a complete reversal of the MDR phenomenon as demonstrated, so that it was a completely sufficient concentration. With the potential to show enhanced MDR reversal effects at higher concentrations than 10 μM we selected both compounds **10c** and **10e** for the liopsomal daunorubicin studies.

At the lowest application concentration of 2.5 μM both inhibitors showed a main decrease in the IC_{50} values of the determined liposomal daunorubicin toxicity. Compound **10c** with the higher P-gp inhibiting activity than **10e** at the lower concentration of 1 μM showed better MDR reversing properties than **10e** with a more than 60% restoration of the MDR sensitivity at this lower concentration of 2.5 μM and a resulting IC_{50} value of 0.88 μM for the liposomal daunorubicin toxicity.

The IC_{50} values further decreased at a concentration of 5 μM and finally led to a complete reversal of the MDR phenomenon at a concentration of 10 μM with almost identical IC_{50} values than in the non-P-gp expressing cell line. So also the observed resistance against liposomal daunorubicin could be successfully reversed by our P-gp inhibitors. Interestingly, also the little weaker inhibitor

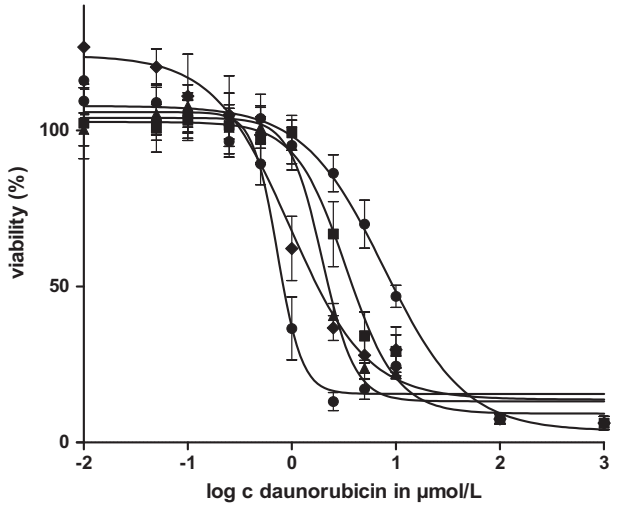


Figure 2. Concentration dependent cell viability profiles (a) with incubated daunorubicin in the mouse T lymphoma cell line without P-gp (left curve ●) and in the mouse T lymphoma cell line with overexpressing P-gp (right curve ●) and (b) with preincubated P-gp inhibitor **10f** concentrations of 2.5 μM (middle curve with ■), of 5 μM (middle curve with▲) and of 10 μM (middle curve with ♦) each in the P-gp overexpressing mouse T lymphoma cell line, number (n) of experiences $n = 3$.

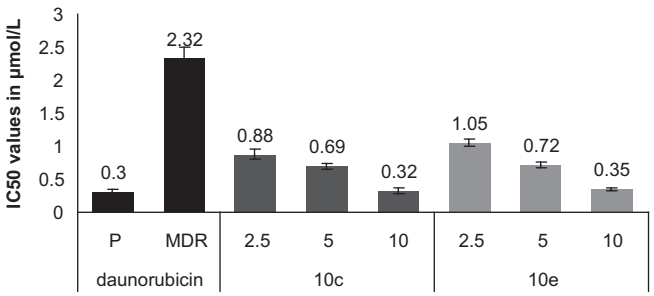


Figure 3. IC_{50} values of liposomal daunorubicin (DaunoXome®) toxicity (a) in the non-P-gp expressing mouse T lymphoma cell line (P) and the P-gp overexpressing mouse T-lymphoma cell line (MDR) shown in the left columns, after preincubation with P-gp inhibitors **10c** (middle columns) and **10e** (right columns) at each increasing concentrations of 2.5, 5 and 10 μM in the P-gp overexpressing mouse T lymphoma cell line, number (n) of experiences $n = 3$.

10e was able to completely reverse the MDR phenomenon similar to the more potent P-gp inhibitor **10c**.

Finally we investigated the cytotoxic effects of our proved MDR reversing compounds in our cellular assay system. We determined the cell viability with increasing compound concentrations up to 80 μM (Fig. 4).

All compounds showed complete MDR reversing activity at 10 μM. Moreover, their potential to reverse MDR has been demonstrated already at the lowest used concentration of 2.5 μM. Most active compounds **10c** and **10e** were completely nontoxic at the concentration 2.5 μM with cell viability data of 95% for **10c** and of 97% for **10e** in the non-P-gp expressing cell line. Both compounds were also nontoxic in the P-gp overexpressing cell line although compound **10c** showed a partly reduced cell viability of 81 %. However, refering to literature such a minimally reduced cell viability >80% under compound application qualifies a compound as nontoxic. At the concentration of 10 μM the cell viability of compound **10c** was already about 75% in both cell lines. Compound **10e** maintained practically nontoxic with cell viabilities of 85% in the P-gp overexpressing and 100% in the non-expressing cell line. Compound **10f** as our third MDR reversing compound showed similar cell viability results after application than compound **10c**. However, the cell viability at the concentration of 10 μM proved to be slightly better with values of about 78% in both cell lines. So we can finally state that a compound toxicity which may contribute to the toxic effects of daunorubicin in our final reversal assay can almost be excluded.

3. Conclusions

Early pyridine-2-ones with P-gp inhibiting activities partly in the range of verpamil were further developed by removing the carbonyl function from the molecular scaffold with a resulting 1,4-dihydropyridine structure. A complex structure-activity discussion was carried out by structural variations of the 4-phenyl, the *N*-benzyl and the 3-ester function.

4-Aryl-1,4-dihydropyridines are known to adopt a boat conformation of the 1,4-dihydropyridine ring which has been demonstrated to flip.[24,25] However, if the ring was substituted in the 3-position the free rotability of the 4-aryl residue was hindered. So taking these facts in account, one may wonder whether the exact positioning of a substituent either in the 4-phenyl or the *N*-benzyl residue may be important with respect to the biological activity. Interestingly, similar tendencies of the aromatic substituent effects were observed for both the 4-phenyl and the *N*-benzyl residues. The 4-position of both aromatic residues was favoured for both a methyl and a methoxy group. The activity data of all of such *N*-benzyl substituted derivatives were much better than the activities of those compounds with the aromatic substitutents at the 4-phenyl residue. The replacement of the 4-phenyl with a 4-(1-naphthyl) residue was of favour for the biological activity. However, a replacement of the *N*-benzyl substituent with the naphthyl residue was less favourable. All ethyl ester compounds were better inhibitors than the corresponding methyl ester compounds, so that the slightly extended alkyl side chain of the 3-ester substituent seemed to be significantly important for the

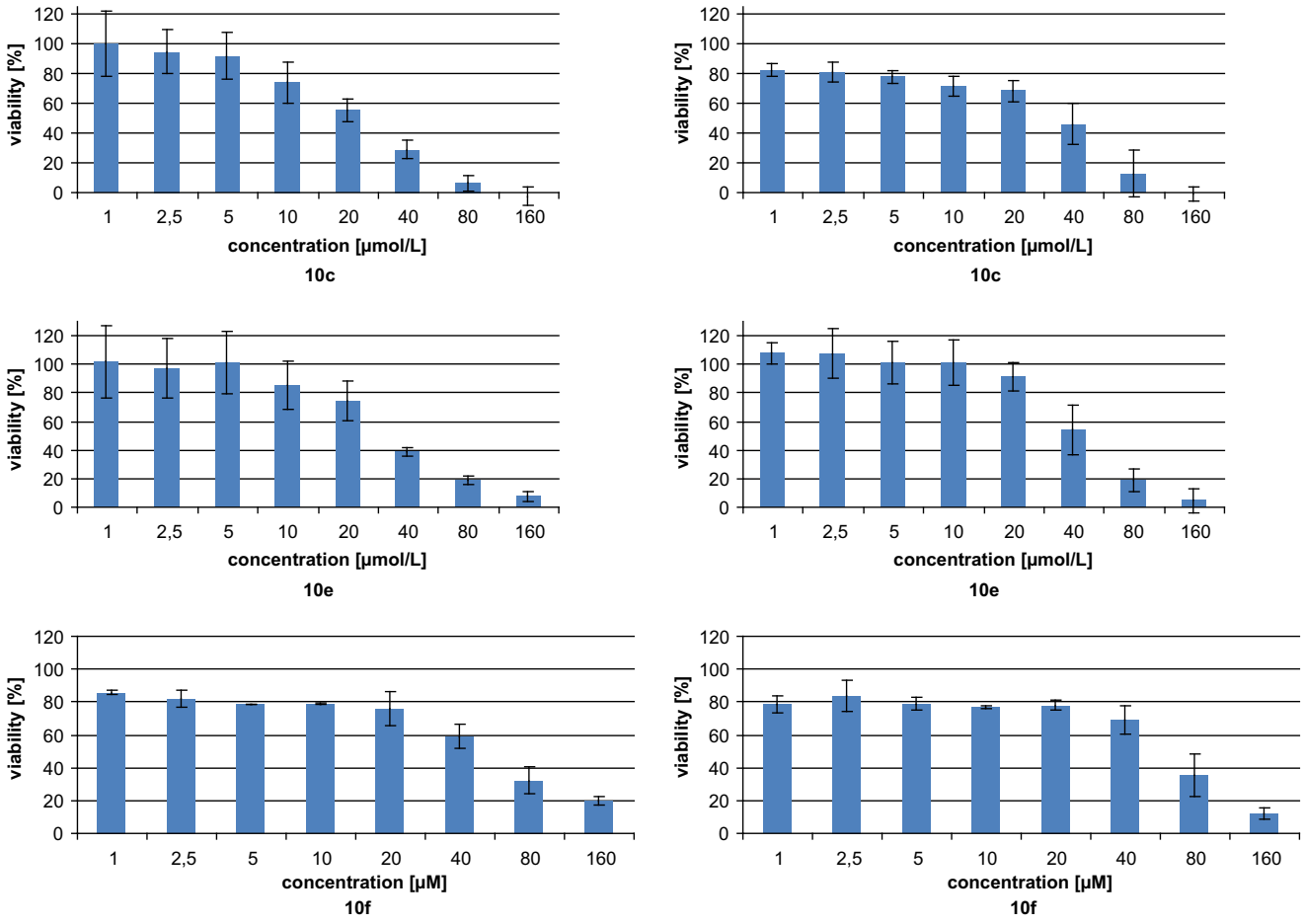


Figure 4. Concentration dependent cell viability profiles of respective compounds **10c**, **10e** and **10f** in both cell lines with the results of the non-P-gp expressing cell line displayed on the left site and those of the P-gp overexpressing cell line displayed on the right site.

molecular properties of the P-gp inhibition. Similar observed substituent effects both in the 4-phenyl and the *N*-benzyl residue may indicate that the inhibitor may show a flexible interaction with the P-gp protein binding site which is supported by the conformational flexibility of such 1,4-dihydropyridines as cited.

