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Structure–activity relationships of novel *N*-acyloxy-1,4-dihydropyridines as *P*-glycoprotein inhibitors

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Abstract—Series of novel *N*-acyloxy-1,4-dihydropyridines have been synthesized and evaluated as *P*-glycoprotein inhibitors in an in vitro assay to estimate their potential to act as multidrug resistance modulators in cancer cells. Structure–activity relationships are discussed and prove a significant and regiospecific influence of certain functional groups.

1. Introduction

Multidrug resistance (MDR) is one emerging problem in cancer therapies of today.[1] Cytostatically active drugs are transported out of the cells by the activity of transmembrane efflux pumps so that intracellular drug levels are too low to reach relevant therapeutic effects.[1,2] *P*-glycoprotein (*P*-gp) belongs to the most important efflux pumps and causes resistances to various cytostatics which belong to different classes of compounds like the vinca alkaloids, anthracycline derivatives or the podophyllotoxins.[1,3]

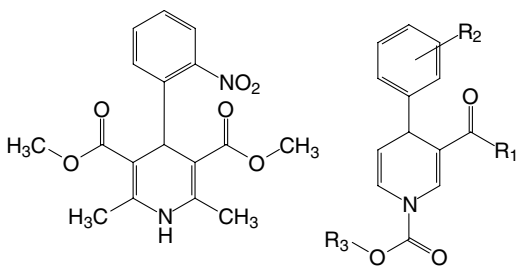
There have been intensive efforts to overcome MDR by the discovery of new cytostatics like the tyrosine kinase inhibitor imatinib or monoclonal antibody gemtuzumab ozagamicine. However, also these structurally new agents turned out to be substrates of *P*-gp and thus are non-effective in MDR resistant cancer cells.[4]

Certain drugs where found to reverse MDR in combination with substrates of *P*-gp. Early compounds like vinblastine or the effective verapamil with own pharmacological effects were dose-limited in therapies so that intracellular effects could not be reached.[1] Moreover, most developed MDR reversers were found to be substrates of the efflux pump themselves.[5]

1,4-Dihydropyridines like nifedipine (Fig. 1) were also found to show MDR reversal effects. However, they pharmacologically act as calcium antagonists like verapamil and so were also not suitable for the therapy of MDR resistant cancer.[6]

Pharmacologically essential structural elements in these calcium antagonists have been the 2- and 6-methyl substitutions and the carbonyl functions at both the 3- and the 5-positions of the 1,4-dihydropyridine ring.[7]

We synthesized novel *N*-acyloxy 1,4-dihydropyridines (Fig. 1) lacking the methyl substitutions and, additionally, the carbonyl function at the 5-position. These 1,4-dihydropyridines have been structurally modified and the biological activities as MDR reversers have been characterized.



nifedipine **N - acyloxy 1,4 - dihydropyridine**

Figure 1.

Keywords: Multidrug resistance; *P*-glycoprotein; Structure–activity relationship; 1,4-Dihydropyridines; Binding region.
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2. Results and discussion

2.1. Chemistry

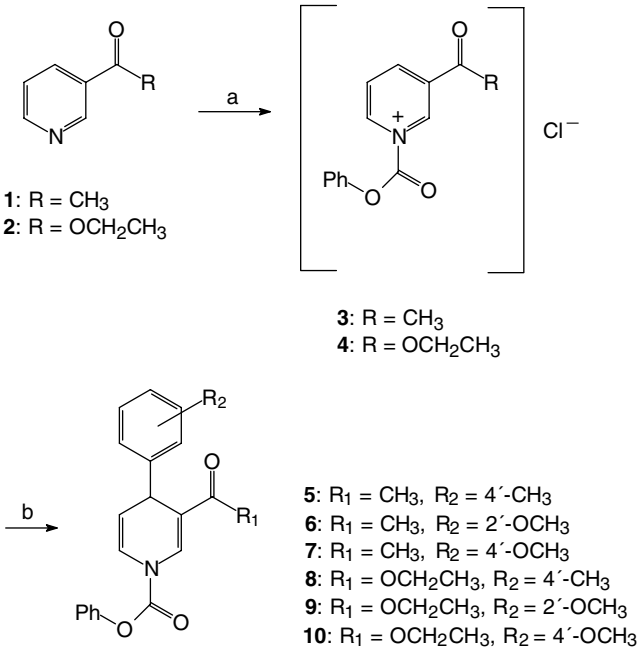
The 1,4-dihydropyridine synthesis started from the 3-carbonyl substituted compounds (**1**, **2**) which have been acylated using phenylchloroformate in THF at low temperatures (Scheme 1).