The MDR reversal studies of various anticancer drug applications were carried out with the most active P-gp inhibitors. We found increased cytotoxic effects for the liposomal daunorubicin application in our P-gp-expressing cell line if compared to the application of the sole daunorubicin. So the liposomal application was more effective but still it was affected by the MDR phenomenon as demonstrated.

Low concentrations of all our best P-gp inhibitors led to a main restoration of more than 50% of the anticancer drug activity of both the liposomal and the sole daunorubicin application. At higher concentrations the restoration was complete and the MDR was overcome even by the compound which showed a saturation effect of the P-gp inhibition at this concentration.

So the best P-gp inhibiting compounds convinced as MDR reversal agents thus giving hope for coming studies of further improving activity by combined aromatic substitutions.

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. ^{1}H 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 *N*-benzyl pyridinium bromides (3a, b).

One equivalent of the respective nicotinic acid ester **2a**, **b** was dissolved in isopropanol and one and a half equivalent of benzyl bromide were added dropwise under stirring. Stirring continued for 18 h at rt. Then the solution was extracted with diethyl ether and finally evaporated. The remaining product was washed with diethyl ether and kept in vacuum in an exsicator.

4.1.1.1. 1-Benzyl-3-methoxycarbonyl-pyridinium bromide (3a). Bright-pink semi-solid product; yield 95%; MS (EI) *m*/*z* (cation) 228 (M^+, 100%), 137 (M^+-Bn, 50%), 91 (Bn, 80%).

^{1}H NMR (acetone-d_6) δ 4.00 (s, 3H, CH_3), 6.48 (s, 2H, *N*–CH_2), 7.42–7.86 (m, 5H, CH_2–C_6H_5), 8.40 (t, *J* = 6.8 Hz, 1H, 5-H), 9.07 (d, *J* = 6.8 Hz, 1H, 4-H), 9.94 (s, 1H, 2-H), 10.12 (d, *J* = 6.8 Hz, 1H, 6-H). Anal. Calcd for $C_{14}H_{14}NO_2Br$: C, 54.17; H, 4.58; N, 4.55. Found: C, 53.99; H, 4.47; N, 4.38.

4.1.1.2. 1-Benzyl-3-ethoxycarbonyl-pyridinium bromide (3b). White-yellow semi-solid product; yield 90%; MS (EI) *m*/*z* (cation) 242 (M^+, 100%), 151 (M^+-Bn, 40%), 91 (Bn, 85%). ^{1}H NMR (acetone-d_6) δ 1.40 (t, *J* = 7.2 Hz, 3H, CH_2–CH_3), 4.48 (q, *J* = 7.2 Hz, 2H, CH_2–CH_3), 6.46 (s, 2H, *N*–CH_2), 7.44–7.85 (m, 5H, CH_2–C_6H_5), 8.40 (t, *J* = 6.8 Hz, 1H, 5-H), 9.09 (d, *J* = 6.8 Hz, 1H, 4-H), 9.97 (s, 1H, 2-H), 10.04 (d, *J* = 6.8 Hz, 1H, 6-H). Anal. Calcd for $C_{15}H_{16}NO_2Br$: C, 55.92; H, 5.01; N, 4.35. Found: C, 55.77; H, 4.88; N, 4.32.

4.1.2. General procedure for the preparation of the 4-phenyl substituted 1,4-dihydropyridines (4a–l).

One equivalent of the nicotinic acid ester salts **3a**,**b** were suspended in dried THF under addition of 0.2 equiv of lithium chloride and 0.1 equiv of copper(I) iodide. One and a half equivalent of the Grignard reagent were added dropwise at −20 °C. In the case of the 3-halogen substituted derivatives the Grignard reagent was freshly prepared from one equivalent of the 3-halogen substituted iodobenzol and 1.2 equiv of isopropyl magnesium chloride in dried THF at −40 °C by stirring under argon atmosphere for 1 h. After addition of the grignard reagent stirring continued at rt until no more starting compound was detectable by tlc. Then the remaining excess of reagent was hydrolysed by the addition of an ammonium chloride solution in water (20%). Extraction with portions of diethyl ether followed. The unified organic layer was then extracted with an ammonium chloride solution in water (20%), with a concentrated solution of ammonia in water (1:1), with water, a hydrochloric acid solution in water (10%), water and, finally, with a saturated sodium chloride solution in water. Then the organic layer was dried over sodium sulfate, filtered and evaporated. The remaining oil was purified by column chromatography using a mixture of dichloromethane and heptane (90:10).

4.1.2.1. Methyl 1-benzyl-4-(3-chlorophenyl)-1,4-dihydropyridine-3-carboxylate (4a). Yellow oil; yield 72%; MS (ESI) *m*/*z* 340 ($M+H^+$, 100%). ^{1}H NMR ($CDCl_3$) δ 3.57 (s, 3H, CH_3), 4.43 (s, 2H, *N*–CH_2), 4.51 (d, *J* = 4.7 Hz, 1H, 4-H), 4.86 (dd, *J* = 7.8, 4.7 Hz, 1H, 5-H), 5.93 (d, *J* = 7.8 Hz, 1H, 6-H), 7.10–7.17 (m, 4H, C_6ClH_4), 7.25–7.39 (m, 6H, 2-H, CH_2–C_6H_5). ^{13}C NMR ($CDCl_3$) δ 43.5 (d, C-4), 52.3 (q, COO**C**H_3), 54.8 (t, *N*–CH_2), 109.8 (s, C-3), 112.0 (d, C-5), 125.8 (d, C4-4-Ph), 126.8 (d, C-4–*N*–CH_2–Ph), 126.9 (d, C-2, –6-*N*–CH_2–Ph), 127.1 (d, C-6–4-Ph), 128.6 (d, C-3, -5-*N*–CH_2–Ph), 128.8 (d, C-2-4-Ph), 129.3 (d, C-6), 130.1 (d, C-5-4-Ph), 134.2 (s, C-3–4-Ph), 141.6 (s, C-1–*N*–CH_2–Ph), 143.6 (s, C-1-4-Ph), 144.6 (d, C-2), 167.2 (s, CO). Anal. Calcd for $C_{20}H_{18}ClNO_2$: C, 70.84; H, 5.35; N, 4.13. Found: C, 70.55; H, 5.23; N, 3.95.

4.1.2.2. Methyl 1-benzyl-4-(3-bromophenyl)-1,4-dihydropyridine-3-carboxylate (4b). Yellow oil; yield 65%; MS (ESI) *m*/*z* 384 ($M+H^+$, 100%). ^{1}H NMR ($CDCl_3$) δ 3.57 (s, 3H, CH_3), 4.43 (s, 2H, *N*–CH_2), 4.49 (d, *J* = 4.7 Hz, 1H, 4-H), 4.86 (dd, *J* = 7.7, 4.7 Hz, 1H, 5-H), 5.91 (d, *J* = 7.7 Hz, 1H, 6-H), 7.01–7.19 (m, 4H, C_6BrH_4), 7.28–7.49 (m, 6H, 2-H, CH_2-C_6H_5).

^{13}C NMR ($CDCl_3$) δ 43.3 (d, C-4), 52.2 (q, COOCH_3), 54.9 (t, *N*–CH_2), 109.9 (s, C-3), 112.5 (d, C-5), 123.0 (s, C-3–4-Ph), 126.7 (d, C-4–*N*–CH_2–Ph), 126.9 (d, C-2, –6-*N*–CH_2–Ph), 128.0 (d, C-6–4-Ph), 128.5 (d, C-3, –5-*N*–CH_2–Ph), 128.6 (d, C4–4-Ph), 129.1 (d, C-6), 130.9 (d, C-5–4-Ph), 133.8 (d, C-2–4-Ph), 141.5 (s, C-1–*N*–CH_2–Ph), 144.4 (s, C-1–4-Ph), 144.7 (d, C-2), 167.1 (s, CO). Anal. Calcd for $C_{20}H_{18}BrNO_2$: C, 62.71; H, 4.74; N, 3.66. Found: C, 62.67; H, 4.70; N, 3.41.

4.1.2.3. Methyl 1-benzyl-4-(3-trifluorophenyl)-1,4-dihydropyridine-3-carboxylate (4c). Yellow oil; yield 70%; MS (ESI) *m*/*z* 374 ($M+H^+$, 100%). ^{1}H NMR ($CDCl_3$) δ 3.56 (s, 3H, CH_3), 4.44 (s, 2H, *N*–CH_2), 4.60 (d, *J* = 4.7 Hz, 1H, 4-H), 4.88 (dd, *J* = 7.7, 4.7 Hz, 1H, 5-H), 5.95 (d, *J* = 7.7 Hz, 1H, 6-H), 7.19–7.53 (m, 10H, 2-H, $C_6CF_3H_4$,CH_2–C_6H_5). ^{13}C NMR ($CDCl_3$) δ 44.3 (d, C-4), 52.3 (q, COOCH_3), 54.8 (t, *N*–CH_2), 109.8 (s, C-3), 112.5 (d, C-5), 122.1 (d, C4–4-Ph), 124.5 (s, CF_3), 126.6 (d, C-4–*N*–CH_2–Ph), 126.7 (d, C-2, -6-*N*–CH_2–Ph), 127.0 (d, C-2–4-Ph), 128.6 (d, C-3, –5-*N*–CH_2–Ph), 129.1 (d, C-6), 129.2 (d, C-5–4-Ph), 130.9 (s, C-3-4-Ph), 132.3 (d, C-6-4-Ph), 141.7 (s, C-1-*N*–CH_2–Ph), 142.5 (s, C-1-4-Ph), 144.5 (d, C-2), 167.3 (s, CO). Anal. Calcd for $C_{21}H_{18}F_3NO_2$: C, 67.60; H, 3.75; N, 3.66. Found: C, 67.42; H, 3.54; N, 3.39.

4.1.2.4. Ethyl 1-benzyl-4-(3-chlorophenyl)-1,4-dihydropyridine-3-carboxylate (4d). Yellow oil; yield 62%; MS (ESI) *m*/*z* 354 (M+H$^+$, 100%). ^{1}H NMR ($CDCl_3$) δ 1.13 (AB*X*$_3$, *J* = 7.1 Hz, 3H, CH_2–C*H*$_3$), 3.95–4.10 (*AB*X$_3$, *J* = 10.3, 7.1 Hz, 2H, C*H*$_2$–CH_3), 4.43 (s, 2H, *N*–CH_2), 4.51 (d, *J* = 4.7 Hz, 1H, 4-H), 4.86 (dd, *J* = 7.8, 4.7 Hz, 1H, 5-H), 5.91 (d, *J* = 7.8 Hz, 1H, 6-H), 7.10–7.19 (m, 4H, C_6ClH_4), 7.26–7.39 (m, 6H, 2-H, CH_2–C_6H_5). ^{13}C NMR ($CDCl_3$) δ 14.2 (q, $COOCH_2CH_3$), 43.5 (d, C-4), 54.6 (t, *N*–CH_2), 61.7 (t, $COOCH_2CH_3$), 108.0 (s, C-3), 112.4 (d, C-5), 125.6 (d, C4-4-Ph), 126.3 (d, C-4–*N*–CH_2–Ph), 126.5 (d, C-2,–6-*N*–CH_2–Ph), 127.1 (d, C-6–4-Ph), 128.6 (d, C-3,–5-*N*–CH_2–Ph), 128.7 (d, C-2–4-Ph), 129.5 (d, C-6), 130.1 (d, C-5–4-Ph), 134.2 (s, C-3–4-Ph), 141.6 (s, C-1–*N*–CH_2–Ph), 143.6 (s, C-1–4-Ph), 144.6 (d, C-2), 167.2 (s, CO). Anal. Calcd for $C_{21}H_{20}ClNO_2$: C, 71.43; H, 5.71; N, 3.97. Found: C, 71.22; H, 5.67; N, 3.92.

4.1.2.5. Ethyl 1-benzyl-4-(3-bromophenyl)-1,4-dihydropyridine-3-carboxylate (4e). Yellow oil; yield 68%; MS (ESI) *m*/*z* 398 (M+H$^+$, 100%). ^{1}H NMR ($CDCl_3$) δ 1.13 (AB*X*$_3$, *J* = 7.1 Hz, 3H, CH_2–C*H*$_3$), 3.95-4.09 (*AB*X$_3$, *J* = 10.3, 7.1 Hz, 2H, C*H*$_2$–CH_3), 4.43 (s, 2H, *N*–CH_2), 4.49 (d, *J* = 4.7 Hz, 1H, 4-H), 4.86 (dd, *J* = 7.7, 4.7 Hz, 1H, 5-H), 5.91 (d, *J* = 7.7 Hz, 1H, 6-H), 7.01–7.19 (m, 4H, C_6BrH_4), 7.28–7.48 (m, 6H, 2-H, CH_2–C_6H_5). ^{13}C NMR ($CDCl_3$) δ 14.3 (q, $COOCH_2CH_3$), 43.1 (d, C-4), 54.6 (t, *N*–CH_2), 61.9 (t, $COOCH_2CH_3$), 108.3 (s, C-3), 112.2 (d, C-5), 123.4 (s, C-3–4-Ph), 126.7 (d, C-4–*N*–CH_2–Ph), 126.9 (d, C-2, –6-*N*–CH_2–Ph), 128.8 (d, C-6–4-Ph), 128.9 (d, C-3, –5-*N*–CH_2–Ph), 128.9 (d, C4–4-Ph), 129.2 (d, C-6), 130.7 (d, C-5–4-Ph), 133.6 (d, C-2–4-Ph), 141.6 (s, C-1–*N*–CH_2–Ph), 144.8 (s, C-1-4-Ph), 146.1 (d, C-2), 167.2 (s, CO). Anal. Calcd for $C_{21}H_{20}BrNO_2$: C, 63.52; H, 5.08; N, 3.53. Found: C, 63.42; H, 5.18; N, 3.63.

4.1.2.6. Ethyl 1-benzyl-4-(3-trifluorophenyl)-1,4-dihydropyridine-3-carboxylate (4f). Yellow oil; yield 71%; MS (ESI) *m*/*z* 410 (M+Na$^+$, 100%). ^{1}H NMR ($CDCl_3$) δ 1.13 (AB*X*$_3$, *J* = 7.1 Hz, 3H, CH_2–C*H*$_3$), 3.95–4.09 (*AB*X$_3$, *J* = 10.3, 7.1 Hz, 2H, C*H*$_2$–CH_3), 4.44 (s, 2H, *N*–CH_2), 4.60 (d, *J* = 4.7 Hz, 1H, 4-H), 4.87 (dd, *J* = 7.7, 4.7 Hz, 1H, 5-H), 5.94 (d, *J* = 7.7 Hz, 1H, 6-H), 7.26–7.53 (m, 10H, 2-H, $C_6CF_3H_4$, CH_2–C_6H_5). ^{13}C NMR ($CDCl_3$) δ 14.2 (q, $COOCH_2CH_3$), 44.1 (d, C-4), 54.8 (t, *N*–CH_2), 61.8 (t, $COOCH_2CH_3$), 108.2 (s, C-3), 112.1 (d, C-5), 122.6 (d, C4–4-Ph), 124.0 (s, CF_3), 126.6 (d, C-4–*N*–CH_2–Ph), 126.8 (d, C-2, –6-*N*–CH_2–Ph), 127.8 (d, C-2–4-Ph), 128.9 (d, C-3, –5-*N*–CH_2–Ph), 129.3 (d, C-6), 129.7 (d, C-5–4-Ph), 130.9 (s, C-3–4-Ph), 132.1 (d, C-6–4-Ph), 141.6 (s, C-1–*N*–CH_2–Ph), 142.4 (s, C-1–4-Ph), 146.6 (d, C-2), 167.4 (s, CO). Anal. Calcd for $C_{22}H_{20}F_3NO_2$: C, 68.26; H, 5.21; N, 3.62. Found: C, 68.16; H, 5.20; N, 3.54.

4.1.2.7. Methyl 1-benzyl-4-(4-tolyl)-1,4-dihydropyridine-3-carboxylate (4g). Yellow semi-solid product; yield 68%; MS (ESI) *m*/*z* 320 (M+H$^+$, 100%). ^{1}H NMR (acetone-d_6) δ 2.23 (s, 3H, C_6H_4–C*H*$_3$), 3.48 (s, 3H, $COOCH_3$), 4.41 (d, *J* = 4.9 Hz, 1H, 4-H), 4.59 (s, 2H, *N*-CH_2), 4.86 (dd, *J* = 7.7, 4.9 Hz, 1H, 5-H), 6.11 (d, *J* = 7.7 Hz, 1H, 6-H), 7.03–7.09 (m, 4H, C_6H_4–CH_3), 7.30–7.41 (m, 6H, 2-H, CH_2–C_6H_5). ^{13}C NMR ($CDCl_3$) δ 24.3 (q, C-4–Ph-*C*H$_3$), 44.0 (d, C-4), 52.3 (q, $COOCH_3$), 53.9 (t, *N*–CH_2), 108.7 (s, C-3), 111.9 (d, C-5), 126.5 (d, C-4–*N*–CH_2–Ph), 126.6 (d, C-2, –6-*N*–CH_2–Ph), 128.6 (d, C-3, –5-*N*–CH_2–Ph), 128.9 (d, C-6, –2-4-Ph), 129.1 (d, C-6), 129.2 (d, C-5, –3–4-Ph), 135.4 (s, C4–4-Ph), 139.2 (s, C-1–4-Ph), 141.9 (s, C-1–*N*–CH_2–Ph), 144.6 (d, C-2), 167.2 (s, CO). Anal. Calcd for $C_{21}H_{21}NO_2$: C, 79.04; H, 6.63; N, 4.39. Found: C, 78.87; H, 6.46; N, 4.21.