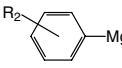
Phenylpyridium salt intermediates (**3**, **4**) resulted and have been regioselectively arylated at the 4-position of the pyridinium ring under catalysis of copper(I) iodide (Scheme 1). After work up procedure the final yields of 1,4-dihydropyridine target compounds (**5**–**10**) made between and 68% and 87%.

2.2. MDR reversal in tumor cells

The biological evaluation was carried out in both, a mouse T-lymphoma parental cell line and the MDR-resistant subline. Rhodamine 123 was used as *P*-gp specific substrate and the fluorescence uptake of this substrate was determined in both cell lines using flow cytometry. By the use of effective *P*-gp inhibitors the fluorescence uptake rates in the MDR-resistant cell line was increased due to the inhibited efflux of the *P*-gp substrate rhodamine. So finally, the comparison of each uptake rates in inhibitor-treated versus untreated cell lines resulted in fluorescence uptake ratios (*R*) which prove compounds with *R*-values >1.0 to be active as *P*-gp inhibitors and so as reversers of MDR in tumor cells. Compounds with *R*-values >10 were found to be very active as MDR reversers.[8]

All of the reported MDR reversing 1,4-dihydropyridine calcium antagonists are unsubstituted at the nitrogen atom. Although they can be oxidized by oxidants like cer(IV) salts or manganese(IV) oxide, they are relatively stable under oxygen atmosphere.[9]



Scheme 1. Reagents and conditions: (a) Cu(I)J (5%), $(CH_3)_2S$, PhOCOCl, THF, −20 °C; (b)  , THF, −20 °C - rt.

Our investigated 1,4-dihydropyridines with a lacking of the 2- and 6-methyl substitution and the 5-carbonyl function are structurally similar to nicotinamide adenine dinucleotide (NAD) model compounds which are easily oxidized, especially without a nitrogen substituent.[10] The enhanced stability of the 1,4-dihydropyridine calcium antagonists towards oxidants results from a steric shielding effect of the methyl functions and a conjugation stability over the second carbonyl substituent in the 5-position of the dihydropyridine ring.[9]

So we introduced a phenoxyacyl substituent at the nitrogen to further stabilize our 1,4-dihydropyridine compounds towards oxidation. This phenoxyacyl substituent allows enhanced conjugation stability of the dihydropyridine system. In consequence, the resulting 1,4-dihydropyridine target compounds (**5**–**10**) show certain stability towards oxygen and could easily be handled in the in vitro studies.

We structurally varied the 3-carbonyl substituent with an ethoxycarbonyl and an acetyl residue. Furthermore, the 4-phenyl substitution which is found being substituted in all of the calcium antagonistic 1,4-dihydropyridines was consequently substituted with a para-methyl function (**5**, **8**) and varied positions of a methoxy function, both in ortho- and in para-position, of the 4-phenyl ring.

All target compounds were found active as MDR reversers at low concentrations of 1 μM with *R*-values >1.0 (Table 1). The standard compound verapamil was found inactive at this concentration.

At 10 μM differences in the biological activities of the structurally varied compounds have been found: A consequent increase in activity is found from the para-methyl to the ortho- and finally the para-methoxy function of the 4-phenyl ring within the 3-acetyl series (compounds **5**–**7**).

Comparing the differences in activity of the compounds from the acetyl series to those of the ethoxycarbonyl series (compounds **8**–**10**) we find almost similar activities for the para-methyl functions in **5** and **8**.

The compound from the ethoxycarbonyl series with the ortho-methoxy function (**9**) indicates slight improved activities compared to that with the acetyl function in **6** at the low concentration, but significant differences were found at the high concentration where the activity of **9** is more than sevenfold higher than that of compound **6**. So compound **9** proves to be highly active with an *R*-value of practically 40.

A similar difference in activity is also found between the para-methoxy substituted derivatives **7** and **10** of both series. The 3-ethoxycarbonyl substituted compound (**10**) is found more active than the 3-acetyl substituted compound **7** at the high concentration.

Table 1. Concentration dependent *P*-gp inhibiting properties of target compounds (**5**–**10**)

Compound	*R*-values	
	1 μM	10 μM
5	1.41	3.58
6	1.01	5.15
7	1.81	7.10
8	1.04	3.09
9	1.60	39.98
10	1.30	19.87
Verapamil	0.66	7.27

The methoxy function mainly increases the activity, especially within the 3-ethoxycarbonyl series. This increase may be caused by the fact that a methoxy function serves as additional hydrogen bond acceptor function within this compound class of MDR reversers.