4.1.2.8. Methyl 1-benzyl-4-(2-methoxyphenyl)-1,4-dihydropyridine-3-carboxylate (4h). Yellow semi-solid product; yield 73%; MS (ESI) *m*/*z* 336 (M+H$^+$, 100%). ^{1}H NMR (acetone-d_6) δ 3.46 (s, 3H, $COOCH_3$), 3.81 (s, 3H, C_6H_4–OCH_3), 4.55 (s, 2H, *N*–CH_2), 4.88 (d, *J* = 4.8 Hz, 1H, 4-H), 4.94 (dd, *J* = 7.7, 4.8 Hz, 1H, 5-H), 5.94 (d, *J* = 7.7 Hz, 1H, 6-H), 7.82–7.10 (m, 4H, C_6H_4–OCH_3), 7.26–7.38 (m, 5H, CH_2–C_6H_5), 7.57 (s, 1H, 2-H). ^{13}C NMR ($CDCl_3$) δ 38.1 (d, C-4), 52.3 (q, $COOCH_3$), 54.8 (t, *N*–CH_2), 56.1 (q, C-2–Ph–OCH_3), 108.9 (s, C-3), 111.8 (d, C-5), 114.2 (d, C-3–4–Ph), 121.0 (d, C-5–4-Ph), 121.4 (s, C-1–4-Ph), 126.7 (d, C-4–*N*–CH_2–Ph), 126.8 (d, C4–4-Ph), 126.9 (d, C-2, –6-*N*–CH_2–Ph), 128.4 (d, C-3, –5-*N*–CH_2–Ph), 129.1 (d, C-6), 130.0 (d, C-6–4-Ph), 141.6 (s, C-1–*N*–CH_2–Ph), 144.6 (d, C-2), 158.6 (s, C-2–4-Ph), 167.2 (s, CO). Anal. Calcd for $C_{21}H_{21}NO_3$: C, 75.20; H, 6.31; N, 4.18. Found: C, 74.94; H, 6.61; N, 4.06.

4.1.2.9. Methyl 1-benzyl-4-(4-methoxyphenyl)-1,4-dihydropyridine-3-carboxylate (4i). Yellow semi-solid product; yield 75%; MS (ESI) *m*/*z* 358 (M+Na$^+$, 100%). ^{1}H NMR (acetone-d_6) δ 3.47 (s, 3H, $COOCH_3$), 3.72 (s, 3H, C_6H_4–OCH_3), 4.38 (d, *J* = 4.9 Hz, 1H, 4-H), 4.58 (s, 2H, *N*–CH_2), 4.85 (dd, *J* = 7.7, 4.9 Hz, 1H, 5-H), 6.10 (d, *J* = 7.7 Hz, 1H, 6-H), 6.77–7.11 (m, 4H, C_6H_4–OCH_3), 7.29–7.51 (m, 6H, 2-H, CH_2–C_6H_5). ^{13}C NMR ($CDCl_3$) δ 44.0 (d, C-4), 52.3 (q, $COOCH_3$), 54.7 (t, *N*–CH_2), 55.8 (q, C-4–Ph–OCH_3), 109.4 (s, C-3), 112.3 (d, C-5), 114.2 (d, C-3, –5-4-Ph), 126.8 (d, C-4–*N*–CH_2–Ph), 126.9 (d, C-2, –6–*N*–CH_2–Ph), 128.4 (d, C-3, –5-*N*–CH_2––Ph), 129.3 (d, C-6), 130.0 (d, C-2, –6-4-Ph), 134.5 (s, C-1–4-Ph), 141.6 (s, C-1–*N*–CH_2–Ph), 144.6 (d, C-2), 157.6 (s, C4–4-Ph), 167.2 (s, CO). Anal. Calcd for $C_{21}H_{21}NO_3$: C, 75.20; H, 6.31; N, 4.18. Found: C, 74.87; H, 6.51; N, 4.10.

4.1.2.10. Ethyl 1-benzyl-4-(4-tolyl)-1,4-dihydropyridine-3-carboxylate (4j). Yellow oil; yield 68%; MS (ESI) *m*/*z* 334 (M+H$^+$, 100%). ^{1}H NMR (acetone-d_6) δ 1.08 (AB*X*$_3$, *J* = 7.1 Hz, 3H, CH_2–C*H*$_3$), 2.24 (s, 3H, C_6H_4–C*H*$_3$), 3.88–3.99 (*AB*X$_3$, *J* = 10.9, 7.1 Hz, 2H, C*H*$_2$–CH_3), 4.43 (d, *J* = 4.9 Hz, 1H, 4-H), 4.55 (s, 2H, *N*–CH_2), 4.85 (dd, *J* = 7.7, 4.9 Hz, 1H, 5-H), 5.94 (d, *J* = 7.7 Hz, 1H, 6-H), 7.01–7.11 (m, 4H, C_6H_4–CH_3), 7.30–7.42 (m, 6H, 2-H, CH_2–C_6H_5). ^{13}C NMR ($CDCl_3$) δ 14.2 (q, $COOCH_2$**C**H_3), 24.2 (q, C-4–Ph–**C**H_3), 44.3 (d, C-4), 54.8 (t, *N*–CH_2), 61.7 (t, COO**C**H_2CH_3), 108.0 (s, C-3), 111.7 (d, C-5), 126.8 (d, C-4–*N*–CH_2–Ph), 126.8 (d, C-2, –6-*N*–CH_2–Ph), 128.6 (d, C-3, –5-*N*–CH_2–Ph), 128.9 (d, C-2, –6–4-Ph), 129.3 (d, C-6), 129.4 (d, C-3, –5–4-Ph), 139.1 (s, C-1–4-Ph), 135.4 (s, C4–4-Ph), 141.7 (s, C-1–*N*–CH_2–Ph), 146.1 (d, C-2), 167.2 (s, CO). Anal. Calcd for $C_{22}H_{23}NO_2$: C, 79.31; H, 6.96; N, 4.20. Found: C, 79.11; H, 6.75; N, 4.11.

4.1.2.11. Ethyl 1-benzyl-4-(2-methoxyphenyl)-1,4-dihydropyridine-3-carboxylate (4k). Yellow semi-solid product; yield 61%; MS (ESI) *m*/*z* 350 (M+H$^+$, 100%). ^{1}H NMR (acetone-d_6) δ 1.06 (AB*X*$_3$, *J* = 7.1 Hz, 3H, CH_2–C*H*$_3$), 3.81 (s, 3H, C_6H_4–OCH_3), 3.94–4.04 (*AB*X$_3$, *J* = 10.7, 7.1 Hz, 2H, C*H*$_2$–CH_3), 4.39 (s, 2H, *N*–CH_2), 4.98–5.25 (m, 2H, 4-H, 5-H), 5.75 (d, *J* = 6.9 Hz, 1H, 6-H), 6.80–7.35 (m, 9H, C_6H_4–OCH_3, CH_2–C_6H_5), 7.47 (s, 1H, 2-H).

^{13}C NMR ($CDCl_3$) δ 14.2 (q, $COOCH_2CH_3$), 38.1 (d, C-4), 54.6 (t, *N*–CH_2), 56.4 (q, C-2–Ph–O**C**H_3), 61.8 (t, COO**C**H_2CH_3), 108.0 (s, C-3), 111.9 (d, C-5), 114.4 (d, C-3–4-Ph), 121.0 (s, C-1–4-Ph), 121.6 (d, C-5–4-Ph), 126.6 (d, C4–4-Ph), 126.7 (d, C-4–*N*–CH_2–Ph), 126.9 (d, C-2, –6–*N*-CH_2–Ph), 128.6 (d, C-3, –5–*N*–CH_2–Ph), 129.3 (d, C-6), 130.3 (d, C-2–4-Ph), 141.5 (s, C-1–*N*-CH_2–Ph), 146.1 (d, C-2), 158.8 (s, C-2–4-Ph), 167.3 (s, CO). Anal. Calcd for $C_{22}H_{23}NO_3$: C, 75.62; H, 6.63; N, 4.01. Found: C, 75.61; H, 6.74; N, 3.71.

4.1.2.12. Ethyl 1-benzyl-4-(4-methoxyphenyl)-1,4-dihydropyridine-3-carboxylate (4l). Yellow oil; yield 72%; MS (ESI) *m*/*z* 350 (M+H$^+$, 100%). ^{1}H NMR (acetone-d_6) δ 1.10 (AB*X*$_3$, *J* = 6.9 Hz, 3H, CH_2–C*H*$_3$), 3.52–3.57 (*AB*X$_3$, *J* = 13.9, 6.9 Hz, 2H, C*H*$_2$–CH_3), 3.77 (s, 3H, C_6H_4–OCH_3), 4.38 (d, *J* = 4.9 Hz, 1H, 4-H), 4.59 (s, 2H,

N–CH_2), 4.86 (dd, *J* = 7.7, 4.9 Hz, 1H, 5-H), 6.11 (d, *J* = 7.7 Hz, 1H, 6-H), 6.76-7.12 (m, 4H, C_6H_4–OCH_3), 7.27–7.42 (m, 6H, 2-H, CH_2–C_6H_5). ^{13}C NMR ($CDCl_3$) δ 14.0 (q, $COOCH_2CH_3$), 44.2 (d, C-4), 54.8 (t, *N*–CH_2), 55.9 (q, C-4–Ph–OCH_3), 61.7 (t, $COOCH_2CH_3$), 109.0 (s, C-3), 112.0 (d, C-5), 114.6 (d, C-3, –5–4-Ph), 126.8 (d, C-4–*N*–CH_2–Ph), 126.9 (d, C-2, –6-*N*–CH_2–Ph), 128.4 (d, C-3, –5-*N*–CH_2–Ph), 129.1 (d, C-6), 131.0 (d, C-2,–6–4-Ph), 134.9 (s, C-1–4-Ph), 141.3 (s, C-1–*N*–CH_2–Ph), 146.6 (d, C-2), 158.3 (s, C4–4-Ph), 167.1 (s, CO).Anal. Calcd for $C_{22}H_{23}NO_3$: C, 75.62; H, 6.63; N, 4.01. Found: C, 75.33; H, 6.47; N, 3.83.