Furthermore, the great difference in activity of the ortho- and para-introduction of this function suggests a strong sensitivity of a potential *P*-gp binding region for this compound class to the possible binding *via* this additional methoxy function.

3. Conclusions

We synthesized novel 1,4-dihydropyridine compound series and evaluated their MDR reversal properties in an in vitro assay which specifically proves *P*-gp inhibiting properties by the direct comparison of parental and MDR subline fluorescence uptake rates of a specific *P*-gp substrate. The compounds partly showed significantly higher activities than verapamil. The methoxy function within the phenyl moiety leads to highest activity ratios and, furthermore, showed remarkable regiospecific effects on the observed activities indicating an interesting conserved interaction between *P*-gp inhibitor function and *P*-gp binding region. At all, our strong MDR reversers are perspective *P*-gp inhibiting compounds to overcome MDR in cancer caused by the expression of *P*-gp.

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 spectra were recorded on a Varian Gemini 2000 at 400 MHz. Chemical shifts are reported in ppm units with tetramethylsilane as internal reference standard. Infrared spectra were recorded either on a Bruker or a Perkin-Elmer FITIR-spectrometer named IFS 28 and Spectrum BX, respectively. Mass spectra were recorded on a AMD INTEGRA AMD 402 mass spectrometer.

4.2. General procedure for the preparation of the 4-aryl-3-carbonyl-substituted 1,4-dihydropyridines (5–10)

Either 3-acetylpyridine (**1**) (1,21 g, 10 mmol) or ethyl nicotinate (**2**) (1.51 g, 10 mmol) were dissolved in dried THF (50 mL). Then copper(I) iodide (0.095 g, 0.5 mmol) and dried dimethyl sulfide (3 mL) were added and the mixture was cooled down to −20 °C. Then phenylchloroformate (1.57 g, 10 mmol) was added dropwise under stirring and the solution was kept stirring for additional 15 min at the low temperature.[11] Then the corresponding grignard reagents *p*-tolylmagnesium chloride (1.51 g, 10 mmol), *o*- or *p*-methoxyphenylmagnesium chloride, respectively, (1.66 g, 10 mmol) were added dropwise under stirring at the maintaining temperature. Stirring was continued for 20 min and then without cooling for additional 30 min until rt was reached. Then the reaction mixture was hydrolysed using a solution of 20% ammonium chloride (50 mL). The water phase was extracted with diethyl ether (50 mL) for three times. The organic layer was then washed twice with each 50 mL of a 20% solution mixture (1/1) of ammonium chloride/ammonia, water, 10% hydrochloric acid and, finally, with a saturated solution of sodium chloride. After drying of the organic layer over sodium sulfate the solution was filtered and evaporated to dryness in the vacuum. The resulting oil was purified by silica gel column chromatography using a mixture of toluene/methanol (85/15) as eluent.

4.2.1. 3-Acetyl-4-(4-methylphenyl)-4*H*-pyridine-1-carboxylic acid phenyl ester (5). White solid; mp 60–65 °C (methanol); yield 87%; IR (ATR) *ν* 1729 (CO); MS(EI) *m*/*z* 333 (M^+, 100%). ^{1}H NMR ($CDCl_3$) *δ* 2.24 (s br, 3H, $COCH_3$), 3.46 (s, 3H, 4′-CH_3), 4.57 (d, J = 4.6 Hz, 1H, 4-H), 5.31 (s br, 1H, 5-H), 7.00 (s br, 1H, 6-H), 7.43–7.08 (m, 9H, ArH), 8.08 (s br, 1H, 2-H).

4.2.2. 3-Acetyl-4-(2-methoxyphenyl)-4*H*-pyridine-1-carboxylic acid phenyl ester (6). Yellow solid; mp 141–145 °C (methanol); yield 71%; IR (ATR) *ν* 1742 (CO); MS(EI) *m*/*z* 349 (M^+, 62%). ^{1}H NMR ($CDCl_3$) *δ* 2.25 (s br, 3H, $COCH_3$), 3.88 (s, 3H, 2′-OCH_3), 5.01 (d, J = 4.7 Hz, 1H, 4-H), 5.35–5.39 (m br, 1H, 5-H), 6.85–6.92 (m, 3H, 6-H, 3′-, 5′-H), 7.01–7.04 (m, 1H, ArH), 7.15–7.30 (m, 4H, 4′-, 6′-H, ArH), 7.39–7.44 (m, 2H, ArH), 8.22 (s br, 1H, 2-H).

4.2.3. 3-Acetyl-4-(4-methoxyphenyl)-4*H*-pyridine-1-carboxylic acid phenyl ester (7). Yellow solid; mp 72–76 °C (methanol); yield 75%; IR (ATR) *ν* 1742 (CO); MS(EI) *m*/*z* 349 (M^+, 26%). ^{1}H NMR ($CDCl_3$) *δ* 2.25 (s br, 3H, $COCH_3$), 3.88 (s, 3H, 4′-OCH_3), 4.56 (d, J = 4.8 Hz, 1H, 4-H), 5.31 (s br, 1H, 5-H), 6.80–6.85 (m, 2H, 3′-, 5′-H), 6.88–6.96 (m br, 1H, 6-H), 7.00–7.05 (m, 1H, ArH), 7.19–7.22 (m, 2H, 2′-, 6′-H), 7.25–7.45 (m, 4H, ArH), 8.07 (s br, 1H, 2-H).

4.2.4. 3-Ethoxycarbonyl-4-(4-methylphenyl)-4*H*-pyridine-1-carboxylic acid phenyl ester (8). White solid; mp 72–78 °C (methanol); yield 70%; IR (ATR) *ν* 1742 (*N*COOPh), 1701 ($COOC_2H_5$); MS (EI) *m*/*z* 363 (M^+, 25%). ^{1}H NMR ($CDCl_3$) *δ* 1.15 (X_3 of ABX_3,

J = 7.1 Hz, 3H, 3-$COOCH_2CH_3$), 2.30 (s, 3H, 4′-CH_3), 4.05, 4,11 (AB of ABX_3, J = 10.8, 7.1 Hz, 2H, 3-$COOCH_2CH_3$), 4.46 (d, J = 4.6 Hz, 1H, 4-H), 5.24 (s br, 1H, 5-H), 6.94–6.99 (m, br, 1H, 6-H), 7.09–7.13 (m, 2H, 3′-, 5′-H), 7.16–7.29 (m, 5H, 2′-, 6′-H, ArH), 7.38–7.43 (m, 2H, ArH), 8.15 (s br, 1H, 2-H).

4.2.5. 3-Ethoxycarbonyl-4-(2-methoxyphenyl)-4*H*-pyridine-1-carboxylic acid phenyl ester (9). Yellow solid; mp 98–103 °C (methanol); yield 68%; IR (ATR) ν 1740 (*N*COOPh), 1700 ($COOC_2H_5$); MS (EI) m/z 379 (M^+, 31%). ^{1}H NMR ($CDCl_3$) δ 1.12 (X_3 of ABX_3, J = 7.1 Hz, 3H, 3-$COOCH_2CH_3$), 3.87 (s, 3H, 2′-OCH_3), 4.01, 4,13 (AB of ABX_3, J = 10.8, 7.1 Hz, 2H, 3-$COOCH_2CH_3$), 4.96 (d, J = 4.4 Hz, 1H, 4-H), 5.30 (s br, 1H, 5-H), 6.84–6.96 (m, 3H, 3′-, 5′-H, 6-H), 7.11–7.32 (m, 5H, 4′-, 6′-H, ArH), 7.38–7.42 (m, 2H, ArH), 8.28 (s br, 1H, 2-H).

4.2.6. 3-Ethoxycarbonyl-4-(4-methoxyphenyl)-4*H*-pyridine-1-carboxylic acid phenyl ester (9). Yellow solid; mp 60–63 °C (methanol); yield 81%; IR (ATR) ν 1736 (*N*COOPh), 1701 ($COOC_2H_5$); MS (EI) m/z 379 (M^+, 36%). ^{1}H NMR ($CDCl_3$) δ 1.17 (X_3 of ABX_3, J = 7.1 Hz, 3H, 3-$COOCH_2CH_3$), 3.77 (s, 3H, 4′-OCH_3), 4.02, 4,14 (AB of ABX_3, J = 10.8, 7.1 Hz, 2H, 3-$COOCH_2CH_3$), 4.45 (d, J = 4.5 Hz, 1H, 4-H), 5.25 (s br, 1H, 5-H), 6.82–6.86 (m, 2H, 3′-, 5′-H), 6.95–7.00 (m br, 1H, 6-H), 7.18–7.29 (m, 5H, 2′-, 6′-H, ArH), 7.38–7.43 (m, 2H, ArH), 8.14 (s br, 1H, 2-H).