4.1.3. General procedure for the preparation of the β-enaminocarbonyl compounds (7a–j)

One equivalent of the respective propiolic acid ester **5a**, **b** and one equivalent of the benzylamine compound **6a**–**d** were stirred in THF for 5 h at rt.[23] Then the solvent was removed in vacuum and the resulting yellow oil of compounds **7a**–**j** was used without further purification.

4.1.3.1. *E*/*Z*-Methyl 3-(benzylamino)acrylat (7a). Yellow oil; yield 98%; MS (ESI) *m*/*z* 214 ($M+Na^+$, 100%). ^{1}H NMR ($CDCl_3$) δ 3.63 (s, 3H, *Z*–CH_3), 3.64 (s, 3H, *E*–CH_3), 4.21 (d, *J* = 5.2 Hz, 2H, *E*–CH_2), 4.34 (d, *J* = 6.0 Hz, 2H, *Z*–CH_2), 4.54 (d, *J* = 8.0 Hz, 1H, *Z*–1-H), 4.82 (d, *J* = 13.2 Hz, 1H, *E*–1-H), 6.67 (dd, *J* = 12.8, 8.0 Hz, 1H, *Z*–2-H), 7.22–7.34 (m, 10H, C_6H_5), 7.57 (dd, *J* = 13.2, 8.1 Hz, 1H, *E*–2-H), 8.10 (br s, 2H, *N*H). Anal. Calcd for $C_{11}H_{13}NO_2$: C, 69.13; H, 6.86; N, 7.33. Found: C, 68.86; H, 6.75; N, 7.24.

4.1.3.2. *E*/*Z*-Methyl 3-(2-methoxybenzylamino)acrylat (7b). Yellow oil; yield 95%; MS (ESI) *m*/*z* 222 ($M+H^+$, 100%). ^{1}H NMR ($CDCl_3$) δ 3.61 (s, 3H, *Z*–$COOCH_3$), 3.63 (s, 3H; *E*–$COOCH_3$), 3.82 (s, 3H, *Z*–C_6H_4–OCH_3), 3.83 (s, 3H, *E*–C_6H_4–OCH_3), 4.20 (d, *J* = 5.7 Hz, 2H, *Z*–CH_2), 4.30 (d, *J* = 6.2 Hz, 2H, *E*–CH_2), 4.47 (d, *J* = 8.0 Hz, 1H, *Z*–1-H), 4.81 (d, *J* = 13.2 Hz, 1H, *E*–1-H), 6.69 (dd, *J* = 13.1, 8.0 Hz, 1H, *Z*–2-H), 6.83–7.27 (m, 8H, C_6H_4–OCH_3), 7.53 (dd, *J* = 13.2, 8.5 Hz, 1H, *E*–2-H), 8.07 (br s, 2H, *N*H). Anal. Calcd for $C_{12}H_{15}NO_3$: C, 65.18; H, 6.84; N, 6.33. Found: C, 64.97; H, 6.63; N, 6.25.

4.1.3.3. *E*/*Z*-Methyl 3-(4-methoxybenzylamino)acrylat (7c). Yellow oil; yield 98%; MS (ESI) *m*/*z* 222 ($M+H^+$, 100%). ^{1}H NMR ($CDCl_3$) δ 3.62 (s, 3H, *Z*–$COOCH_3$), 3.64 (s, 3H; *E*–$COOCH_3$), 3.77 (s, 3H, *Z*–C_6H_4–OCH_3), 3.78 (s, 3H, *E*–C_6H_4–OCH_3), 4.12 (d, *J* = 5.1 Hz, 2H, *Z*–CH_2), 4.27 (d, *J* = 5.9 Hz, 2H, *E*–CH_2), 4.52 (d, *J* = 8.0 Hz, 1H, *Z*–1-H), 4.82 (d, *J* = 13.2 Hz, 1H, *E*–1-H), 6.66 (dd, *J* = 13.1, 8.0 Hz, 1H, *Z*–2-H), 6.83–7.18 (m, 8H, C_6H_4–OCH_3), 7.53 (dd, *J* = 13.2, 8.0 Hz, 1H, *E*–2-H), 8.04 (br s, 2H, *N*H). Anal. Calcd for $C_{12}H_{15}NO_3$: C, 65.18; H, 6.84; N, 6.33. Found: C, 65.08; H, 6.75; N, 6.30.

4.1.3.4. *E*/*Z*-Methyl 3-(4-methylbenzylamino)acrylat (7d). Yellow oil; yield 93%; MS (ESI) *m*/*z* 205 ($M+H^+$, 100%). ^{1}H NMR ($CDCl_3$) δ 2.26 (s, 3H, *Z*–C_6H_4–CH_3), 2.27 (s, 3H, *E*–C_6H_4–CH_3), 3.57 (s, 3H, *Z*–$COOCH_3$), 3.59 (s, 3H; *E*–$COOCH_3$), 4.10 (d, *J* = 5.3 Hz, 2H, *E*–CH_2), 4.24 (d, *J* = 5.9 Hz, 2H, *Z*–CH_2), 4.47 (d, *J* = 7.9 Hz, 1H, *Z*–1-H), 4.76 (d, *J* = 13.2 Hz, 1H, *E*–1-H), 6.61 (dd, *J* = 13.2, 8.0 Hz, 1H, *Z*–2-H), 7.07–7.18 (m, 8H, C_6H_4–CH_3), 7.53 (dd, *J* = 13.2, 8.0 Hz, 1H, *E*–2-H), 8.01 (br s, 2H, *N*H). Anal. Calcd for $C_{12}H_{15}NO_2$: C, 70.27; H, 7.37; N, 6.83. Found: C, 69.95; H, 7.18; N, 6.75.

4.1.3.5. *E*/*Z*-Methyl 3-(1-naphthylamino)acrylat (7e). Yellow oil; yield 94%; MS (ESI) *m*/*z* 242 ($M+H^+$, 100%). ^{1}H NMR ($CDCl_3$) δ 3.63 (s, 3H, *Z*–CH_3), 3.64 (s, 3H; *E*–CH_3), 4.56 (d, *J* = 8.1 Hz, 1H, *Z*–1-H), 4.60 (br s, 2H, *Z*–CH_2), 4.80 (d, *J* = 5.7 Hz, 2H, *E*–CH_2), 4.94 (d, *J* = 13.3 Hz, 1H, *E*–1-H), 6.73 (dd, *J* = 13.1, 8.1 Hz, 1H, *Z*–2-H), 7.41–7.55 (m, 8H, *E*/*Z*–$C_{10}H_7$), 7.61 (dd, *J* = 13.3, 6.9 Hz, 1H, *E*–2-H), 7.78–7.93 (m, 6H, *E*/*Z*–$C_{10}H_7$), 8.21 (br s, 2H, *N*H). Anal. Calcd for $C_{15}H_{15}NO_2$: C, 74.72; H, 6.27; N, 5.81. Found: C, 74.55; H, 6.23; N, 5.67.

4.1.3.6. *E*/*Z*-Ethyl 3-(benzylamino)acrylat (7f). Yellow oil; yield 90%; MS (ESI) *m*/*z* 205 ($M+H^+$, 100%). ^{1}H NMR ($CDCl_3$) δ 1.24–1.26 (m, 6H, *E*/*Z*–CH_2–CH_3), 4.06–4.12 (*AB*X_3, *J* = 14.3, 7.2 Hz, 4H, *E*/*Z*–CH_2–CH_3), 4.20 (d, *J* = 5.2 Hz, 2H, *E*–*N*–CH_2), 4.34 (d, *J* = 6.0 Hz, 2H, *Z*–*N*–CH_2), 4.53 (d, *J* = 8.1 Hz, 1H, *Z*–1-H), 4.82 (d, *J* = 13.2 Hz, 1H, *E*–1-H), 6.67 (dd, *J* = 13.2, 8.1 Hz, 1H, *Z*–2-H), 7.28–7.34 (m, 10H, C_6H_5), 7.57 (dd, *J* = 13.2, 8.1 Hz, 1H, *E*–2-H), 8.11 (br s, 2H, *N*H). Anal. Calcd for $C_{12}H_{15}NO_2$: C, 70.16; H, 7.37; N, 6.83. Found: C, 69.95; H, 7.24; N, 6.72.

4.1.3.7. *E*/*Z*-Ethyl 3-(2-methoxybenzylamino)acrylat (7g). Yellow oil; yield 91%; MS (ESI) *m*/*z* 236 ($M+H^+$, 100%). ^{1}H NMR ($CDCl_3$) δ 1.21–1.25 (m, 6H, *E*/*Z*–CH_2–CH_3), 3.82 (s, 3H, *Z*–C_6H_4–OCH_3), 3.83 (s, 3H, *E*–C_6H_4–OCH_3), 4.05–4.12 (m, 4H, *E*/*Z*–CH_2–CH_3), 4.20 (d, *J* = 5.7 Hz, 2H, *Z*–*N*–CH_2), 4.30 (d, *J* = 6.2 Hz, 2H, *E*–*N*–CH_2), 4.46 (d, *J* = 8.0 Hz, 1H, *Z*–1-H), 4.80 (d, *J* = 13.2 Hz, 1H, *E*–1-H), 6.68 (dd, *J* = 13.1, 8.0 Hz, 1H, *Z*–2-H), 6.83–7.27 (m, 8H, C_6H_4–OCH_3), 7.53 (dd, *J* = 13.2, 8.4 Hz, 1H, *E*–2-H), 8.07 (br s, 2H, *N*H). Anal. Calcd for $C_{13}H_{17}NO_3$: C, 66.41; H, 7.29; N, 5.96. Found: C, 66.25; H, 7.23; N, 5.77.