4.3. Cell culture

L5178Y mouse T-lymphoma cell line which was a gift from the National Cancer Institute (NCI) has been infected with the PHA *mdr-1*/A retrovirus as described.[8,12] *P*-gp expressing cells have been selected by culturing the infected cells in 60 ng of colchicine. Both the L5178Y *mdr* subline and the parental cell line L5178 Y were grown in McCoys 5A medium with 10% heat activated horse serum, L-glutamine (2 nM) and antibiotics.

4.4. In vitro MDR reversal assay for *P*-gp inhibition

Cultured cells were adjusted to a concentration of 2×10^6/mL, then resuspended in serum free McCoys 5A medium and distributed into 0.5 mL aliquots in Eppendorf centrifuge tubes. After the addition of test compounds from stock solutions (1.0 mg/mL) the samples were incubated for 10 min at room temperature. Then *P*-gp substrate rhodamine 123 was added with a final concentration of 5.2 μM and then cells were incubated for additional 20 min at 37 °C. Then they were washed twice and resuspended in 0.5 mL phosphate-buffered saline (PBS). For analysis fluorescence of 1×10^4 cells were measured by flow cytometry with a Beckton Dickinson FACScan instrument. From the fluorescence data the uptake ratios have been calculated.

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References and notes

1. (a) Teodori, E.; Scapecchi, S.; Gualtieri, F. *Farmaco* **2002**, *57*, 385; (b) Krishna, R.; Mayer, L. D. *Eur. J. Pharmacol. Sci.* **2000**, *11*, 265; (c) Ambudkar, S. V.; Dey, S.; Hrycyna, C. A.; Ramachandra, M.; Pastan, I.; Gottesmann, M. M. *Annu. Rev. Pharmacol. Toxicol.* **1999**, *39*, 161.
2. (a) Dantzig, A. H.; Law, K. L.; Cao, J.; Starling, J. J. *Curr. Med. Chem.* **2001**, *8*, 39; (b) Gottesmann, M. M.; Pastan, I. *Annu. Rev. Biochem.* **2003**, *62*, 385.
3. Penzotti, J. E.; Lamb, M. L.; Evensen, E.; Grootenhuis, P. D. J. *J. Med. Chem.* **2002**, *45*, 1737.
4. (a) Mahon, F. X.; Belloc, F.; Lagarde, V.; Chollet, C.; Moreau-Gaudry, F.; Reiffers, J.; Goldmann, J. M.; Melo, J. V. *Blood* **2003**, *101*, 2368; (b) Matsui, H.; Takeshita, A.; Naito, K.; Shinjo, K.; Shigeno, K.; Maekawa, M.; Yamakawa, Y.; Tanimoto, M.; Kobayashi, M.; Ohnishi, K.; Ohno, R. *Leukemia* **2002**, *16*, 813.
5. Pauli-Magnus, C. H.; Von Richter, O.; Burk, O.; Ziegler, A.; Mettang, T.; Eichelbaum, M.; Fromm, F. W. *J. Pharmacol. Exp. Ther.* **2000**, *293*, 376.
6. Nogae, I.; Kohno, K.; Kikuchi, J.; Kuwano, M.; Akiyama, S.; Kiue, A.; Suzuki, K.; Yoshida, Y.; Cornwell, M. M.; Pastan, I.; Gottesmann, M. M. *Biochem. Pharmacol.* **1989**, *38*, 519.
7. Triggle, D. J. *Cell. Mol. Neurobiol.* **2003**, *23*, 293.
8. (a) Weaver, J. L.; Szabo, D.; Pine, P. S.; Gottesmann, S.; Goldberg, A.; Aszalos, A. *Int. J. Cancer* **1993**, *54*, 456; (b) Sharples, D.; Hajos, G.; Riedel, Z.; Csanyi, D.; Molnár, J. *Arch. Pharm. Pharm. Med. Chem.* **2001**, *334*, 269.
9. Eisner, U.; Williams, J. R.; Matthews, B. W.; Ziffer, H. *Tetrahedron* **1970**, *26*, 899.
10. Chaykin, S. *Annu. Rev. Biochem.* **1967**, *36*, 149.
11. Comins, J. D.; Stroud, E. D.; Herrick, J. J. *Heterocycles* **1984**, *22*, 151.
12. Cornwell, M. M.; Pastan, I.; Gottesmann, M. M. *J. Biol. Chem.* **1987**, *262*, 2166.