4.1.3.8. *E*/*Z*-Ethyl 3-(4-methoxybenzylamino)acrylat (7h). Yellow oil; yield 88%; MS (ESI) *m*/*z* 236 ($M+H^+$, 100%). ^{1}H NMR ($CDCl_3$) δ 1.23–1.26 (m, 6H, *E*/*Z*–CH_2–CH_3), 3.78 (s, 3H, *Z*–C_6H_4–OCH_3), 3.87 (s, 3H, *E*–C_6H_4–OCH_3), 4.06–4.14 (m, 6H, *E*/*Z*–CH_2–CH_3, *E*–*N*–CH_2), 4.27 (d, *J* = 5.8 Hz, 2H, *Z*–*N*–CH_2), 4.51 (d, *J* = 8.1 Hz, 1H, *Z*–1-H), 4.82 (d, *J* = 13.3 Hz, 1H, *E*–1-H), 6.66 (dd, *J* = 13.2, 8.1 Hz, 1H, *Z*–2-H), 6.84–7.19 (m, 8H, C_6H_4–OCH_3), 7.55 (dd, *J* = 13.3, 7.8 Hz, 1H, *E*–2-H), 8.05 (br s, 2H, *N*H). Anal. Calcd for $C_{13}H_{17}NO_3$: C, 66.41; H, 7.29; N, 5.96. Found: C, 66.05; H, 7.10; N, 5.88.

4.1.3.9. E/Z-Ethyl 3-(4-Methylbenzylamino)acrylat (7i). Yellow oil; yield 83%; MS (ESI) *m*/*z* 220 ($M+H^+$, 100%). ^{1}H NMR ($CDCl_3$) δ 1.16-1.20 (m, 6H, *E*/*Z*–CH_2–CH_3), 2.26 (s, 3H, *Z*-C_6H_4–CH_3), 2.27 (s, 3H, *E*-C_6H_4–CH_3), 4.00–4.09 (m, 6H, *E*/*Z*–CH_2–CH_3, *E*–*N*–CH_2), 4.22 (d, *J* = 5.9 Hz, 2H, *Z*–*N*–CH_2), 4.44 (d, *J* = 8.1 Hz, 1H, *Z*–1-H), 4.72 (d, *J* = 13.2 Hz, 1H, *E*–1-H), 6.59 (dd, *J* = 13.1, 8.1 Hz, 1H, *Z*–2-H), 7.07–7.18 (m, 8H, C_6H_4–CH_3), 7.55 (dd, *J* = 13.2, 7.9 Hz, 1H, *E*–2-H), 8.03 (br s, 2H, *N*H). Anal. Calcd for $C_{13}H_{17}NO_2$: C, 71.26; H, 7.82; N, 6.39. Found: C, 70.95; H, 7.69; N, 6.27.

4.1.3.10. *E*/*Z*-Ethyl 3-(1-naphthylylamino)acrylat (7j). Yellow oil; yield 98%; MS (ESI) *m*/*z* 256 ($M+H^+$, 100%). ^{1}H NMR ($CDCl_3$) δ 1.22–1.28 (m, 6H, *E*/*Z*–CH_2–CH_3), 4.06–4.17 (m, 4H, *E*/*Z*–CH_2–CH_3), 4.55 (d, *J* = 8.1 Hz, 1H, *Z*–1-H), 4.60 (br s, 2H, *Z*–*N*–CH_2), 4.80 (d, *J* = 5.7 Hz, 2H, *E*–*N*–CH_2), 4.95 (d, *J* = 13.3 Hz, 1H, *E*–1-H), 6.72 (dd, *J* = 13.1, 8.1 Hz, 1H, *Z*–2-H), 7.41–7.55 (m, 8H, *E*/*Z*–$C_{10}H_7$), 7.62 (dd, *J* = 13.3, 6.9 Hz, 1H, *E*–2-H), 7.77–7.93 (m, 6H, *E*/*Z*–$C_{10}H_7$), 8.21 (br s, 2H, *N*H). Anal. Calcd for $C_{16}H_{17}NO_2$: C, 75.33; H, 6.72; N, 5.49. Found: C, 74.99; H, 6.65; N, 5.38.

4.1.4. General procedure for the preparation of the cinnamyl aldehydes (9a,b).

One equivalent of the corresponding aryl iodide **8a**, **b** was dissolved in DMF and then 3 equiv of acrolein diethyl acetale, one equivalent of tetrabutyl ammonium acetate, one and a half equivalents of potassium carbonate and, finally, 1 equiv of potassium chloride were added together with 0.03 equiv of palladium(II) acetate. The mixture was stirred for 2–3 h at 90 °C. After cooling to rt 2 M hydrochloric acid was added and stirring continued for 10 min.

Then the mixture was extracted with diethyl ether for several times and the unified organic layer was dried over sodium sulfate, filtered and evaporated in vacuum. The remaining oily product was purfied by column chromatography using an eluent mixture of hexane and acetic acid ethyl ester (90/10).

4.1.4.1. Cinnamyl aldehyde (9a). Yellow oil; yield 75%; MS (ESI) *m*/*z* 132 ($M+H^+$, 100%). ^1H NMR ($CDCl_3$) δ 6.68 (dd, *J* = 16.0, 7.4 Hz, 1H, 2-H), 7.23–7.44 (m, 6H, 3-H, C_6H_5), 9.69 (d, *J* = 7.4 Hz, 1H, 1-H). Anal. Calcd for C_9H_8O: C, 82.20; H, 6.13. Found: C, 81.90; H, 5.95.

4.1.4.2. 3-Naphthylacrylaldehyde (9b). Yellow oil; yield 90%; MS (ESI) *m*/*z* 183 ($M+H^+$, 100%). ^1H NMR ($CDCl_3$) δ 6.84 (dd, *J* = 15.7, 7.6 Hz, 1H, 2-H), 7.49–8.16 (m, 7H, $C_{10}H_7$), 8.29 (d, *J* = 15.7 Hz, 1H, 3-H), 9.83 (d, *J* = 7.6 Hz, 1H, 1-H). Anal. Calcd for $C_{13}H_{10}O$: C, 85.79; H, 6.37. Found: C, 85.55; H, 6.25.

4.1.5. General procedure for the preparation of the *N*-benzyl and 4-naphthyl substituted 1.4-dihydropyridines (10a–j)

One equivalent of the corresponding cinnamyl aldehyde **9a**, **b** and one equivalent of the corresponding β-enaminocarbonyl compound **7a**–**j** were dissolved in dichloromethane. After addition of three equivalents of sodium sulfate and 0.05 equiv of iron(II) chloride hexahydrate (5%) the solution was stirred at 40 °C for 12 h. After both starting products disappeared as monitored by tlc the solution was filtered and the solvent was removed in vacuum. The remaining oil was purified by MPLC using cyclohexane, *t*-butylmethylether and dichloromethane in a mixture of 80/15/5.

4.1.5.1. Methyl 1,4-dihydro-1-(2-methoxybenzyl)-4-phenylpyridine-3-carboxylate (10a). Yellow oil; yield 85%; MS (ESI) *m*/*z* 336 ($M+H^+$, 100%). ^1H NMR ($CDCl_3$) δ 3.81 (s, 3H, $COOCH_3$), 3.94 (s. 3H, C_6H_4–OCH_3), 4.05–4.08 (m, 1H, 4-H), 4.09–4.15 (m, 2H, *N*–CH_2), 4.22–4.35 (m, 1H, 5-H), 6.23–6.33 (m, 1H, 6-H), 6.83–7.71 (m, 10H, 2-H, C_6H_5, C_6H_4–OCH_3).

^{13}C NMR ($CDCl_3$) δ 44.0 (d, C-4), 52.2 (q, $COOCH_3$), 48.9 (t, *N*–CH_2), 56.1 (q, C-2–*N*–CH_2– Ph–OCH_3), 109.8 (s, C-3), 111.9 (d, C-5), 114.1 (d, C-3–*N*–CH_2–Ph), 120.9 (d, C-5–*N*–CH_2–Ph), 125.8 (d, C-4–4-Ph), 127.3 (s, C-1–*N*–CH_2–Ph), 127.8 (d, C4–*N*–CH_2–Ph), 127.9 (d, C-6–*N*–CH_2–Ph), 128.7 (d, C-3, –5–4-Ph), 129.0 (d, C-2, –6–4-Ph), 129.3 (d, C-6), 142.2 (s, C-1–4- Ph), 144.5 (d, C-2), 156.5 (s, C-2–*N*–CH_2–Ph), 167.2 (s, CO). Anal. Calcd for $C_{21}H_{21}NO_3$: C, 75.26; H, 6.32; N, 4.18. Found: C, 75.21; H, 6.28; N, 4.04.

4.1.5.2. Methyl 1,4-dihydro-1-(4-methoxybenzyl)-4-phenylpyridine-3-carboxylate (10b). Yellow oil; yield 80%; MS (ESI) *m*/*z* 336 ($M+H^+$, 100%). ^1H NMR ($CDCl_3$) δ 3.33 (s, 3H, $COOCH_3$), 3.80 (s. 3H, C_6H_4–OCH_3), 4.35 (s, 2H, *N*–CH_2), 4.50 (d, *J* = 4.8 Hz, 1H, 4-H), 4.89 (dd, *J* = 7.7, 4.8 Hz, 1H, 5-H), 5.88 (d, *J* = 7.8 Hz, 1H, 6-H), 6.90–7.49 (m, 10H, 2-H, C_6H_5, C_6H_4–OCH_3). ^{13}C NMR ($CDCl_3$) δ 44.2 (d, C-4), 52.4 (q, $COOCH_3$), 54.8 (t, *N*–CH_2), 55.8 (q, C-4–*N*–CH_2– Ph–OCH_3), 109.5 (s, C-3), 112.5 (d, C-5), 114.3 (d, C-3, –5-*N*–CH_2–Ph), 125.5 (d, C-4–4-Ph), 127.6 (d, C-2, –6-*N*–CH_2–Ph), 128.3 (d, C-3, –5–4-Ph), 129.0 (d, C-6), 129.4 (d, C-2, –6–4–Ph), 133.9 (s, C-1–*N*–CH_2–Ph), 142.0 (s, C-1–4-Ph), 144.1 (d, C-2), 158.6 (s, C4–*N*–CH_2–Ph), 167.1 (s, CO). Anal. Calcd for $C_{21}H_{21}NO_3$: C, 75.26; H, 6.32; N, 4.18. Found: C, 75.21; H, 6.28; N, 4.04.

4.1.5.3. Methyl 1,4-dihydro-1-(4-methylbenzyl)-4-phenylpyridine-3-carboxylate (10c). Yellow oil; yield 77%; MS (ESI) *m*/*z* 320 ($M+H^+$, 100%). ^1H NMR ($CDCl_3$) δ 2.25 (s, 3H, C_6H_4–CH_3), 3.48 (s, 3H, $COOCH_3$), 4.24-4.38 (m, 1H, 4-H), 4.42-4.46 (m, 3H, 5-H, *N*–CH_2), 5.78-5.95 (m, 1H, 6-H), 6.81–7.49 (m, 9H, C_6H_5, C_6H_4–OCH_3), 7.63 (s, 1H, 2-H). ^{13}C NMR ($CDCl_3$) δ 24.3 (q, C-4–*N*–CH_2– Ph-CH_3), 44.1 (d, C-4), 52.3 (q, $COOCH_3$), 54.0 (t, *N*–CH_2), 109.8 (s, C-3), 112.3 (d, C-5), 125.0 (d, C-4–4-Ph), 126.8 (d, C-2, –6-*N*–CH_2-Ph), 128.7 (d, C-3, –5–4-Ph), 129.0 (d, C-2, –6–4-Ph), 129.2 (d, C-6), 128.8 (d, C-3, –5-*N*–CH_2–Ph), 136.4 (s, C4–*N*–CH_2–Ph), 138.5 (s, C-1–*N*–CH_2–Ph), 142.1 (s, C-1–4-Ph), 145.3 (d, C-2), 167.2 (s, CO). Anal. Calcd for $C_{21}H_{21}NO_2$: C, 79.02; H, 6.63; N, 4.39. Found: C, 78.89; H, 6.45; N, 4.25.

4.1.5.4. Ethyl 1,4-dihydro-1-(2-methoxybenzyl)-4-phenylpyridine-3-carboxylate (10d). Yellow oil; yield 89%; MS (ESI) *m*/*z* 350 ($M+H^+$, 100%). ^1H NMR ($CDCl_3$) δ 1.07 (ABX$_3$, *J* = 7.0 Hz, 3H, CH_2–CH_3), 3.78 (s. 3H, C_6H_4–OCH_3), 3.49–4.03 (m, 2H, CH_2–CH_3), 4.20–4.35 (m, 1H, 4-H), 4.41–4.49 (m, 3H, 5-H, *N*–CH_2), 6.21–6.38 (m, 1H, 6-H), 6.83–7.72 (m, 10H, 2-H, C_6H_5, C_6H_4–OCH_3). ^{13}C NMR ($CDCl_3$) δ 14.2 (q, $COOCH_2CH_3$), 43.8 (d, C-4), 49.2 (t, *N*–CH_2), 56.3 (q, C-2–*N*–CH_2– Ph–OCH_3), 61.7 (t, $COOCH_2CH_3$), 109.4 (s, C-3), 112.0 (d, C-5), 114.4 (d, C-5– *N*–CH_2–Ph), 121.2 (d, C-5–*N*–CH_2–Ph), 128.0 (s, C-1–*N*–CH_2–Ph), 125.8 (d, C-4–4-Ph), 127.4 (d, C4–*N*-CH_2–Ph), 129.3 (d, C-2, –6–4-Ph), 129.1 (d, C-3, –5–4-Ph), 129.3 (d, C-6), 127.4 (d, C-6–*N*–CH_2–Ph), 142.2 (s, C-1–4-Ph), 144.9 (d, C-2), 156.7 (s, C-2–*N*–CH_2–Ph), 167.1 (s, CO). Anal. Calcd for $C_{22}H_{23}NO_3$: C, 75.68; H, 6.64; N, 4.01. Found: C, 75.44; H, 6.53; N, 3.88.

4.1.5.5. Ethyl 1,4-dihydro-1-(4-methoxybenzyl)-4-phenylpyridine-3-carboxylate (10e). Yellow oil; yield 71%; MS (ESI) *m*/*z* 350 ($M+H^+$, 100%). ^1H NMR ($CDCl_3$) δ 1.10 (ABX$_3$, *J* = 7.0 Hz, 3H, CH_2–CH_3), 3.79 (s. 3H, C_6H_4–OCH_3), 3.97–4.03 (ABX$_3$, *J* = 14.3, 7.0 Hz, 2H, CH_2–CH_3), 4.35 (s, 2H, *N*-CH_2), 4.50 (d, *J* = 4.9 Hz, 1H, 4-H), 4.88 (dd, *J* = 7.7, 4.9 Hz, 1H, 5-H), 5.88 (d, *J* = 7.7 Hz, 1H, 6-H), 6.89–7.49 (m, 10H, 2-H, C_6H_5, C_6H_4–OCH_3). ^{13}C NMR ($CDCl_3$) δ 14.5 (q, $COOCH_2CH_3$), 39.8 (d, C-4), 55.1 (t, *N*-CH_2), 55.8 (q, C-4–*N*–CH_2–Ph–OCH_3), 62.1 (t, $COOCH_2CH_3$), 108.0 (s, C-3), 112.0 (d, C-5), 114.5 (d, C-3, –5-*N*–CH_2–Ph), 125.9 (d, C-4–4-Ph), 127.8 (d, C-2, –6-*N*–CH_2–Ph), 129.0 (d, C-2, –6–4-Ph), 129.1 (d, C-3, –5–4-Ph), 129.3 (d, C-6), 134.1 (s, C-1–*N*–CH_2–Ph), 142.2 (s, C-1–4-Ph), 146.1 (d, C-2), 158.6 (s, C4–*N*–CH_2–Ph), 167.2 (s, CO). Anal. Calcd for $C_{22}H_{23}NO_3$: C, 75.68; H, 6.64; N, 4.01. Found: C, 75.38; H, 6.54; N, 4.03.

4.1.5.6. Ethyl 1,4-Dihydro-1-(4-methylbenzyl)-4-phenylpyridine-3-carboxylate (10f). Yellow oil; yield 73%; MS (ESI) *m*/*z* 334 ($M+H^+$, 100%). ^1H NMR ($CDCl_3$) δ 1.13 (ABX$_3$, *J* = 7.0 Hz, 3H, CH_2–CH_3), 2.25 (s, 3H, C_6H_4–CH_3), 3.97–4.03 (ABX$_3$, *J* = 14.2, 7.0 Hz, 2H, CH_2–CH_3), 4.13–4.23 (m, 1H, 4-H), 4.25–4.44 (m, 1H, 5-H), 4.51 (s, 2H, *N*–CH_2), 5.77-5.90 (m, 1H, 6-H), 6.73–7.59 (m, 9H, C_6H_5, C_6H_4–CH_3), 7.63 (s, 1H, 2-H). ^{13}C NMR ($CDCl_3$) δ 14.5 (q, $COOCH_2CH_3$), 24.2 (q, C-4–*N*–CH_2–Ph–CH_3), 44.0 (d, C-4), 54.4 (t, *N*–CH_2), 62.0 (t, $COOCH_2CH_3$), 109.4 (s, C-3), 112.0 (d, C-5), 125.5 (d, C-4–4-Ph), 128.0 (d, C-3, –5-*N*–CH_2–Ph), 129.3 (d, C-2, –6–4-Ph), 128.7 (d, C-3, –5–4-Ph), 129.0 (d, C-6), 126.4 (d, C-2, –6-*N*–CH_2–Ph), 136.7 (s, C4–*N*–CH_2–Ph), 138.9 (s, C-1–*N*–CH_2–Ph), 142.4 (s, C-1–4-Ph), 145.9 (d, C-2), 167.1 (s, CO). Anal. Calcd for $C_{22}H_{23}NO_2$: C, 79.31; H, 6.96; N, 4.20. Found: C, 79.25; H, 6.76; N, 4.15.

4.1.5.7. Methyl 1,4-dihydro-1-(1-naphthylmethyl)-4-phenylpyridine-3-carboxylate (10g). Yellow crystals; mp 135-137 °C; yield 87%; MS (ESI) *m*/*z* 356 ($M+H^+$, 100%). ^1H NMR ($CDCl_3$) δ 3.54 (s, 3H, CH_3), 4.55 (d, *J* = 4.7 Hz, 1H, 4-H), 4.89–4.93 (m. 3H, 5-H, *N*–CH_2), 5.94 (d, *J* = 7.8 Hz, 1H, 6-H), 7.11–7.91 (m, 13H, 2-H, C_6H_5, $C_{10}H_7$). ^{13}C NMR ($CDCl_3$) δ 45.1 (d, C-4), 53.1 (q, $COOCH_3$), 53.1 (t, *N*–CH_2), 108.8 (s, C-3), 113.2 (d, C-5), 124.2 (d, C-2, –8-*N*–CH_2–Nph), 125.0 (d, C-4–4-Ph), 125.6 (d, C-6–*N*–CH_2–Nph), 125.8 (d, C7–*N*–CH_2–Nph), 126.5 (d, C-4–*N*–CH_2–Nph), 126.9 (d, C-3-*N*-CH_2-Nph), 128.6 (d, C-5-*N*–CH_2–Nph), 129.1 (d, C-3, –5–4-Ph),

129.6 (d, C-6), 129.8 (d, C-2, –6–4-Ph), 132.6 (s, C-8a–*N*–CH_2–Nph), 133.5 (s, C-4a–*N*–CH_2–Nph), 133.8 (s, C-1–*N*–CH_2–Nph), 142.1 (s, C-1–4-Ph), 145.3 (d, C-2), 167.2 (s, CO). Anal. Calcd for $C_{24}H_{21}NO_2$: C, 81.16; H, 5.96; N, 3.94. Found: C, 81.05; H, 6.04; N, 3.94.

4.1.5.8. Ethyl 1,4.dihydro-1-(1-naphthylmethyl)-4-phenylpyridine-3-carboxylate (10h). Yellow crystals; mp 145–148 °C; yield 84%; MS (ESI) *m*/*z* 370 ($M+H^+$, 100%). ^1H NMR ($CDCl_3$) δ 1.09 (ABX$_3$, *J* = 7.1 Hz, 3H, CH_2–CH_3), 3.93–4.07 (*AB*X$_3$, *J* = 10.6, 7.1 Hz, 2H, CH_2–CH_3), 4.55 (d, *J* = 4.8 Hz, 1H, 4-H), 4.89–4.92 (m, 3H, 5-H, *N*–CH_2), 5.93 (d, *J* = 7.9 Hz, 1H, 6-H), 7.11–7.92 (m, 13H, 2-H, C_6H_5, $C_{10}H_7$). ^{13}C NMR ($CDCl_3$) δ 14.0 (q, $COOCH_2CH_3$), 44.8 (d, C-4), 53.3 (t, *N*–CH_2), 61.7 (t, $COOCH_2CH_3$), 108.0 (s, C-3), 112.2 (d, C-5), 124.5 (d, C-2, –8-*N*–CH_2–Nph), 125.5 (d, C-6–*N*–CH_2–Nph), 125.6 (d, C-4–4-Ph), 125.9 (d, C7–*N*–CH_2–Nph), 126.3 (d, C-4–*N*–CH_2–Nph), 126.7 (d, C-3–*N*–CH_2–Nph), 128.4 (d, C-5–*N*–CH_2–Nph), 129.1 (d, C-6), 129.3 (d, C-3, –5-4-Ph), 129.4 (d, C-2, –6-4-Ph), 132.3 (s, C-8a–*N*–CH_2–Nph), 133.4 (s, C-4a–*N*–CH_2–Nph), 133.9 (s, C-1–*N*–CH_2–Nph), 142.3 (s, C-1–4-Ph), 145.8 (d, C-2), 167.1 (s, CO). Anal. Calcd for $C_{25}H_{23}NO_3$: C, 81.27; H, 6.27; N, 3.79. Found: C, 80.95; H, 6.11; N, 3.76.

4.1.5.9. Methyl 1-benzyl-1,4-dihydro-4-(1-naphthyl)pyridine-3-carboxylate (10i). Yellow crystals; mp 125–127 °C; yield 85%; MS (ESI) *m*/*z* 356 ($M+H^+$, 100%). ^1H NMR ($CDCl_3$) δ 3.47 (s, 3H, CH_3), 4.45 (s, 3H, *N*–CH_2), 5.04 (dd, *J* = 7.8, 4.7 Hz, 1H, 5-H), 5.37 (d, *J* = 4.7 Hz, 1H, 4-H), 5.80 (d, *J* = 7.8 Hz, 1H, 6-H), 7.26-8.24 (m, 13H, 2-H, C_6H_5, $C_{10}H_7$). ^{13}C NMR ($CDCl_3$) δ 42.3 (d, C-4), 53.2 (q, $COOCH_3$), 54.8 (t, *N*–CH_2), 109.2 (s, C-3), 113.3 (d, C-5), 124.1 (d, C-2, –8–4-Nph), 125.6 (d, C-6–4-Nph), 125.9 (d, C7–4-Nph), 126.4 (d, C-4–4-Nph), 126.6 (d, C-3–4-Nph), 126.8 (d, C-4–*N*–CH_2–Ph), 126.9 (d, C-2, –6-*N*–CH_2–), 128.5 (d, C-5–4-Nph), 128.6 (d, C-3, –5-*N*–CH_2–Ph), 129.6 (d, C-6), 132.7 (s, C-8a–4-Nph), 133.5 (s, C-4a–4-Nph), 134.0 (s, C-1–4-Nph), 141.6 (s, C-1-*N*–CH_2–Ph), 145.3 (d, C-2), 167.2 (s, CO). Anal. Calcd for $C_{24}H_{21}NO_2$: C, 81.16; H, 5.96; N, 3.94. Found: C, 81.10; H, 6.30; N, 3.78.

4.1.5.10. Ethyl 1-benzyl-1,4-dihydro-4-(1-naphthyl)pyridine-3-carboxylate (10j). Yellow oil; yield 79%; MS (ESI) *m*/*z* 370 ($M+H^+$, 100%). ^1H NMR ($CDCl_3$) δ 0.93 (ABX$_3$, *J* = 7.0 Hz, 3H, CH_2–CH_3), 3.90–4.05 (m, *AB*X$_3$, *J* = 13.6, 7.0 Hz, 3H, 4-H, CH_2–CH_3), 4.45 (s, 2H, *N*–CH_2), 5.02 (dd, *J* = 7.7, 5.0 Hz, 1H, 5-H), 5.80 (d, *J* = 7.7 Hz, 1H, 6-H), 7.18–8.26 (m, 13H, 2-H, C_6H_5, $C_{10}H_7$). ^{13}C NMR ($CDCl_3$) δ 14.2 (q, $COOCH_2CH_3$), 42.3 (d, C-4), 61.5 (q, $COOCH_2CH_2$), 54.6 (t, *N*–CH_2), 109.1 (s, C-3), 112.8 (d, C-5), 124.5 (d, C-2, –8–4-Nph), 126.5 (d, C-4–*N*–CH_2–Ph), 125.6 (d, C–6–4-Nph), 125.7 (d, C8–4-Nph), 126.4 (d, C-4–4-Nph), 126.6 (d, C-3–4-Nph), 126.9 (d, C-2, –6-*N*–CH_2–Ph), 128.5 (d, C-5–4-Nph), 128.6 (d, C-3, –5-*N*–CH_2–Ph), 129.6 (d, C-6), 132.7 (s, C-8a–4-Nph), 133.5 (s, C-4a–4-Nph), 134.0 (s, C-1–4-Nph), 141.6 (s, C-1-*N*–CH_2–Ph), 146.0 (d, C-2), 167.2 (s, CO). Anal. Calcd for $C_{25}H_{23}NO_2$: C, 81.33; H, 6.28; N, 3.79. Found: C, 81.28; H, 6.24; N, 3.67.

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[26,27] were cultured at 37 °C under carbon dioxide containing atmosphere (5%) in McCoys 5A medium containing 10% of fetal calf serum, L-glutamine (2 mM) and 5 mL of a gentamicin solution (5 mg/mL). The medium used for culturing the P-gp expressing subline L5178Y has been additionally supplemented with colchicine (60 ng/mL) to ensure a stable P-gp expression.

4.3. P-gp inhibition assay

Cells of both cell lines were taken in an adjusted concentration of 1 x 10^6 cells per mL medium and resuspended in serum free Mc Coys 5A medium. 0.5 mL Aliquots were filled into Eppendorf centrifuge tubes. The test compounds taken from prepared stock solutions in DMSO (1.0 mg/mL) were added in a volume of 5 μL. After 10 min of inhibitor preincubation at rt the P-gp substrate rhodamine 123 was added using 5 μL of a 0.5 mM solution in water. Incubation was continued for 40 min at 37 °C. After that the cells were centrifuged and washed twice with phosphate-buffered saline (PBS). Then they were resuspended in PBS for measurement. The non-inhibitor containing cells were treated in the same way as the inhibitor preincubated cells. The fluorescence uptake of rhodamine 123 within a number of 1×10^4 counted cells was determined by flow cytometry using a Becton Dickinson FACScan flow cytometer. 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.

4.4. MDR reversal studies

Cells of both cell lines were corrected to a cell number of 5×10^5 cells per mL. Daunorubicin both as pure compound and as liposomal DaunoXome® preparation was added from stock solutions reaching a final DMSO concentration of 1%. The daunorubicin has been used in concentrations of 0.01 μM to 10 μM. Then 100 μL of the cell and daunorubicin containing suspension were divided into 96-well plates and additionally supplemented with the P-gp inhibitor in the respective concentration. Cells were incubated for 24 h at 37 °C under the carbon dioxide containing atmosphere (5%). Then 10 μL of a MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) solution (5 mg/mL) in PBS were added to each well. After additional four hours of incubation 100 μL of lysis buffer containing 50 g of sodium dodecyl sulfate (SDS), 125 mL of acetic acid and 375 mL of DMSO each per liter were added and cells were shaken for 30 min for a complete cell lysis. Each plate was measured spectrophotometrically with a microplate reader PolarStar Galaxy at 560 nm. The experiments were repeated in triplicate and the IC_{50} values were determined from the mean percent growth inhibitions.

4.5. Cytotoxicity studies

Cells, cell numbers and both incubation as well as workup procedure were exactly the same as used for the determination of the daunorubicin toxicity except the use of the respective 1,4-dihydropyridines instead of the daunorubicin. The used concentration range of the 1,4-dihydropyridines varied from 1 μM up to 80 μM as the final concentration. The spectrophotometrical analysis followed the described protocol. The experiments were repeated in triplicate.

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